International Journal of Physical Medicine & Rehabilitation

Iwamoto, et al., Int J Phys Med Rehabil 2019, 7:6

Research Article Open Access

Effect of Frequency-Modulated Repetitive Peripheral Magnetic Stimulation (rPMS) on Leg Muscle Atrophy in Animal Model

Yosuke Iwamoto[1*], Naohisa Miyakoshi[1], Toshiki Matsunaga[2], Daisuke Kudo[1], Kimio Saito[2], Junpei Iida[1], Yasuhiro Takahashi[1], Junichi Inoue[1], Motoki Mita[1], Shohei Murata[1], Rena Wakabayashi[1] and Yoichi Shimada[1]

[1]Department of Orthopaedic Surgery, Akita University Graduate School of Medicine, Akita, Japan

[2]Department of Rehabilitation Medicine, Akita University Hospital, Akita, Japan

[*]**Corresponding author:** Yosuke Iwamoto, Department of Orthopaedic Surgery, Akita University Graduate School of Medicine, 1-1-1, Hondo, Akita-shi, Akita, 010-8543, Japan, Tel: +81-18-884-6148; E-mail: iwa_motio55@med.akita-u.ac.jp

Received date: November 21, 2019; **Accepted date**: December 06, 2019; **Published date**: December 12, 2019

Abstract

Objective: To investigate the effect of frequency-modulated repetitive peripheral magnetic stimulation (rPMS) for disuse muscle atrophy in the animal model.

Methods: Thirty-five 6-week-old male SD rats were randomly assigned to 5 groups: the control group (C group), 20-Hz constant-stimulation group (20 Hz group), 5-Hz constant-stimulation group (5 Hz group), 20-Hz/5-Hz modulated-stimulation group (20 Hz/5 Hz group), and non-stimulation group (N group). Atrophy of hind limb muscles was induced by tail suspension for 5 weeks. For the stimulation groups, rPMS to the right sciatic nerve were performed for 30 min a day for 2 weeks. Variables assessed were isometric maximum muscle contraction force and the degree of muscle fatigue of the soleus muscle, muscle weight of tibialis anterior.

Results: The isometric maximum muscle tension was 28.5 ± 3.8 in the 20 Hz group, 20.1 ± 3.8 in the 5 Hz group, 30.6 ± 2.8 in the 20 Hz/5 Hz group, and 21.1 ± 2.3 in the N group. Muscle fatigue in the 20 Hz and the 20 Hz/5 Hz group was significantly lower than in the N group ($p<0.05$). Muscle weight was 0.56 ± 0.09 in the 20 Hz group, 0.45 ± 0.91 in the 5 Hz group, 0.67 ± 0.79 in the 20 Hz/5 Hz group, and 0.42 ± 0.71 in the N group.

Conclusion: The 20-Hz/5-Hz frequency-modulated rPMS for atrophied leg muscles in the rat tail suspension model improved muscle atrophy equal to or better than 20-Hz constant-frequency stimulation and more than 5-Hz constant-frequency stimulation.

Keywords: Muscle atrophy; Repetitive peripheral magnetic stimulation; Frequency; Animal study

Introduction

Disuse syndrome is a collective term for disorders caused by reduced physical and mental activity due to long-term bed rest [1]. Disuse syndrome has various adverse effects on the musculoskeletal, respiratory, circulatory, urinary/digestive, metabolic/endocrine, immune, and cognitive/behavioural systems [2]. In particular, when muscle activity decreases due to inactivity, muscle atrophy (disuse muscle atrophy) occurs, leading to a decline in motor function, thus promoting additional inactivity. The function of various body systems declines in such conditions, and inactivity thus progresses further; as a result, the state of the disease may worsen or require more time for recovery [3]. Therefore, it is important to suppress the progression of disuse muscle atrophy and obtain more rapid recovery to improve the prognosis of disuse syndrome.

