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Korean J Phys Anthropol
21(2): 149～165, 2008

The Effects of Sciatic Neurectomy on Rat Gastrocnemius Muscle

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Abstract : The effects of unilateral sciatic neurectomy on gastrocnemius muscle were studied in adult male rats.

The morphological changes of both gastrocnemius muscles were observed by light and electron microscopy. Western blot analysis was performed to study the protein expression.

Following the denervation, the affected muscle weights decreased significantly than normal. And the denervation led to a significant reduction in the area and diameter of muscle fibers on light microscopy. The affected muscle fibers showed the decreasing in size and the irregularity of myofibrilar arrangement on electron microscopy. On transverse view, the area of affected muscle fibers were smaller than normal. Their myofibrils were smaller and very irregular in size. The thin and thick myofilaments were not regular and partially lost. Mitochondria between myofibrils and subsarcolemmal area in affected muscle fibers were damaged and partially lost. The sacoplasmic reticulum and T-tubules were mostly lost and irregular. Some satellite cells were observed adjacent the muscle fiber, but they were inactive morphologically. On longitudinal view, most of myofibrils in affected muscle fibers were lost generally or partially although the their most sarcomeres were regularly arranged. Z line and myofilaments were lost partially and were partially irregularly arranged. The loss of thin myofilaments was more than that of thick myofilaments. Much debris of cell organelles were scattered among myofibrils. The expression of MyoD and myogenin were decrease significantly and the expression of p-mTOR and p-FOXO1 were decreased, too. On the other hand, MuRF1 was increased significantly.

Taken together, the main effect of gastrocnemius muscle by sciatic neurectomy is the atrophy as a result of the loss of myofilaments and cell organelles through the decrease of protein synthesis and the increase of protein degradation.

Key words : Sciatic nerve, Neurectomy, Denervation, Atrophy, Muscle fiber, Gastrocnemius muscle

Introduction

Skeletal muscle form, function, viability, and differentiation are dependent on intact innervation (Batt et al. 2006).

Skeletal muscle atrophy, characterized by a profound reduction in skeletal muscle mass and functional capacity, occurs as a result of the conditions of disuse such as denervation (Jackman and Kandarian 2004). It is known that the modelings of muscular atrophy are not only the denervation (neurectomy) but also the immobilization as the wrapping (Sun et al. 2004), external fixation (Reznick 2003) or casting (Dupont et al. 2003), unloading (Carlson et al. 1999), space flight (Zhou et al. 1995), and tenotomy (William et al. 2000).

The denervation leads to the morphological changes of vascular beds, reduction of capillary/muscle fibers ratio, significant myonuclear loss and finally myofiber death (Borisov et al. 1996, Tyml et al. 1999, Borisov et al. 2000). In skeletal muscle, the intimate association between muscle fibers and the surrounding con-

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nective tissue is important for maintenance of the integrity and proper function of the entire muscle (Cohn and Campbell 2000, Takala and Virtanen 2000). Muscle satellite cells provide injured skeletal muscle the ability to regenerate and repair by proliferating, differentiation, and replacing dead muscle fibers, and are responsible for the hypertrophy of exercising muscle through the formation of new muscle fibers. The general consensus is that satellite cells undergo repetitive proliferation and differentiation over the first 3～4 weeks after acute denervation (Lu et al. 1997, Hawke and Garry 2001, Jejurikar and Kuzon 2003).

The disuse conditions according to the reduced muscle tension trigger several signaling factors, Akt, mTOR, p70S6 kinase, 4E-BP1, glucocorticoids, myostatin, NF-kappa B, and reactive oxygen species (Robert et al. 2003, Leger et al. 2006). Myogenic differentiation 1 (MyoD) and myogenin of myogenic regulation factors play a major role in coordinating the muscle developmental program and the process of adaptation in adult skeletal muscles (Eftimie et al. 1991, Buonanno et al. 1992, Sakuma et al. 1999, Hyatt et al. 2003, 2006). Muscle-specific RING finger protein 1 (MuRF1) is known as a sarcomere-associated protein in skeletal muscles. MuRF1 interacts with the myofibrilar giant spring protein titin at the M line, resulting in disruption of the subdomain that binds MuRF1.

Gastrocnemius can be divided into a superficially “white” portion and a deeper “red” portion. The white portion of gastrocnemius muscle were composed of type IIb fibers, and the red portion of gastrocnemius muscle were composed of type I (about 20%), type IIa (about 30%), and type IIb (about 50%) (Batt et al. 2006).

The aim of this study was to elucidate the morphological changes of gastrocnemius muscle by the unilateral sciatic neurectomy in the adult rats through light and electron microscopy, and to understand its mechanisms by Western blot analysis.

