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Original Article

Differences in strength-duration curves of electrical diagnosis by physiotherapists between DJ-1 homozygous knockout and wild-type mice: a randomized controlled pilot trial

Ju-Hyun Kim, PT, PhD[1)a], Won-Deok Lee, PT, MS[2)a], Mee-Young Kim, PT, PhD[2)], Lim-Kyu Lee, PT, PhD[2, 3)], Byoung-Sun Park, PT, MS[2)], Seung-Min Yang, PT, MS[2)], Ji-Woong Noh, PT, MS[2)], Yong-Sub Shin, PT, MS[2)], Jeong-Uk Lee, PT, PhD[4)], Taek-Yong Kwak, PhD[5)], Tae-Hyun Lee, PhD[6)], Jaehong Park, PhD[7)], Bokyung Kim, DVM, PhD[8)], Junghwan Kim, PT, PhD[9)*]

[1)] *Department of Physical Therapy, College of Health Welfare, Wonkwang Health Science University, Republic of Korea*
[2)] *Laboratory of Health Science and Nanophysiotherapy, Department of Physical Therapy, Graduate School, Yongin University, Republic of Korea*
[3)] *Commercializations Promotion Agency for R&D Outcomes, Republic of Korea*
[4)] *Department of Physical Therapy, College of Health Science, Honam University, Republic of Korea*
[5)] *Department of Taekwondo Instructor Education, College of Martial Arts, Yongin University, Republic of Korea*
[6)] *Department of Combative Martial Arts Training, College of Martial Arts, Yongin University, Republic of Korea*
[7)] *Department of Social Welfare, College of Public Health and Welfare, Yongin University, Republic of Korea*
[8)] *Department of Medicine, Institute of Functional Genomics, School of Medicine, Konkuk University, Republic of Korea*
[9)] *Department of Physical Therapy, College of Public Health and Welfare, Yongin University: Yongin 17092, Republic of Korea*

Abstract. [Purpose] Strength-duration (SD) curves are used in electrical diagnosis by physiotherapists to confirm muscle degeneration. However, the usefulness of SD curves in comparing muscle degeneration in DJ-1 homozygous knockout (DJ-$1^{-/-}$) and wild-type mice (DJ-$1^{+/+}$) is not yet fully understood. The electrical properties of the gastrocnemius muscles of DJ-$1^{-/-}$ and DJ-$1^{+/+}$ mice were compared in the current study. [Subjects and Methods] The electrode of an electrical stimulator was applied to the gastrocnemius muscle to measure the rheobase until the response of contractive muscle to electrical stimulation became visible in mice. [Results] The rheobase of DJ-$1^{-/-}$ mice showed a significant increase in a time-dependent manner, compared to that of DJ-$1^{+/+}$ mice. [Conclusion] These results demonstrate that the DJ-1 protein may be implicated in the regulation of neuromuscular activity of gastrocnemius muscles of mice.

Key words: DJ-1, Strength-duration (SD) curves, Physiotherapist

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*Corresponding author. Junghwan Kim (E-mail: junghwankim3@yongin.ac.kr)
[a)]The first two authors (Kim JH and Lee WD) contributed equally to this work.

INTRODUCTION

DJ-1 (~20 kDa), also called PARK7 and CAP-1, is a small conserved protein associated with autosomal-recessive early onset Parkinson's disease[1–3]. The human DJ-1 protein contains 189 amino acid residues[1–3] and acts as a redox sensor, which the thiol group of cysteines of DJ-1 can be oxidized to SOH, S_2OH, and S_3OH[4–6]. It was proposed that DJ-1 may prevent reactive oxygen species (ROS) accumulation by regulating the levels of other antioxidants. Furthermore, DJ-1 has multiple cellular functions such as transcriptional regulation, protease or redox-dependent chaperone activity, apoptosis and sumoylation[5–8], all of which have been shown to play a role in skeletal muscle activity. Therefore, DJ-1 appears to be an important player in skeletal muscle function during the life-span, although its mechanistic roles are yet to be determined. Skeletal muscle atrophy has proven to be a significant orthopedic problem in the area of physical therapy[9–11]. Muscle atrophy has generally been reported that immobilization-induced atrophy, especially of physiotherapeutic area, decreased muscle volume via decrease of contractile proteins, resulting in muscle weakness and disorder of activities of daily living (ADL)[9, 10, 12, 13]. In our previous study, it was demonstrated that cast-immobilization- and undernutrition-induced atrophy are correlated with each other via DJ-1 protein in skeletal muscle[13]. Furthermore, electrical properties such as rheobase and chronaxie are important factors in electrodiagnosis by physiotherapists for measurement of muscle degeneration such as total or partial muscle paralysis[14, 15]. Rheobase is measured as the threshold stimulus current for an active response with a long-duration pulse and chronaxie is the pulse width at twice the rheobase threshold current[14, 15]. However, the tendency of change in electrical activities on knockout of DJ-1 is not fully understood. Therefore, in the present study, the electrical properties of the gastrocnemius muscle of DJ-1 knockout (DJ-$1^{-/-}$) and wild-type (DJ-$1^{+/+}$) mice were compared.

