Effects of therapeutic magnetic stimulation on acute muscle atrophy in rats after hindlimb suspension

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ABSTRACT

In most subjects with spinal cord injury, the spinal neurons below the level of injury are spared. Therefore, it is conceivable that the skeletal muscles innervated by these spinal nerves can be activated by applying therapeutic magnetic stimulation along the dorsal spine. The purpose of this study was to evaluate the ability of magnetic stimulation to prevent acute muscle atrophy in rats after hindlimb suspension. Forty adult male Wistar rats were randomly assigned to stimulated and non-stimulated (control) groups. Their hindlimbs were unweighted using a suspension method, causing muscle atrophy. In the stimulation group, magnetic stimulation (20 Hz, 60 min per day) was applied to the sciatic nerve for 10 days. After the stimulation period, the tibialis anterior (TA) and extensor digitorum longus (EDL) were surgically removed and histologically measured. The lesser diameters of type 1, 2A, and 2B muscle fibers were significantly greater in the stimulated group than in the non-stimulated group for both the TA and EDL (p < 0.05). The mean difference in lesser fiber diameter was 20% (range, 14%-27%). These results suggest that therapeutic magnetic stimulation is an effective method of preventing muscle atrophy.

Functional electrical stimulation (FES) is a method of restoring functionality to upper or lower extremities by electrically stimulating the lower motor neurons of patients with paraplegia, quadriplegia or hemiplegia. FES has been successfully used to restore eating and writing ability in quadriplegics, and walking ability in paraplegics (1, 8, 12, 15, 19, 20). However, many patients with upper motoneuron injury have difficulty performing useful muscle contractions (e.g., standing, walking) because their muscles are atrophied and weak (22).

To prevent muscle atrophy and contracture of joints, it is important to preserve muscle power

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when restoring function of paralyzed extremities with FES (13). To restore ambulatory function in paraplegic patients, therapeutic electric stimulation (TES) has been performed to restore muscle strength and to determine the tolerance of muscle fatigue before FES is applied (9). One principle difference among the various approaches to restoration of paralyzed muscles is the use of surface versus internal electrodes for stimulation. The disadvantages of surface electrodes include loosening, skin irritation, poor cosmetic appearance, and the inability to stimulate deep muscles (e.g., iliopsoas muscle). Surface electrodes can also be unreliable due to habituation of the reflex and because small differences in electrode placement can create large differences in response. The advantages of percutaneous or implantable electrodes include 1) selective activation of individual paralyzed muscles for control of fine, precise movements with high reliability and repro-

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ducibility, 2) ease of use when implanted in the body, and 3) stimulation of deep muscles. However, because intramuscular electrical stimulation requires electrode placement under general anesthesia, it carries the risk of infection in the penetrated region as the electrodes migrate, and it can be painful to patients with sensation below the level of injury. Another disadvantage of implantable systems is that additional surgery is required in the case of functional loss due to breakage or movement of the electrodes.

Recently, functional magnetic stimulation (FMS) has been developed into a non-invasive technique for stimulating the brain (2), and has been used to stimulate phrenic nerves, spinal nerves (10), and the cerebral cortex (3, 14). Studies indicate that FMS has potential as a tool for treating depression and other central nervous system disorders.

In most subjects with spinal cord injury, the spinal motor neurons below the level of injury are preserved. Therefore, it is conceivable that the skeletal muscles innervated by these spinal nerves can be activated by applying therapeutic magnetic stimulation (TMS) along the dorsal spine. The purpose of the present study was to evaluate the ability of magnetic stimulation to prevent acute muscle atrophy in rats.

MATERIALS AND METHODS

We used 40 adult male Wistar ST rats with an average body weight of 236 g (range, 210–249 g). The animals were assigned to 2 groups: the stimulated group (n = 20), and the non-stimulated (control) group (n = 20). All rats were suspended by their tails through the silks, and their hindlimbs were unweighted to cause their muscles to atrophy. For the hindlimb suspension, the rats were deeply anaesthetized with an intraperitoneal injection of pentobarbital sodium (40 mg/kg).

TMS was performed using a commercially available magnetic stimulator (Daiya Industry Co., Tokyo, Japan), with magnetic coils with an average diameter of 30 mm. This stimulator can generate a maximum field strength of 1.0 tesla near the magnetic coil. A computer with an interactive program written specifically for activation of the stimulator was used to control the frequency and length of the stimulation. The continuous stimulation parameters were set at 750 V (about 93% of maximum intensity of this system) and 20 Hz. The magnetic coil was supported by an adjustable frame. We were able to keep the center of the coil positioned at L3–L5 beside the midline for initial lumbosacral stimulation, which was varied to determine optimal stimulation (causing maximal movement of the hindlimbs) (Fig. 1).

Stimulation was performed for 60 min/day, for 10 days. The animal care protocol for this study conformed to Akita University's guidelines for institutional animal care and the guidelines of the US National Institutes of Health (NIH). Magnetic stimulation of the stimulated group began 1 day after the operation. The day after the stimulation ended, the rats were sacrificed by anaesthesia, and the tibialis anterior (TA) and extensor digitorum longus (EDL) muscles were surgically removed from both legs. Muscle samples (10-mm-thick cross-sections) were taken from the region of maximum circumference of each muscle. Cross-sections of those samples were also obtained. All samples were rapidly frozen in 2-methylbutane (isopentan), and were stored in liquid nitrogen at -80°C until they were analyzed.