Materials and Methods

1. Experimental animals

The experimental animals were male Sprague-Dawely (SD) rats (specific pathogen-free; age, 9 weeks; body weight, 280～300 g) in this study. The animals were purchased from Damul science (Daejeon, Korea). The experiments were begun after the animals had 1 week of in-house acclimation. The animals were housed in standard cages and provided with food and water *ad libitum*. Two rats were housed in a cage. The room temperature was maintained at 21～23°C with a 12 : 12 h light-dark cycle throughout the experiments. For experimentation, we divided rats into two groups. One group were performed the only sciatic neurectomy, and the other group were performed the sciatic neurectomy and immobilization into left legs. Normal rats were same age as experimental rats.

2. Surgical procedure

1) Sciatic neurectomy

Totally 37 rats were anesthetized by an injection of ketamine hydrochloride (50 mg/mL, 160 mg/kg, Yuhan corporation, Korea), and the sciatic nerve was identified and lifted through an incision on the lateral aspect of the upper-thigh of left leg. A segment (about 1cm) of left sciatic nerve was excised, whereas the right leg was kept intact. The surgical incisions were closed with 3-0 silk and the animals were returned to their cages.

2) Sciatic neurectomy and immobilization by external fixation

The immobilization on the same side of sciatic neurectomy was achieved just after the neurectomy and by the tie of skin using the monofilament 316 L stainless steel wires (Tapercut V-37, Ethicon, USA) driven in pairs through the lateral plane of the femur and tibia.

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Totally 37 rats were used.

3. Body and muscle weight

At 2, 4, 8, 12, and 16 weeks after operation, 5 rats per time point in each group were sacrificed, and the rats were weighed. At the times, the gastrocnemius muscles from both the operated (left) and contralateral non-operated (right) legs in each group were rapidly dissected out and weighed in wet condition.

4. Light and electron microscopy

At 8 weeks after operation, rats were sacrificed with ether, and a transcardial perfusion was performed with 4% paraformaldehyde (Yakuri pure chemicals co., Ltd., Japan). Gastrocnemius muscle were removed from the both legs, were divided red (GR) and white (GW) portion, and they were cut into small blocks in the mid belly.

For light microscopy, blocks of muscle (about 0.3 × 0.3 × 0.3 cm) were fixed in 10% formalin (Samchun pure chemical co., LTD, Korea). The fixed muscle specimens were washed in water, dehydrated in a graded ethanol series, cleared in xylene, embedded in paraffin and cut into 5-μm-thick sections. The sectional tissue stained with Masson's trichrome kit (Sigma-aldrich, USA) for collagen fiber, and the images were observed under light microscopy.

For electron microscopy, blocks of muscle (about 1 × 1 × 1 mm) were fixed by immersion in a solution containing 2.5% glutaraldehyde and 4% paraformaldehyde at pH 7.4 (half-Karnovsy solution, Karnovsky 1965) for two hours. The muscle specimens were postfixed for two hours in 1% osmium tetroxide, dehydrated in a graded series of ethanol, and embedded in Epon-812 resin, mixed by Luft's method (Luft 1961). Semithin sections, cut in both transverse and longitudinal planes, were stained with 0.1% toluidine blue for light microscopy. Thin sections (about 80 nm thick) for transmission electron microscopy were cut in both transverse and longitudinal planes by NOVA ultramicrotome (LKB, Vienna, Austria), and picked up on 100-mesh grids. After staining with uranyl acetate and lead citrate, the specimens were viewed with a electron microscope (JEM-1200 EX II, accelerating voltage, 80 kV, Japan).

5. Morphological analysis

The toluidine blue stained sections were observed under light microscopy, and this images were taken with the Nikon digital sight DS-U1 (Japan). The transverse sectional areas and the longitudinal sectional diameters of muscle fibers were measured using image analysis computer software (Analysis pro ver. 3.2, Soft Imaging System GmbH, Germany). The mean area and diameter of muscle fibers was calculated from measurements of 100～150 fibers.

6. Western blot analysis

100 μg of gastrocnemius muscles were homogenized in 1 mL modified RIPA buffer [150 mM NaCl, 5 mM EDTA, 50 mM Tri-HCl (pH 8.0), 1% NP 40, 1 mM aprotinin, 0.1 mM leupeptin, 1 mM pepstatin] by using homogenizer (Ultra-Turrax T8, Germany). Aliquots of protein (20 μg) were mixed with 20 μL SDS gel loading solution, and the mixtures were denatured at 100°C for 5 min. Denatured protein were separated on 10% SDS polyacrylamide gel, at 100 volt for 2 hours, and transfered onto PVDF membranes, at 200 volt for 4 hours. The blots were blocked with PBS containing 5% skimmilk for 1 hour, and were then incubated with specific antibodies against myoD (Santa Cruz Biotechnology, USA), myogenin (Santa Cruz Biotechnology, USA), phospho-mTOR (Cell Signaling Technology, USA), phospho-FOXO1 (Cell Signaling Technology, USA), and MuRF1 (Muscle-specific RING finger protein 1, Santa Cruz Biotechnology, USA) for 1 hour. They were incubated with goat anti-mouse or goat anti-rabbit conjugated secondary antibody for 60 min.