Management for disuse muscular atrophy includes strengthening exercises [4], electrical stimulation [5,6], and magnetic stimulation [7-9]. Magnetic stimulation generates a magnetic field around a pulsed current through a magnetic stimulation coil and stimulates the central nervous system or peripheral motor nerves [10]. In contrast to electrical stimulation, magnetic stimulation can cause muscle contractions in a non-contact manner via neuromuscular stimulation with magnetic pulses from a distant area [11]. Magnetic stimulation has been shown to prevent, aid recovery from, and improve muscle atrophy through continuous muscle contractions induced via the application of continuous magnetic stimulation to peripheral motor nerves. Sakuraba et al. reported that intermittent repeated magnetic stimulation with a frequency of 20 Hz prevented muscle atrophy on disused muscles in the rat tail suspension model. Baek et al. performed 3 weeks of intermittent repeated magnetic stimulation with a frequency of 10 Hz to the vastus lateralis muscle of patients after hip replacement surgery, and reported improved muscle strength, standing balance, and walking function compared to the control group [12]. In recent years, such a stimulation method had been referred to as "repetitive peripheral magnetic stimulation (rPMS)," and reports of animal experiments and clinical studies are increasing. However, previous reports only covered constant-frequency stimulation [7-9,12-14].

Skeletal muscle is composed of fast and slow muscle fibres, and the optimal contractile response frequency of each muscle is different [15]. In electrical stimulation, slow muscles are mainly excited by low-frequency stimulation of 5-20 Hz, and fast muscles are mainly excited by high-frequency stimulation of 20-60 Hz [16]. Therefore, it is possible that the stimulation effect may be higher when combining different stimulation frequencies than when using a constant

frequency. We hypothesized that a frequency-modulated stimulation method would be more effective in suppressing atrophy of the disused atrophy muscle than the constant-frequency stimulation method.

The purpose of this study was to investigate the effect of frequency-modulated rPMS on atrophied muscles due to disuse of lower limbs in a rat tail suspension model.

Materials and Methods

Animals

Thirty-five 6-week-old male Sprague-Dawley (SD) rats (Japan SLC, Shizuoka, Japan) with an average body weight of 202 g (range, 180-230 g) were used in these experiments. Animals were assigned to 1 of 5 groups: group 1, control group (C group); group 2, 20-Hz constant-stimulation group (20 Hz group); group 3, 5-Hz constant-stimulation group (5 Hz group), group 4, 20-Hz/5-Hz modulated-stimulation group (20 Hz/5 Hz group), and group 5, non-stimulation group (N group) (Figure 1). The animals were individually housed under a 12-h light/dark cycle and given ad libitum access to food and water.

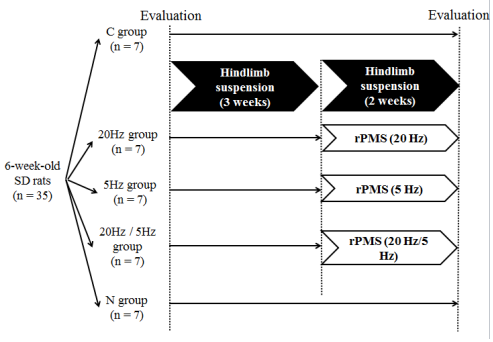

Figure 1: Experimental protocol. C: Control; N: Non-stimulation; rPMS: repetitive Peripheral Magnetic Stimulation.

Hind limb suspension

In groups 2 through 5, the tail suspension procedure was performed in accordance with the recommendations by Holton and Globus [17] using the tail suspension system (RTC2010 type, Yamashita Giken, Tokushima, Japan). The overhead trolley system allowed 360° of rotation and was adjusted to keep the rat trunk at 30° of inclination, thus permitting the animal to move around to reach for food and water, but not touching the cage floor or walls with the hind limbs. The rats were kept in suspension for total 5 weeks to create disuse muscle atrophy in the hind limb muscles.

Protocol of the rPMS

From 3 weeks after tail suspension, rPMS were performed for (2-4) groups (20 Hz, 5 Hz, and 20 Hz/5 Hz groups) for 2 weeks. General anaesthesia was induced by intraperitoneal injection of xylazine hydrochloride (Sederac; Nippon Zenyaku Kogyo, Fukushima, Japan) and ketamine hydrochloride (Ketalar; Daiichi Sankyo Propharma, Tokyo, Japan). Under general anaesthesia, rPMS (stimulator: MagPro R100 MCF-B65, butterfly coil: MagVenture) was continued daily for 30 minutes for the right sciatic nerve. Stimulation was provided at an intensity of 1.0 T and was verified by ankle motion on the stimulated side. The cycle of stimulation was as follows: 20 Hz group, 20 Hz of magnetic stimulation for 10 sec, rest for 5 sec, repeat; 5 Hz group: 5 Hz of magnetic stimulation for 10 sec, rest for 5 sec, repeat; 20 Hz/5 Hz group, 20 Hz for 10 sec, rest for 5 sec, then 5 Hz of magnetic stimulation for 10 sec, rest for 5 sec (Figure 2). After the rPMS, the rats were returned to the suspension.

