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An herbal medicine, Go-sha-jinki-gan (GJG), increases muscle weight in severe muscle dystrophy model mice

Yusei Takemoto [a], Shoya Inaba [a], Lidan Zhang [a], Kousuke Baba [b], Keisuke Hagihara [b], So-ichiro Fukada [a, *]

[a] *Laboratory of Molecular and Cellular Physiology, Graduate School of Pharmaceutical Sciences, Osaka University, 1-6 Yamada-oka, Suita, Osaka 565-0871, Japan*

[b] *Department of Kampo Medicine, Graduate School of Medicine, Osaka University, 2-2 Yamada-oka, Suita, Osaka 565-0871, Japan*

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SUMMARY

Go-sha-jinki-gan (GJG), a traditional Japanese herbal medicine has a clinical implication to alleviate age-related symptoms, especially in some motor disorders. However, the scientific evidence is limited, and there is a possibility to expand the medical application range of GJG. Using senescence-accelerated mice, our group showed that GJG exerted an effect to prevent sarcopenia, the aged-related loss of skeletal muscle. Because muscular dystrophy is characterized by a progressive loss of skeletal muscle, we examined the effects of GJG on a mouse model of muscular dystrophy. Using a newly established mouse model for Duchenne muscular dystrophy (DMD), DBA/2-*mdx*, we showed that GJG significantly increased the body and skeletal muscle weights in comparison to the control DBA/2-*mdx* mice, regardless of gender. The increased skeletal muscle mass resulted from an increment in the myofiber size, but not from the myofiber number. Both the skeletal muscle regenerative ability and the accumulation of fibrosis (the dystrophic pathology) in GJG-fed DBA/2-*mdx* mice were comparable to those in control DBA/2-*mdx* mice, suggesting that the cellular target of GJG is myofibers, with no contribution from the muscle satellite cells neither in an direct nor in an indirect manner. Taken together, GJG increased the skeletal muscle mass in a mouse model of muscular dystrophy, in addition to our previously tested sarcopenia mouse model.

* Corresponding author.
E-mail address: fukada@phs.osaka-u.ac.jp (S.-i. Fukada).

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1. Introduction

Skeletal muscle consists mainly of multinuclear myofibers and is known for a remarkable regenerative ability. In mammalians, myofibers are the terminally differentiated cells in an irreversible cell cycle state; therefore, the regenerative ability depends on the myogenic-primed mononuclear cells [1], called muscle satellite cells. Muscle satellite cells are localized in a unique anatomical position and are defined by Pax7 (Paired box 7) expression [2,3].

New myofiber generation depends completely on the muscle satellite cells. While the size of myofibers is reversible, and the states are known as muscle atrophy or hypertrophy with no satellite cell contribution [4–6]. Immobilization and some diseases, including cancer cachexia, type I diabetes, and sepsis, often lead to the atrophy of myofibers [7]. The aged-related loss and atrophy of skeletal muscle mass is known as sarcopenia. Conversely, resistance training increases skeletal muscle mass by causing hypertrophy of myofibers. Besides resistance training, some intrinsic factors regulate the size of myofibers. For example, a well-known promoting factor of muscle hypertrophy is insulin-like growth factor (IGF1), and an inhibiting factor is myostatin. IGF-1 signaling induces muscle hypertrophy via phosphatidylinositol-3-kinase (PI3K) and protein kinase B (PKB, also known as Akt). Akt signaling eventually promotes protein synthesis. Negative signaling of myostatin, a member of the transforming growth factor β (TGF-β) superfamily, regulates skeletal muscle mass via activin receptor IIB/activin-like kinase (ActRIIB/ALK4/5) receptors. Myostatin-null animals show remarkable increases in skeletal muscle mass and size of myofibers [8,9]. In myogenic cells, myostatin acts on myofibers but does not influence satellites cell/progenitors activity, suggesting that myostatin-inhibition directly affects myofibers and that the increased muscle mass play beneficial roles in preventing muscular dystrophy [10].

One representative muscular dystrophy is Duchenne muscular dystrophy (DMD), cause by a mutation in the *Dystrophin* gene. *Mdx* mice (C57BL/10ScSn-*Dmd*^{mdx}, hereafter B10-*mdx*) also have a mutation in the *dystrophin* gene and have been widely used as a mouse model for DMD. B10-*mdx* has been used in a large number of studies on pathologies of and therapeutic approach for DMD [11]. The effect of myostatin has been also evaluated using B10-*mdx* mice [12]. The diaphragm, the tissue most frequently used in pathological studies, undergoes extensive progression of degeneration, mineralization, and replacement by fibrosis and fat. In contrast, the limb muscles of B10-*mdx* show little fibrosis and fat replacement. Furthermore, in contrast to the DMD patients, the body and skeletal muscle weights of B10-*mdx* are reported heavier than those of control mice, meaning that B10-*mdx* does not adequately model to elucidate the therapeutic effects especially on the weight of skeletal muscle. Based on the background, a more suitable model had been required for investigating DMD treatments [13].

We previously found that DBA/2 inbred mice exhibited a relatively normal skeletal muscle regenerative ability after a single injury but showed a remarkable loss of skeletal muscle mass after repeated injuries. Because muscle regeneration and degeneration recurred spontaneously in *mdx* mice, we generated DBA/2-*mdx*. Intriguingly, DBA/2-*mdx* showed remarkable decreases in muscle and body weights [14]. Two different laboratories (Children's National Medical Center and The Jackson Laboratory) assessed and reproduced the decrease in the muscle weight, body weight, and a lower regenerative potential of DBA/2-*mdx* compared to B10-*mdx* [15]. Thus, using the DBA/2-*mdx* mice should be a more suitable model in evaluating dystrophic changes, including during the recovery of skeletal muscle mass with new therapeutic approaches than using the B10-*mdx* [16].

Currently, there are few known therapeutic approaches for DMD. Although the effects of herbal medicines on DMD patients have received some attentions [17], little scientific evidence is available. Recently, Kishida et al. reported that Go-sha-jinki-gan (GJG), a traditional Japanese herbal medicine, protected muscle tissues against sarcopenia in a senescence-accelerated mouse, SAMP8 [18], a widely-used model in aging research exhibiting several accelerated aging characteristics [19]. GJG is composed of 10 herbals in a fixed proportion. This medicine has been used to alleviate various types of age-related conditions in locomotion, and no severe adverse effects in humans have been reported. In addition,

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