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Table 1. The changes of the body weights of rats in normal and experimental groups for 16 weeks

Operated (weeks)	Normal	Denervation only		Denervation and immobilization	
	Body weight (g)	Body weight (g)	% change	Body weight (g)	% change
0	313.6±0.9798	318.2±3.200	1.46	318.0±1.803	1.40
2	367.0±4.455	380.9±4.720	3.78	353.1±4.919	−3.78
4	422.4±6.537	419.9±5.839	−0.59	403.8±3.395	−4.40*
8	460.7±7.688	448.7±3.019	−2.6	436.7±4.483	−5.20*
12	475.0±2.640	470.3±9.952	−1.53	466.6±4.946	−1.70
16	484.3±4.333	485.4±11.95	0.20	482.3±2.848	−0.41

Value: Mean (g)±SEM (n)
*Difference between normal and experimental rats are significant from statistical results ($p < 0.05$).

Table 2. The changes of gastrocnemius muscle weights for 16 weeks

Operated (weeks)	Denervation only			Denervation and immobilization		
	Contralateral (mg)	Affected (mg)	% change	Contralateral (mg)	Affected (mg)	% change
2	2505±41.2	1410±36.9***	−43.7	2443±138.6	1419±29.9***	−41.9
4	2741±117.8	858.3±24.7***	−68.6	2741±117.8	893.7±54.4***	−67.3
8	2920±89.6	649.5±46.6***	−77.7	2898±101.5	649.5±46.6***	−77.5
12	3021±71.9	540.4±20.2***	−82.1	3037±318.0	556.1±11.5***	−81.6
16	3105±56.6	533.3±24.7***	−83.0	3037±31.8	548.9±21.1***	−81.9

Value: Mean (mg)±SEM (n)
***Difference between contralateral and affected are significant from statistical results ($p < 0.0001$).

Table 3. The diameters and areas of the red and white gastrocnemius muscle fibers at 8 weeks after denervation

	Gastro-cnemius	Normal	Contralateral	Denervation only		Denervation and immobilization	
				Affected	% change	Affected	% change
Area (μm^2)	Red	3237±117.7	3259±156.0	212.9±14.37*	−93.5	304.4±10.51*	−90.7
	White	3726±167.5	3583±139.1	228.0±6.360*	−93.6	314.1±9.540*	−91.2
Diameter (μm)	Red	36.44±1.348	41.11±1.290	10.11±0.385*	−75.4	16.22±0.4070*	−60.5
	White	56.64±1.165	52.13±0.7917	14.57±0.390*	−72.0	16.40±0.5828*	−68.5

Value: Mean (μm^2 or μm)±SEM (n)
*Difference between contralateral and affected are significant from statistical results ($p < 0.05$).

Then, the bands were developed by 1 mL ECL detection reagent (Amersham, USA) for 1 min. Immunoreactive bands were quantified using the imaging system LAS-300 (Fujifilm, Japan).

7. Statistical analysis

All data were expressed as mean±SEM. The data was analyzed by the student's *t*-test. All significance levels were set at $p < 0.05$.

Results

1. Body and muscle weights

The body weights of animals for 16 weeks after operation were shown in Table 1. The body weights of