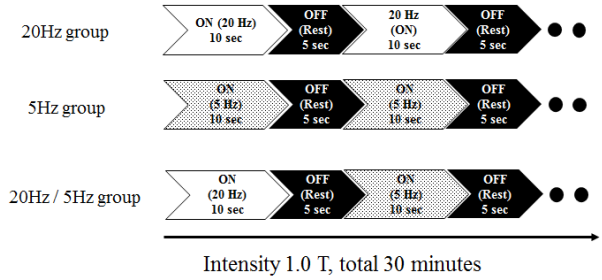

Figure 2: Protocol of the repetitive peripheral magnetic stimulation (rPMS).

Evaluations

Body weight was assessed in all groups. Muscle strength and fatigability of the soleus muscle and weight of the tibialis anterior (TA) muscle were assessed in suspension group (2)-5)).

Body weight was measured at the begging of the suspension (initial) and five weeks later (5w) (Keimaiko; Yamato-scale, Hyogo, Japan).

Muscle strength and fatigability of right soleus muscle were measured at 5 weeks later of the tail suspension under general anaesthesia. After opening the posterior surface of the right leg to expose the sciatic nerve in the gluteal region, a bipolar cuff electrode (inter-electrode distance, 5 mm; MT Giken, Tokyo, Japan) was attached to the sciatic nerve. The rat was immobilized on a platform. The distal end of the Achilles tendon was exposed and cut at the insertion to the calcaneus. A transducer (ZPS-DPU; Imada, Aichi, Japan) was attached and fixed next to the stump with a load of 0.3 N [18]. Signals transmitted from the force transducer during isometric muscular contraction were digitally recorded on a force-time curve (ZP-Recorder; Imada). To prevent muscle desiccation during the study, the exposed area was covered with gauze moistened with saline. The study was conducted at a constant temperature of 25°C-27°C. To obtain a tetanic contraction, muscle contraction was induced by a monophasic rectangular pulse with a frequency of 40 Hz, a pulse width of 0.2 ms, and a stimulation intensity of 4 V. Each stimulation lasted 180 sec, as previously described [19]. We recorded muscle strength every 10 sec after the start of stimulation, and this was defined as the maximum isometric contraction tension of the calf muscles. A strength decrement index (SDI) was used to assess muscle fatigue [20], calculated as follows: SDI (%)=(initial contractile tension in the period of stimulation [Ti]-contractile tension at each second from initial stimulation [Tt]) × 100/Ti. This formula provides the attenuation of torque from the beginning of stimulation (Figure 3). A high SDI indicates greater muscle fatigue.

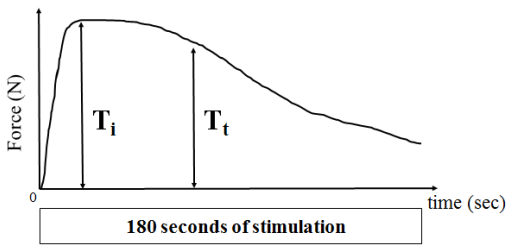

Figure 3: Strength Decrement Index (SDI) calculation method.

Weight and of right tibialis anterior muscle were measured after the evaluation of muscle strength and fatigability of right soleus muscle. Rats were euthanized by an injection of sodium pentobarbital (150 mg/kg body weight), and muscle weight was measured (Keimaiko; Yamato-scale, Hyogo, Japan).

The protocol for all animal experiments was approved in advance by the Animal Research Committee of our institute, and subsequent animal experiments adhered to the "Guidelines for Animal Experimentation" of the university.

Statistical analysis

All statistical analyses were performed with IBM SPSS Statistics software (version 24, Chicago, IL, USA). Continuous variables were expressed as mean ± standard deviation (SD). Differences between groups at each time point were evaluated using one-way analysis of variance. Multiple comparisons were made using Scheffe's and Dunn's post hoc tests, as appropriate. Nonparametric data, including muscle weight and isometric muscle strength and muscle fatigue were analysed by Dunn's method. Parametric data including body weight was analysed by Scheffe's method. Values of $p<0.05$ were considered significant.

Results

Body weight

Initially, there were no statistically significant differences in body weight between groups. At 5 weeks, group C was significantly heavier than all other groups ($p<0.0001$), confirming that there was a change related to muscle disuse due to tail suspension (Table 1).

