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Effect of Exercise on Intermediate Filament Expression in the Skeletal Muscles of Rats with Sciatic Nerve Injury

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Abstract. [Purpose] This study examined the effect of exercise on intermediate filament expression in rat gastrocnemius muscles. [Subjects] After administering a crush injury to the sciatic nerve of male Sprague-Dawley rats, the rats were divided into experiment and control groups. [Methods] The experiment and control groups were made to exercise for 60 minutes daily for 5 days per week. Rats were sacrificed at 12 days, 19 days, 26 days and 61 days after sciatic nerve crush injury. Frozen sections of the gastrocnemius muscle were prepared. Immunohistochemical staining for desmin and vimentin, and enzyme histochemistry for nicotinamide adenine dinucleotide-tetrazolium reductase (NADH-TR) were used to visualize degeneration of skeletal muscles. [Results] Desmin was expressed during muscle degeneration and regeneration, whereas vimentin was expressed only during muscle regeneration. Muscle fibers in the experimental group were normal at 61 days after injury. In the NADH-TR reaction, the control group showed aggregated diformazan but the experimental group showed normal evenly distributed diformazan granules. At reinnervation, target fibers were found by the NADH-TR reaction in animals sacrificed 26 days after injury. [Conclusion] Our results indicate that running is an effective exercise for inducing the expression of intermediate filaments during regeneration of skeletal muscles.
Key words: Desmin, Vimentin, NADH-TR

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INTRODUCTION

During muscle regeneration, intermediate filaments play an important role in the muscle cytoskeleton[1)]. Desmin and vimentin are known to react more strongly with immature muscle fibers than with mature muscle fibers[2)], but there are divergent opinions on the appearance and duration of intermediate filaments in demyelinated muscle fibers[3)]. Exercise is the basic therapy employed to prevent complications such as muscle contracture and atrophy, and to help in functional recovery[4)]. Endurance exercise not only increases the number and density of capillaries and mitochondria but also induces muscle fiber growth and conversion[5)]. In animal models of nerve injury, exercise influences the recovery of motor and sensory functions. Many studies have reported parameters of muscle and nerve function, but the results have varied with the type of exercise, onset of exercise and the intensity and duration of exercise. Occasionally, conflicting results have been published. The present study aimed to examine the regeneration of skeletal muscles after crush injury (CI) to the rat sciatic nerve, which shows intermediate filament expression.

SUBJECTS AND METHODS

Twenty-two male Sprague-Dawley rats (experimental group, 10; control group, 10; normal group, 2) were used in this study. The rats were 1- month- old and their mean body weight was 166 ± 28 g. They were fed with standard laboratory chow and given water ad libitum; they were housed in a temperature-controlled room (21 ± 3°C) with even light-dark cycles (12: 12 h). The procedures conducted on the animals were approved by the Ethical Committee for Animal Experiments of Dong-A University. The experimental group of SD rats was trained on a treadmill (Dual treadmill, model DJ2-2429; Daejong Instrument, Korea) at a speed of 18 m/minute for 30 minutes per day for 1 week. Each group was exercised 60 minutes daily, 5 times a week. The slope of the treadmill was 10°, and its speed was 20 m/min (50% VO2max)[6)]. Sciatic nerve CI was administered to the animals in the experimental group at 12 days (after 5 days of exercise), 19 days (after 10 days of exercise), 26 days (after 15 days of exercise), 33 days(after

20days of exercise) and 61 days (after 40 days of exercise) after injury. In each experimental group, 2 rats were exercised. The control group comprised 10 rats that were not exercised after CI. The normal group consisted of 2 rats with no CI and no exercise. To administer CI, rats were anesthetized. The sciatic nerve was exposed 5–6 mm distal to the ischial tuberosity and crushed constantly for 30 successive seconds using hemostatic forceps[7]. At 12, 19, 26, 33, and 61 days after injury, rats were sacrificed, and the gastrocnemius muscle was excised. The middle region of the gastrocnemius muscle was excised between the muscle origin and insertion. To prepare the tissue section, rats were anesthetized and the muscle was frozen for 10 seconds and excised. Frozen muscle tissue was dissected using Cryocut (Reichert-Jung, Germany) and fixed on poly-L-lysine-coated slides. The tissue sections were completely dried by warming the slides at 40°C for 40 minutes, then fixed in acetone for 15 minutes. The slides were dried for 2 hours in a slide warmer. The tissue sections were rinsed with phosphate-buffered saline (PBS). Methyl alcohol containing 0.3% hydrogen peroxide solution was added to the tissue sections for 20 minutes to block tissue hydrogen peroxide activity. The tissue sections were rinsed with PBS and placed in normal horse serum for 30 minutes. Gently washed tissue was reacted with mouse anti-rat desmin antibodies (1: 200 dilution; Serotec, UK) and mouse anti-rat vimentin antibodies (1: 200; Serotec, UK) at 4°C for 14–16 hours. The primary antibody was added and the section were rinsed. The tissue sections were reacted with biotinylated horse anti-mouse immunoglobulin (Ig)G (Vector Lab, California, USA), the secondary antibody, at room temperature for 1 hour. The tissue sections were rinsed with PBS and reacted with avidin-biotin horseradish peroxidase complex (ABC) solution (Vector Lab, California, USA), which had been prepared 30 minutes earlier. The tissue sections were rinsed with PBS, and a color reaction was performed in a mixed solution of 0.05 M Tris-HCl buffer (Tris buffer, pH 7.6), 0.05% 3,3'-diaminobenzine tetrahydrochloride (DAB; Sigma Co, USA), and 0.01% hydrogen peroxide. After confirming the color of the tissue section, the tissue sections were washed in Tris buffer, PBS, and distilled water for 10 minutes. Counterstaining was performed using Harris hematoxylin, and the sections were mounted with Permount (Polysciences, Warrington, PA, USA). In order to rule out nonspecific binding in the control group, primary antibody reaction or ABC solution was omitted so that no immunological response would be found and to reveal the specificity of the immunological stain. For the enzyme histochemical stain, the tissue sections were dried at room temperature for 20 minutes, reacted with nicotinamide adenine dinucleotide-tetrazolium reductase (NADH-TR) at 37°C for 1 hour, and mounted with Acquamount (Polysciences, USA). Muscle tissues were observed under a light microscope (Axiophot; Zeiss, Germany), and images were captured using a digital camera (Axiocam; Zeiss, Germany).