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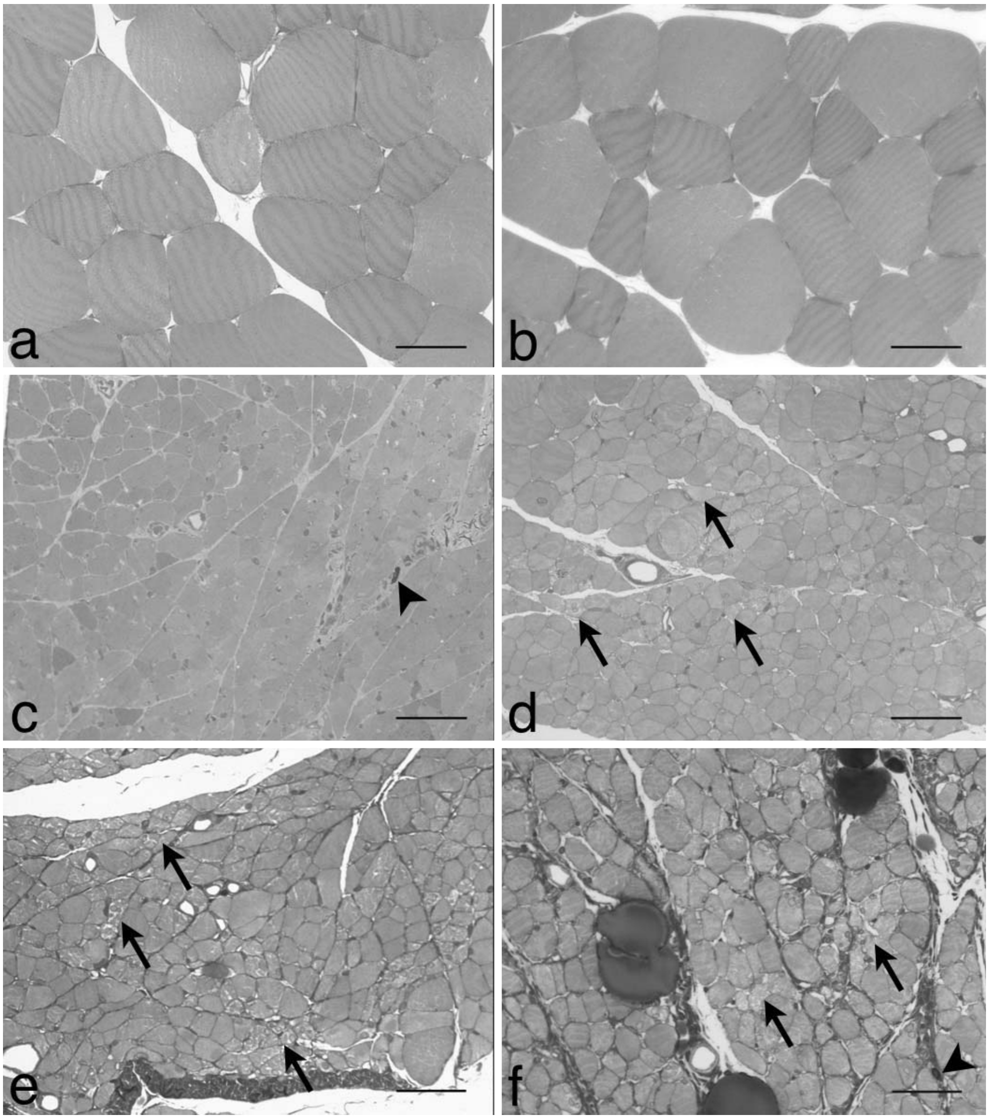

Fig. 1. Light micrographs showing the transverse section of gastrocnemius muscle from normal (a), contralateral (b), denervation (c and d), and denervated and immobilized legs (e and f). Red (deep) portion of gastrocnemius meuscle is c and e, and white (superficial) portion is d and f. The size of a and b muscle fibers is similar. The sizes of affected muscle fibers (c-f) are distinctly smaller than those of a and b. The contents of sacoplasm in some muscle fibers of the affected legs are lost partially (arrows). Perimyosium and endomyosium are found well in affected muscles. Capillaries and mast cell (arrow heads) are found within the connective tissue. Toluidine bule staining, Scale bar=50 μm.

affected rats were slightly lower than those of normal, but that was not significant.

Table 2 showed the changes of gastrocnemius muscle weights for 16 weeks after operation. The muscle weights of the only denervated legs were gradually reduced compared with those of contralateral. Following

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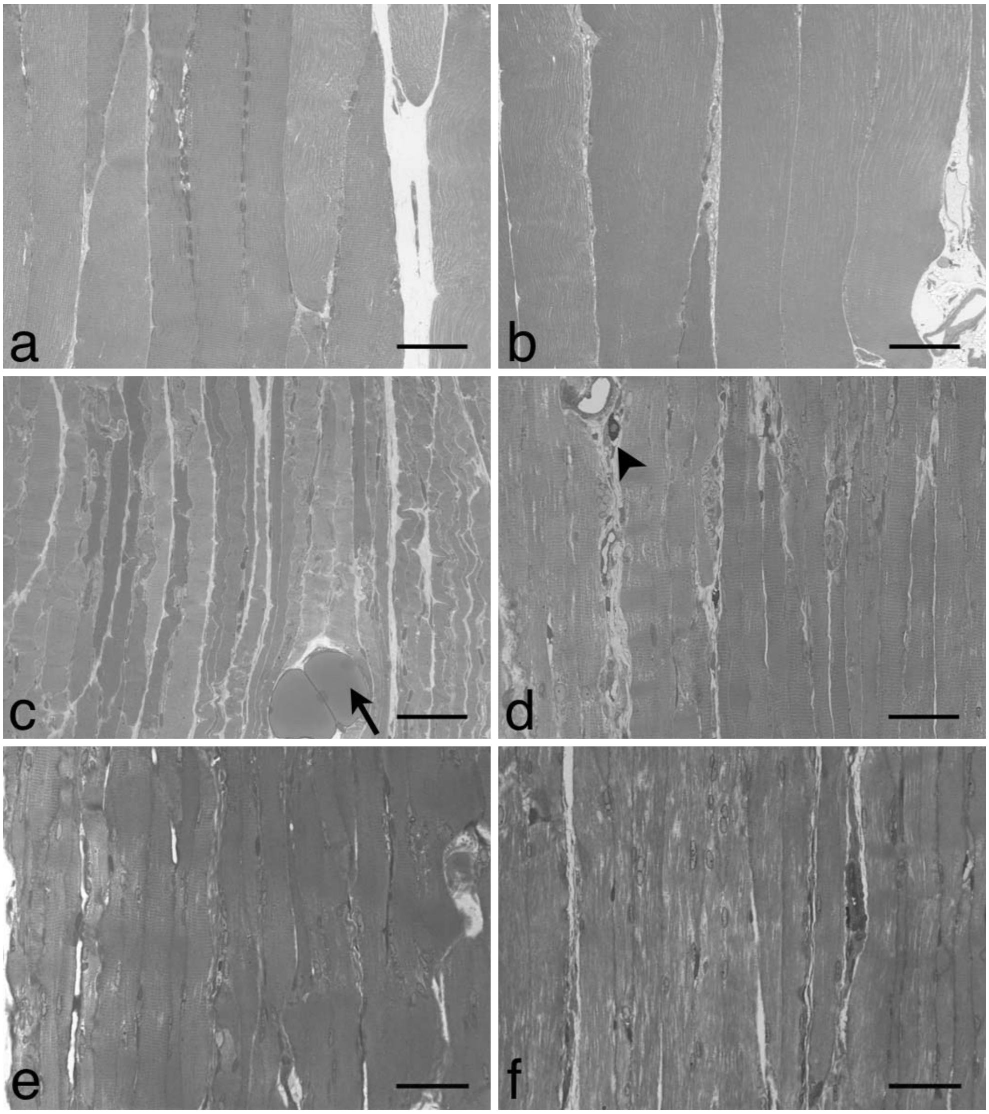