	C	20 Hz	5 Hz	20 Hz/5 Hz	N	ANOVA
Initial	207.1 ± 17.3	193.6 ± 8.5	210.0 ± 15.5	195.7 ± 10.1	202.9 ± 7.6	p=0.08
5 w	309.3 ± 20.1*	222.1 ± 24.5	221.4 ± 17.5	229.3 ± 15.4	236.4 ± 18.1	p<0.0001

ANOVA, one-way analysis of variance

*Significantly different from other groups, p<0.0001 by Scheffe's method.

Table 1: Mean ± SD body weight (g) at baseline and after 5 weeks of suspension.

Muscle strength and fatigability of soleus muscle

The isometric maximum muscle tension was 28.5 ± 3.8 in the 20 Hz group, 20.1 ± 3.8 in the 5 Hz group, 30.6 ± 2.8 in the 20 Hz/5 Hz group, and 21.1 ± 2.3 in the N group. Values in the 20 Hz and 20 Hz/5 Hz groups were significantly higher than in the 5 Hz and N groups ($p<0.05$ for both), but were not significantly different from each other. There was no significant difference between the 5 Hz group and the N group (Figure 4).

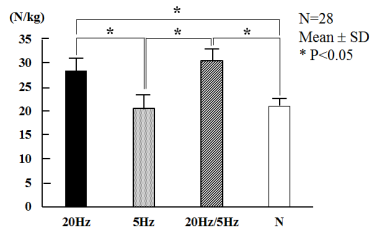

Figure 4: Isometric maximum muscle tension of the rat soleus.

Figure 5 shows the time course of SDI (%) in the muscle fatigue test. Values in the 20 Hz and 20 Hz/5 Hz groups were significantly smaller than in the N groups ($p<0.05$), but were not significantly different from each other. Only in the 20 Hz/5 Hz group, the SDI was significantly smaller than the 5 Hz group ($p<0.05$) (Table 2).

Weight of tibialis anterior muscle

Muscle weight was 0.56 ± 0.09 in the 20 Hz group, 0.45 ± 0.91 in the 5 Hz group, 0.67 ± 0.79 in the 20 Hz/5 Hz group, and 0.42 ± 0.71 in the N group. Values in the 20 Hz and 20 Hz/5 Hz groups were significantly higher than in the N groups ($p<0.05$ for both), but were not significantly different from each other. The 20 Hz/5 Hz group was significantly heavier than the 5 Hz group ($p<0.05$). There was no significant difference between the 5 Hz group and the N group (Figure 6).

Time	20 Hz	5 Hz	20 Hz/5 Hz	N	ANOVA
180 s	59.0 ± 11.2*	65.5 ± 10.0**	50.7 ± 9.4*	74.8 ± 6.0	p=0.0015

Values (%) represent mean ± SD.

ANOVA. One-way analysis of variance.

*Significantly different from non-stimulation group, p<0.05 by Dunn's method.

**Significantly different from 20 Hz/5 Hz group, p<0.05 by Dunn's method.

Table 2: Strength decrement index (SDI) (%) at the end of soleus muscle fatigue tests.

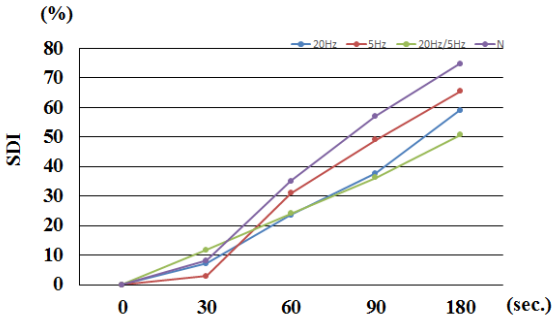

Figure 5: Time course of SDI (%).

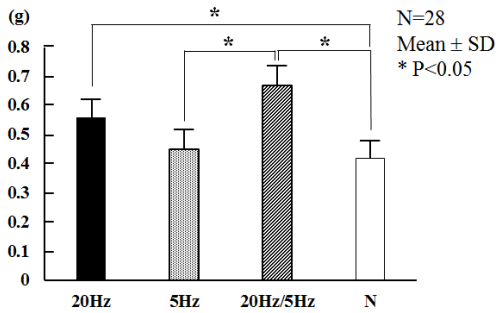

Figure 6: Wet weight of the rat anterior tibial muscle.