SUBJECTS AND METHODS

Male DJ-1 homozygous knockout (DJ-$1^{-/-}$, B6.Cg-Park7tm1shn/J, 25–30 g; n=6) and wild-type (DJ-$1^{+/+}$; n=6) mice with the same background were purchased from Jackson Laboratory[13] (Bar Harbor, ME, USA). To confirm DJ-1 gene depletion in mice, the distal tips of tails were obtained from DJ-$1^{-/-}$ and DJ-$1^{+/+}$ mice. Genotyping using polymerase chain reaction for DJ-$1^{-/-}$ confirmation was performed with the following primers: DJ-1 forward, 5′-GCT GAA ACT GTG CCA TGT GA-3′; DJ-1 reverse, 5′-TGC TAA AGC GCA TGC TCC AGA CT-3′; Mutant Neo, 5′-TGG ATG TGG AAT GTG TGC GAG-3′. The expression of DJ-1 protein was confirmed in aortic strips using western blotting analysis[13, 16, 17]. Our investigation conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996), and all experiments and animal care conformed to the institutional guidelines established by Konkuk University, Korea. The animals were sacrificed using CO_2 inhalation and gastrocnemius skeletal muscles were removed rapidly and carefully from the limbs of animals and placed in cold Krebs solution (containing, in mM: NaCl, 118.0; KCl, 4.8; $CaCl_2$, 2.5; $MgSO_4$, 1.2; $NaHCO_3$, 24.9; glucose 10.0; KH_2PO_4 1.2) with 95% O_2 and 5% CO_2 mixed gas[13]. Rheobase and chronaxie were measured at the regions of the gastrocnemius muscles using an electrical stimulator (Duo 500, Gymnauniphy Co., Belgium). A rheobase measurement pad was applied to the regions of the gastrocnemius muscles of animals until the muscle contraction response became visible[14, 15]. Data have been expressed as the mean ± SEM. The statistical evaluation of data, using GraphPad Prism (GraphPad Software, San Diego, CA, USA), was performed using Student's t-tests for group comparisons and ANOVA for multiple comparisons. A p value of <0.05 was considered to be statistically significant.

RESULTS

The rheobase of DJ-1 knockout mice showed a significant increase in a time-dependent manner, compared to that of the wild-type mice (n=6; Table 1).

DISCUSSION

The skeletal muscle is tissue that has high potential plasticity and comprises approximately 40% of the total body weight[9, 11]. Maintenance of muscle volume and function is important for healthy life, and is important in the rehabilitation of musculoskeletal disease in the field of physical therapy[10, 11, 13]. However, as muscle mass decreases, there is an accompanying loss of muscle strength and use of nutrients which contributes to reduced muscle function and quality of ADL[18–21]. Previous studies and our reports using a model of disuse atrophy induced by cast immobilization indicated loss of muscle mass and cross-sectional area due to a decrease in the rate of protein synthesis[9–11]. The increased degradation of proteins in muscle atrophy is widely coupled with activation of protein ligases such as muscle-specific RING finger-1 (MuRF-1) and the muscle atrophy F-box protein (MAFbx, also called atrogin-1)[9–11]. Our previous data showed that the first to describe the protective functions of DJ-1 against skeletal muscle atrophy are correlated with the MAPK-ubiquitin ligase (both MuRF-1 and atrogin-1) pathway[13]. In the present study, it was found that DJ-$1^{-/-}$ increased the rheobase in a time-dependent manner. Based on these results, it is cautiously speculated that the increment in the rheobase of DJ-$1^{-/-}$ mice

Table 1. Differences in the rheobase of DJ-$1^{-/-}$ and DJ-$1^{+/+}$ mouse gastrocnemius muscles

ST (ms)	Rheobase (mA)		ST (ms)	Rheobase (mA)	
	DJ-$1^{+/+}$	DJ-$1^{-/-}$		DJ-$1^{+/+}$	DJ-$1^{-/-}$
0.1	3.60 ± 0.14	5.93 ± 0.84*	50	1.37 ± 0.05	2.12 ± 0.29*
0.2	2.58 ± 0.08	4.35 ± 0.69*	70	1.37 ± 0.05	2.12 ± 0.29*
0.5	2.12 ± 0.11	3.50 ± 0.48*	100	1.37 ± 0.05	2.10 ± 0.29*
1	1.55 ± 0.10	2.47 ± 0.38*	200	1.37 ± 0.05	2.10 ± 0.29*
2	1.37 ± 0.05	2.22 ± 0.26*	300	1.37 ± 0.05	2.10 ± 0.29*
5	1.37 ± 0.05	2.13 ± 0.28*	500	1.37 ± 0.05	2.10 ± 0.29*
10	1.37 ± 0.05	2.12 ± 0.29*	700	1.37 ± 0.05	2.08 ± 0.29*
20	1.37 ± 0.05	2.12 ± 0.29*	1,000	1.37 ± 0.05	2.07 ± 0.30*
30	1.37 ± 0.05	2.12 ± 0.29*	-	-	-

Mean ± standard error. ST: stimulative time. *Significantly different from DJ-$1^{+/+}$ for controls with $p<0.05$.

aids in decrease of neuromuscular activities and disorder of movements. This result is similar to that of a previous study that reported that 24-month-old DJ-$1^{-/-}$ mice showed shorter stride lengths than wild-type mice[22]. Moreover, DJ-$1^{-/-}$ mice exhibit loss of Ca^{2+} homeostasis, such that decrease in depolarization evoked Ca^{2+} release from the sarcoplasmic reticulum in the DJ-1 null muscle cells, implying that DJ-1 plays a critical role in calcium regulation of skeletal muscle cells[23]. However, further systematic and scientific studies in the fields of physical therapy, such as exercise therapy, electrotherapy, neurophysiotherapy, and hydrotherapy, are needed to confirm the mechanisms underlying the effects of DJ-1 in atrophied muscle strips and cells[24–27]. In summary, the rheobase of DJ-1 knockout mice was significantly higher than that of wild-type mice. The present results suggest that DJ-1 affects the important play a role in muscle activity[13, 22, 23, 26].

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