Fig. 2. Light micrographs showing the longitudinal sections of gastrocnemius muscle from normal (a), contralateral (b), denervation (c and d), and denervated and immobilized legs (e and f). Red portion of gastrocnemius meuscle is c and e, and white portion is d and f. The diameters of a and b muscle fibers are similar, but the diameters of muscle fibers in b are slightly larger than in a. The remarkable decreasing of diameter of affected muscle fiber is found in a and b. The surfaces of a few affected muscle fibers is slightly irregular. Sometimes there are the fat cells (arrow) and mast cells (arrow head) in connective tissue among affected muscle fibers. Toluidine bule staining, scale bar=50 μm.

denervation and immobilization, gastrocnemius muscles shrank compared with those of contralateral. These were significant statistically ($p < 0.0001$). The loss rate of muscle weights in the affected rats was prominent at 8 weeks after operation, and so we observed the morphology and protein expression of gastrocnemius

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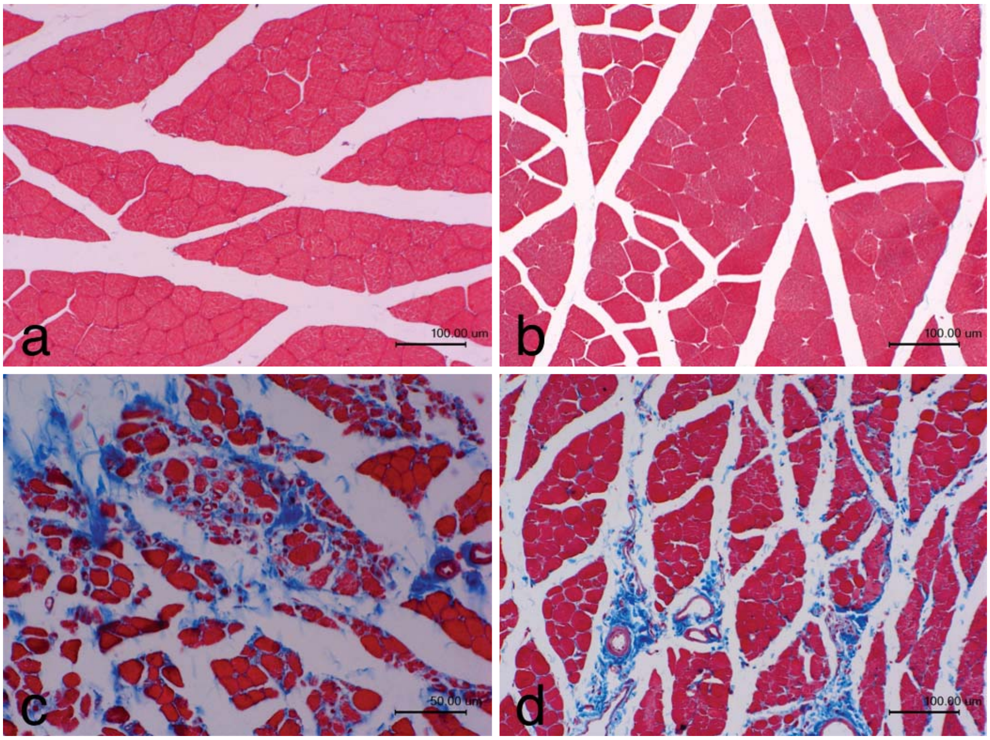