Discussion

This study was the first one to test the effect of frequency-modulated rPMS on the atrophied muscle of the lower limb instead of the conventional constant-frequency stimulus [7,12,14].

Muscle strength and fatigability

Various reports have shown that constant-frequency electrical stimulation [21] and magnetic stimulation [8] have an effect on muscle atrophy. In this study, the isometric maximum muscle tension was significantly higher in the 20 Hz and 20 Hz/5 Hz group than in the 5 Hz and N group. On the other hand, there was no significant difference the 20 Hz group and the 20 Hz/5 Hz group. This result is significant in that it shows that 20-Hz/5-Hz frequency-modulated stimulation can recover atrophy muscle as well as conventional 20-Hz constant-frequency stimulation. In the muscle fatigue test, the SDI of 20 Hz and 20 Hz/5 Hz group were significantly smaller than the N group, suggesting that fatigue resistance had also recovered. On the other hand, there was no significant difference the 20 Hz group and the 20 Hz/5 Hz group. Moreover, the SDI in the 20 Hz /5 Hz group was significantly smaller than the 5 Hz group. From these results, there is a possibility that frequency-modulated rPMS have a potential to suppress atrophy of the disused atrophy muscle than the constant-frequency stimulation method. Future studies are needed to confirm our findings.

Weight analysis of muscle

The statistical results of weight analysis of TA muscle were same as soleus muscle fatigability. From these results, it can be said that the degree of recovery from muscle atrophy was better in the following order: the 20 Hz/5 Hz group, followed by the 20 Hz group, and then the 5 Hz group. Muscle hypertrophy is closely related to the total number of pulses applied [22]. The total number of stimulation pulses was greater in the 20 Hz group than in the 20 Hz/5 Hz group, but the recovery of muscle weight (and hence muscle hypertrophy) was greater in the 20 Hz/5 Hz group. On the other hand, comparison between the constant-frequency stimulation groups showed that the muscle weight of the 20 Hz group was significantly higher than that of the 5 Hz group. This result suggests that frequency-modulated stimulation contributes to the recovery of muscle atrophy more than constant-frequency stimulation. Further studies are needed to confirm our findings.

In a review paper on electrical stimulation, Maffiuletti et al. reported that constant-frequency stimulation of muscle fibres with various frequency response performances with voluntary contractions may limit muscle stimulation effects [23]. Frequency-modulated stimulation may have an effect on disuse muscle atrophy that cannot be obtained by constant-frequency stimulation alone. In addition, rPMS cause less pain during stimulation than electrical stimulation [24] and have the advantage of being able to flexibly change stimulation frequencies. In this study, the combination of 20 Hz and 5 Hz was selected as the frequency-modulated stimulus. Misawa and colleagues examined the electrical stimulation effect on the spinal cord of paralyzed muscles in injured rats, and reported the possibility that the effect was higher at 100 Hz than at 20 Hz. [5]. In the future, it is expected that the optimal stimulation method for disuse muscle atrophy will be established by examining a wider range of frequencies, including 100 Hz.

Sakuraba and colleagues examined the genes expressed in muscle by performing 20 Hz constant frequency rPMA on rat atrophy muscles, and reported that decreased mRNA expression of MHCIβ and MHCIIa, while mRNA of MHCIIB and MHCIId (x) was not decreased [7]. They concluded that the down-regulation of MHCIIB and MHCIId (x) was suppressed by magnetic stimulation. In the future, we would like to elucidate the physiological mechanism of the effect of frequency-modulated stimulation by evaluating the gene expression in disuse muscle atrophy.

Limitations of this study include the evaluation of muscle mass and tissue with the TA muscle and the isometric muscle contraction and muscle fatigue tests with the soleus muscle due to the nature of the experiment. There is a difference in the distribution of muscle composition between the TA muscle and the soleus muscle of the rat, which may have influenced the interpretation of the results.

Conclusion

In the rat tail suspension model, 20-Hz/5-Hz frequency-modulated rPMS for atrophied leg muscles in the rat tail suspension model improved muscle atrophy equal to or better than 20-Hz constant-frequency stimulation and more than 5-Hz constant-frequency stimulation.

Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Acknowledgments

The authors thank the members of the Department of Orthopedic Surgery, Akita University Graduate School of Medicine and the Department of Rehabilitation Medicine, Akita University Hospital for their cooperation.

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