Samples were cut into 10-µm-thick serial sections in a cryostat maintained at -15°C. Transverse serial sections were stained using a histochemical method (adenosine triphosphatase) with preincubation at pH 4.4. Type 1, type 2A, and type 2B fibers were identified according to the criteria of Dubowitz and Brooke (7). The lesser diameters of 150 fibers from each muscle fiber type were measured using a Mac SCOPE (MITANI Co. Tokyo, Japan). We first analyzed the distribution of the muscle types, and then measured the lesser diameters of muscle fibers. This method of measurement was designed to correct for the distortion of a muscle fiber into an oval shape when it was cut obliquely (11). The data were reported as mean ± standard deviation (SD). Differences in lesser diameters between each muscle type



Fig. 1 Experimental position during therapeutic magnetic stimulation

were statistically evaluated using Student's *t*-test. A probability value of p < 0.01 was considered to indicate significance.

RESULTS

Tibialis anterior (TA)

The mean lesser diameters of type 1, type 2A, and type 2B muscle fibers in the stimulated group were $31.4 \pm 9.7 \ \mu\text{m}$ (mean \pm SD), $28.9 \pm 7.2 \ \mu\text{m}$, and $33.0 \pm 7.2 \ \mu\text{m}$, respectively; in the non-stimulated group, they were $24.8 \pm 4.7 \ \mu\text{m}$, $24.6 \pm 4.1 \ \mu\text{m}$, and $28.7 \pm 6.8 \ \mu\text{m}$. For all 3 muscle fiber types, there was a significant difference in fiber diameter between the stimulated and non-stimulated muscles (p = 0.001 for type 1, p < 0.001 for types 2A and 2B) (Fig. 2A).

Extensor digitorum longus (EDL)

The mean lesser diameters of type 1, type 2A, and type 2B muscle fibers in the stimulated group were $30.7 \pm 6.6 \,\mu\text{m}$ (mean \pm SD), $30.2 \pm 6.8 \,\mu\text{m}$, and $35.0 \pm 7.9 \,\mu\text{m}$, respectively; in the non-stimulated group, they were $24.6 \pm 3.9 \,\mu\text{m}$, $22.9 \pm 2.9 \,\mu\text{m}$, and $25.7 \pm 3.9 \,\mu\text{m}$. For all 3 muscle fiber types, there was a significant difference in fiber diameter between the stimulated and non-stimulated muscles (p < 0.001) (Fig. 2B). Figs. 3A and 3B show examples of muscle fibers from the non-stimulated and stimulated groups, respectively.

DISCUSSION

In the past decade, neurophysiologists have used magnetic stimulation clinically as a relatively safe and non-invasive method for stimulating nervous tissues (4). Magnetic stimulation applies Farady's law, which states that a change in a magnetic field induces an electric field that opposes the change in the magnetic field. The induced electric field creates an eddy current that can stimulate nerves or muscles. Magnetic stimulation has been used to stimulate peripheral nerves, spinal nerves, cranial nerves, and the cerebral cortex (2). Recently, studies have been conducted to assess the potential of magnetic stimulation as a tool for treating depression and other central nervous system disorders.

Electrical stimulation requires electrode placement, and can be painful to patients with preserved sensation below the level of injury. Magnetic stimulation has the advantage of being able to stimulate deep structures without discomfort to the patient, and it can be applied relatively easily without physical contact. FMS is a noninvasive, painless, and



Fig. 2 The mean lesser diameter of type 1, type 2A and type 2B muscle fibers of (A) tibials anterior (TA) and (B) extensor digitorum longus (EDL). Values are expressed as mean \pm SD.

simple procedure. It can stimulate the spinal nerves when applied outside the clothing, producing contraction of the innervated musculature (11, 16).

Animal studies show that immobilization of the lower limbs can cause muscle atrophy ranging from 15% to 70%, with most muscle atrophy occurring during the first 7 days of immobilization (17). Therapeutic electrical stimulation (TES) or magnetic stimulation (TMS) can help prevent muscle atrophy after spinal cord injury, if it is performed during the acute phase. In animal models of muscle disuse such as space flight (5), spinal cord transaction (23), and hindlimb suspension (6), muscle atrophy progresses at an extremely high rate during the first several months. In a recent study, thigh girth decreased by 50% within 3 weeks after spinal cord injury, suggesting that nearly all atrophy occurs within the first month after spinal cord injury (21).

Muscle atrophy in patients with spinal cord injury involves a progressive decrease in fiber diameter and changes in fiber type distribution. In the early



Fig. 3 Microscopic view of muscle fibers from non-stimulated (A) and stimulated (B) EDL muscles.

stage after cord transaction, atrophy predominantly occurs in type 2 muscle, whereas later-stage atrophy predominantly occurs in type 1 muscle (18). Restoration of paralyzed muscles using TES requires an increase in muscle fiber diameter. Muscle power should be maintained during TES, which should produce an increase muscle fiber size. Occurrence of muscle atrophy before application of FMS increases the time required for the muscles to return to near-normal condition. In the present study, we found significant differences in muscle fiber diameters of type 1. 2A and 2B fibers of the TA and EDL between non-stimulated and stimulated muscles. These results suggest that TMS is an effective method of reducing muscle atrophy after spinal cord injuries.

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