Fig. 3. Light micrographs showing the transverse sections of gastrocnemius muscle from normal muscle (a), contralateral muscle (b), and denervated red (c) and white (d) portion muscles at 8 weeks after operation. Muscle fibers are red, and collagen fibers are blue. The a and b muscle fibers are evenly distributed with fewer collagen fibers. A dramatic hyperplasia of the collagen fibers are observed between the muscle fibers at 8 weeks after denervation. Mainly collagen fibers are observed around capillaries. Masson's trichrome staining. Scale bars = 100 µm (a, b, and d), 50 µm (c).

muscle at 8 weeks after operation.

2. Morphological analysis

Using image analysis computer software, the area and diameter of gastrocnemius muscle fibers at 8 weeks after operation were measured (Table 3).

1) Transverse sectional areas

The transverse sectional areas of muscle fibers (TSA) in the GR and GW of the normal legs at 8 weeks were 3,237±117 μm^2 and 3,726±167 μm^2, respectively. TSA of the GR and GW in contralateral legs were similar to that of normal. TSA of the GR and GW were decreased about 90% not only the denervated legs but also the denervated and immobilized legs compared with those of contralateral respectably.

2) Diameters

The muscle fiber diameters of the GR and GW in normal legs were 36.44±1.3 µm and 56.64±1.1 µm, respectively. The muscle fiber diameters of the GR and GW in the contralateral legs were 41.11±1.2 µm and 52.13±0.7 µm, respectively.

The diameters of muscle fibers of GR and GW in the affected legs were significantly smaller about 70～75% than those of the contralateral.

3. Light micrography

The morphological studies were performed on the GR and GW at 8 weeks after operation.

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1) Observations on transverse sections

The normal muscle fibers were polygonal in shape. They had full sarcoplasmic contents (Fig. 1a). Also morphology of gastrocnemius muscle fiber in the contralateral legs was similar to that of normal (Fig. 1b). Many affected muscle fibers of the GR and GW showed that shapes were a circle-like appearance, and the contents of sarcoplasm were lost partially and not stained. The perimyosium and endomyosium were seen distinctly. Sometimes, nuclei in affected muscle fibers were observed in the middle of the muscle fiber. The fat cells and mast cells were observed in the connective tissue of the affected gastrocnemius muscle fibers (Fig. 1c-f).

2) Observations on longitudinal sections

The muscle fibers of normal and contralateral legs were fairly similar to each other morphologically (Fig. 2a, b). The affected muscle fibers showed their striations although the diameters were narrow. Sarcolemma of many affected muscle fibers were not straight longitudinally. There were much empty sarcoplasmic area longitudinally between myofibrils. Perinuclear cytoplasm is relatively not or weak stained in many areas. Oval nuclei were located at the sublemmal area, singly or multiply. The size of nuclei in affected muscle fibers were similar to that of the normal and contralateral (Fig. 2c-f).

3) Masson's trichrome staining view

A hyperplasia of the collagen fibers in the affected muscle occurred at 8 weeks after denervation (Fig. 3).

4. Western blot analysis

The extent of protein expression was recoding on the basis of adjuvant volume. An decreases in MyoD and myogenin proteins were 79.2% and 87.9% significantly compared with those of the contralateral. At the same time, p-mTOR and p-FOXO1 proteins decreased 14.6% and 22.7%, but which was not significant. On the contrary, the MuRF1 proteins increased 80.7% significantly compared with that of contralateral (Fig. 4).

5. Electron micrography

The morphology of muscle fibers in the denervated legs was similar to the simultaneowoly denervated and immobilizated legs. And the result of electron micrography was explained the result of gastrocnemius red portion in denervated legs.

1) Normal and contralateral legs

In the transverse sections, normal muscle fibers of GR showed two kind of cells. One had the curvilinear-

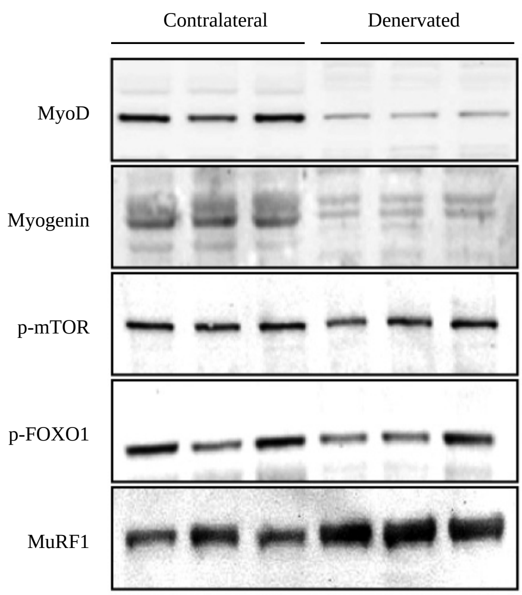

Fig. 4. Western blot analysis was performed by using proteins from gastrocnemius muscle at 8 weeks after denervation, and antibodies against myoD, myogenin, p-mTOR, p-FOXO1, and MuRF1. Expression of MyoD and myogenin in the affected muscle are significant decreased. Expression of phospo-mTOR and phospho-FOXO1 in affected muscles are slightly decreased in comparison with contralateral. On the contrary, Expression of MuRF1 in the affected muscle increases remarkably than that of contralaeral.