RESULTS

From the morphological viewpoint, normal gastrocnemius muscle fibers showed an acute angle (Fig. 1). In the 12-day group, muscle fibers that had atrophied because of nerve injury showed a small diameter and round shape; these findings were also observed in the control group (Fig. 2–6). In the 61-day group, the muscle fibers of the experimental group showed normal morphology (Fig. 6D, E, F) but those of the control group showed an angular shape. In the enzyme and immunohistochemical reactions, muscle fibers in the control group showed a weakly positive reaction to desmin and no reaction to vimentin (Fig. 1A, B). The desmin, vimentin, and NADH-TR reactions after sciatic nerve injury are shown in Figs. 2, 3, 4, 5, and 6.

DISCUSSION

When a nerve is injured, an increase in the number of leukocytes and monocytes splits the muscle fiber into pieces in 2 days, and the pieces are removed by phagocytosis within 2 weeks[8]. Muscle fibers in the experimental group underwent necrosis and contained histiocytes, whereas muscle fibers in the control group showed degenerative changes. Desmin and vimentin are thought to be important factors that control the muscle development process[9]. In the present study, they showed positive immune responses,

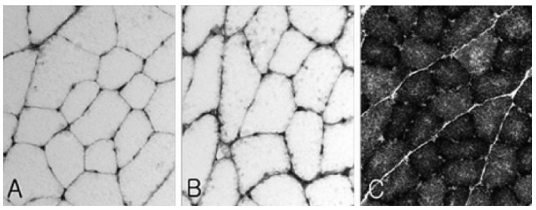

Fig 1. Cross-sections of normal muscle fibers in the rat gastrocnemius muscle. Sections were immunoreacted with desmin (A), vimentin (B) and NADH-TR (C). Muscle fibers showed their normal polygonal appearances. Original magnification x 250.

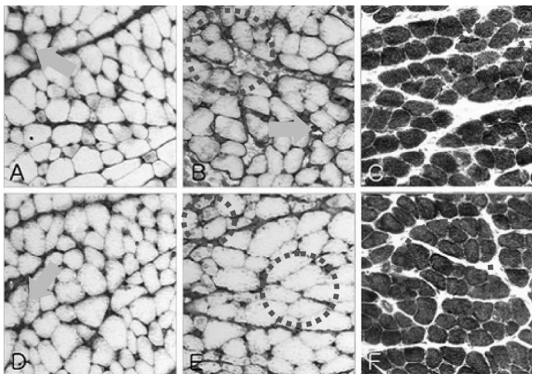

Fig. 2. 12th day after CI. The control group showed a more intensive immunoreactions to desmin and vimentin (A, B) than the experimental group (D, E). Sections were immunoreacted with desmin (A, D), vimentin (B, E) and NADH-TR (C, F). Control groups (A, B, C), experimental groups (D, E, F).

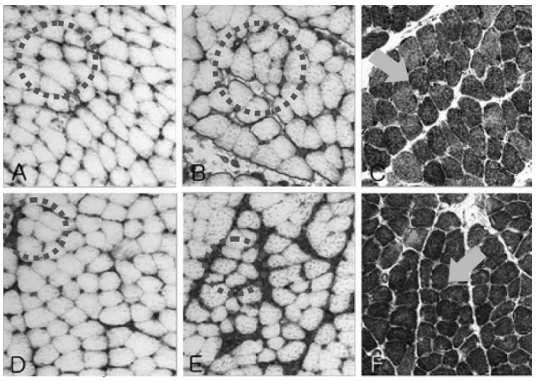

Fig. 3. 19th day after CI. Regeneration of muscle fibers and strong intensity of immunolabelling were observed in the control group. Immunoreactivity of vimentin was stronger than that of the experimental group. Sections were immunoreacted with desmin (A, D), vimentin (B, E), and NADH-TR (C, F). Control groups (A, B, C), experimental groups (D, E, F).