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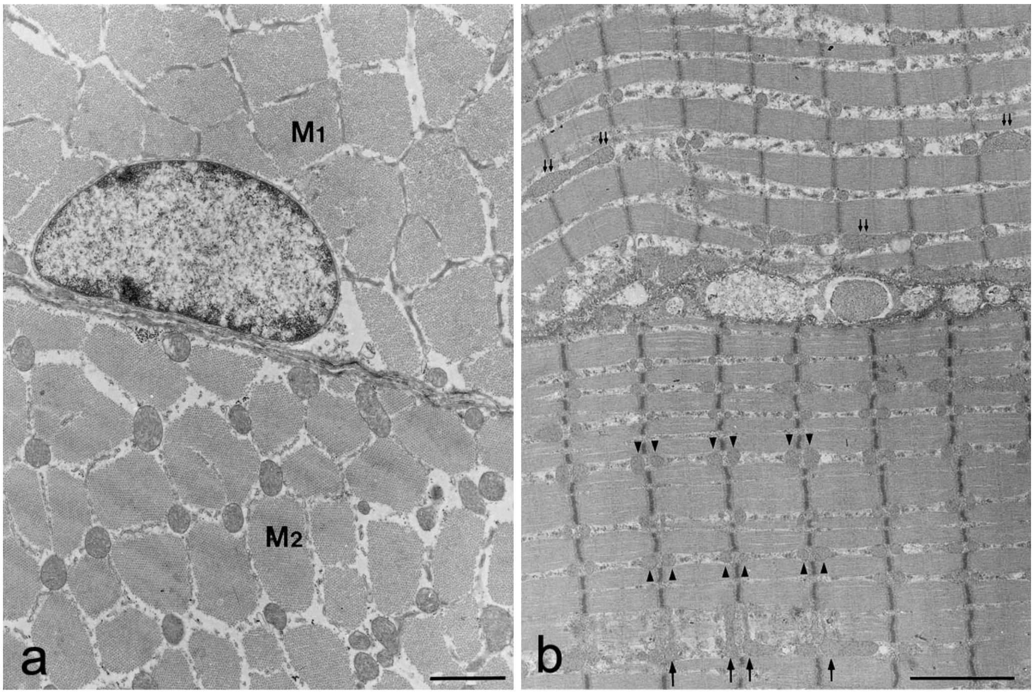

Fig. 5. Electron micrograph of 8 week normal gastrocnemius muscle in transverse (a) and longitudinal (b) sections. In the transverse sectional view, two muscle fibers are shown. One (M1) has the polygonal myofibrils, the another (M2) has the curvilinear-polygonal myofibrils. In the longitudinal view, myofibrils are arranged parallel and show the uniform diameter and regular sarcomeres. Relatively thick mitochondria are found between myofibrils and a pair of round mitochondria (called "I-band limited mitochondria") are located near Z line (arrow heads). Sarcoplasmic reticulum (arrows) were observed mainly between myofibrils, and T-tubules were found between the I-band limited mitochondria. Some subsarcolemmal mitochondria are found (double arrows). bars=1 μm (a), and 2 μm (b).

polygonal myofibrils, and contained the small and few mitochondria. The another had the polygonal myofibrils, and had plenty round and long mitochondria among the myofibrils. Oval nuclei were observed at subsarcolemmal area and showed euchromatic. An arrangement of the thin and thick myofilaments was regular in transverse section (Fig. 5a).

In the logitudinal sections, normal muscle fibers of GR were filled solidly with plenty myofibrils that were arranged parallel and show the uniform diameter and regular sarcomeres. Myofibrils were composed of thick and thin myofilaments. A sarcomere had Z-line, A band, and I band. Z line was most electrodnese. Relatively oval or round mitochondria was I-band limited mitochondria. So this view was as if a pair of mitochondria were located near Z line. And many subsarcolemmal mitochondria were observed. T-tubule were observed between a I-band limited mitochondria. Also sarcoplasmic reticula were observed mainly between myofibrils (Fig. 5b).

Morphology of gastrocnemius muscle fibers in contralateral legs very similar to that of normal.