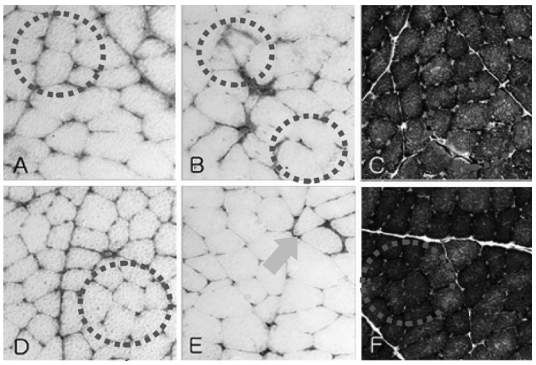

Fig. 5. 33rd day after CI. Most of the muscle fibers revealed regeneration and positive reactions to desmin but not vimentin in the experimental group. Only large fibers showed positive reactions to vimentin in the control group. Sections were immunoreacted with desmin (A, D), vimentin (B, E), and NADH-TR (C, F). Control groups (A, B, C), experimental groups (D, E, F).

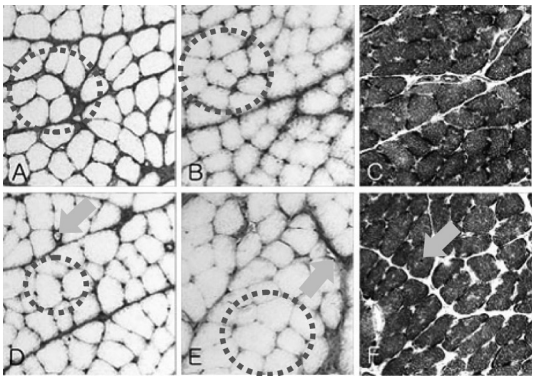

Fig. 4. 26th day after CI. The control group showed strong positive reaction with desmin, vimentin and contained several central nuclei. Target fiber was first observed in the NADH-TR reaction (F) (arrow). Sections were immunoreacted with desmin (A, D), vimentin (B, E), NADH-TR (C, F). Control groups (A, B, C), experimental groups (D, E, F).

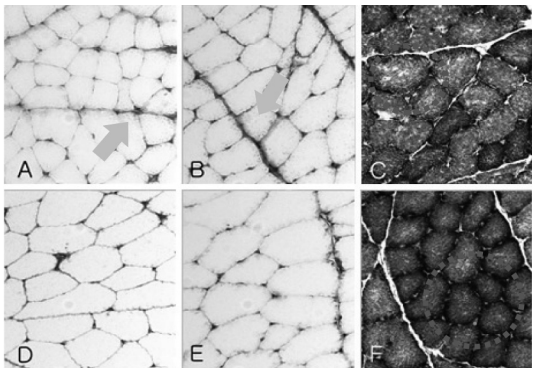

Fig. 6. 61st day after CI. Weak immunoreaction to desmin and vimentin was still present in the control group but not in the experimental group. Diformazan granules showed the shape of fiber type in the control group whereas they were regularly distributed and expressed close to normal staining in the experiment group by NADH-TR reaction. Sections were immunoreacted with desmin (A, D), vimentin (B, E), and NADH-TR (C, F). Control groups (A, B, C), experimental groups (D, E, F).

nucleus condensation, and 2 nuclei. These results can be interpreted as showing both muscle regeneration and degeneration. Bornemann and Schmalbruch[10] reported that demyelinated muscle fibers do not contain desmin and vimentin, but Goebe[11] reported that in adults and children, demyelinated muscle fibers showed the presence of desmin. There are many differing opinions on desmin (Fig. 5, 6), but our results suggest that newly produced muscle fibers show a positive reaction to desmin and vimentin.

In the NADH-TR reaction of the 19-day group, aggregated diformazan granules were observed in the control group because of the presence of muscle fiber clusters, but evenly distributed diformazan granules were observed in the experimental group. For the first time, target fibers were observed during reinnervation in experimental muscle fibers in the NADH-TR reaction (Fig. 6F). Very few muscle fibers showed a positive reaction to desmin at 26 days, but the positive reaction to vimentin indicated that desmin had disappeared before vimentin. Sixty-one days after sciatic nerve injury in the experimental group muscle fibers had recovered normally, few muscle fibers reacted with desmin, and the muscle fibers had the transverse angle observed in normal muscle fibers. Some muscle fibers in the control group showed a positive reaction to vimentin, suggestive of the recovery stage. In the NADH-TR reaction, the control group showed aggregation of diformazan granules, but the experimental group showed evenly distributed diformazan granules on normal staining. The findings of this study show that intermediate filament expression of desmin and vimentin may be used for diagnosis of neuromuscular diseases.

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