2) Affected legs

In the transverse sectional view, area of gastrocnemius muscle fibers were greatly smaller than those of

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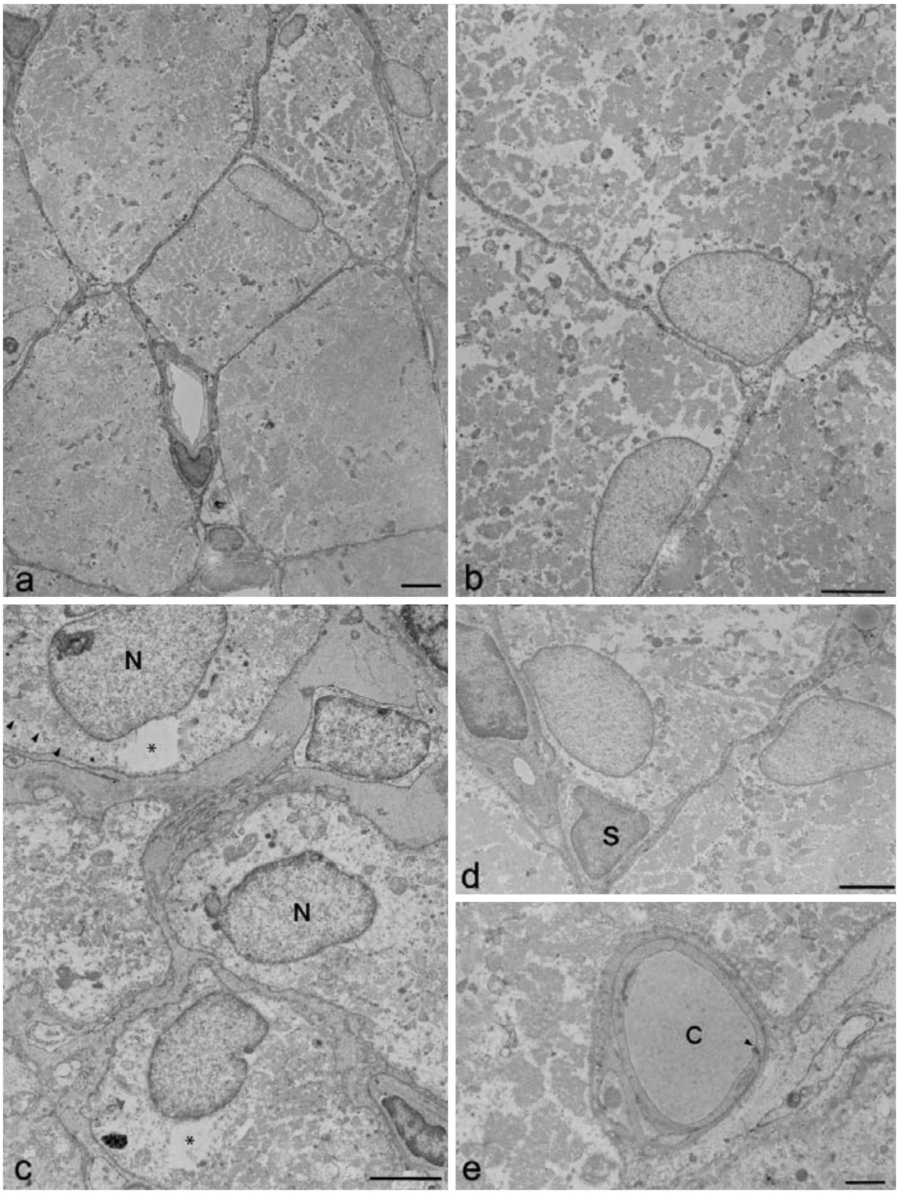

Fig. 6. Electron micrograph of 8 week denervated and immobilized red portion of gastrocnemius muscles in the transverse section. The areas of most of muscle fibers is smaller than that of normal or contralateral. The transverse plane of muscle fibers shows round, triangular, quadrilateral and polygonal (a). The denervated muscle fibers have shapeless and a few myofibrils which diameter is very small and scatted. A part of myofibrils were obliquely section although the most myofibrils were sectioned transversely (arrow heads) (c). And the denervated muscle fibers have condensed and scattered mitochondria, many vesicles, and amorphous materials in their sarcoplasm (b-e). Perinuclear mitochondria are lost and damaged, so sarcoplasmic matrixes are empty (c). See the thick connective tissue, endomyosium. Some electrodensed and condensed mitochondria were fused. Oval shaped nuclei show the euchromatin. The space among myofibrils is irregularly arranged. Some nuclei (N) are located far from sarcolemma. A portion of perinuclear sarcoplasm is empty (asterisk) (c). A satellite cell (S) is shown adjacent to muscle fiber (d). A capillary (C) is located at the invaginated portion of muscle fiber that show damaged. Endothelial cells have many vesicles and endothelial junction (arrow head) (e). bars=1 μm (e), and 2 μm (a, b, c, d, and e).

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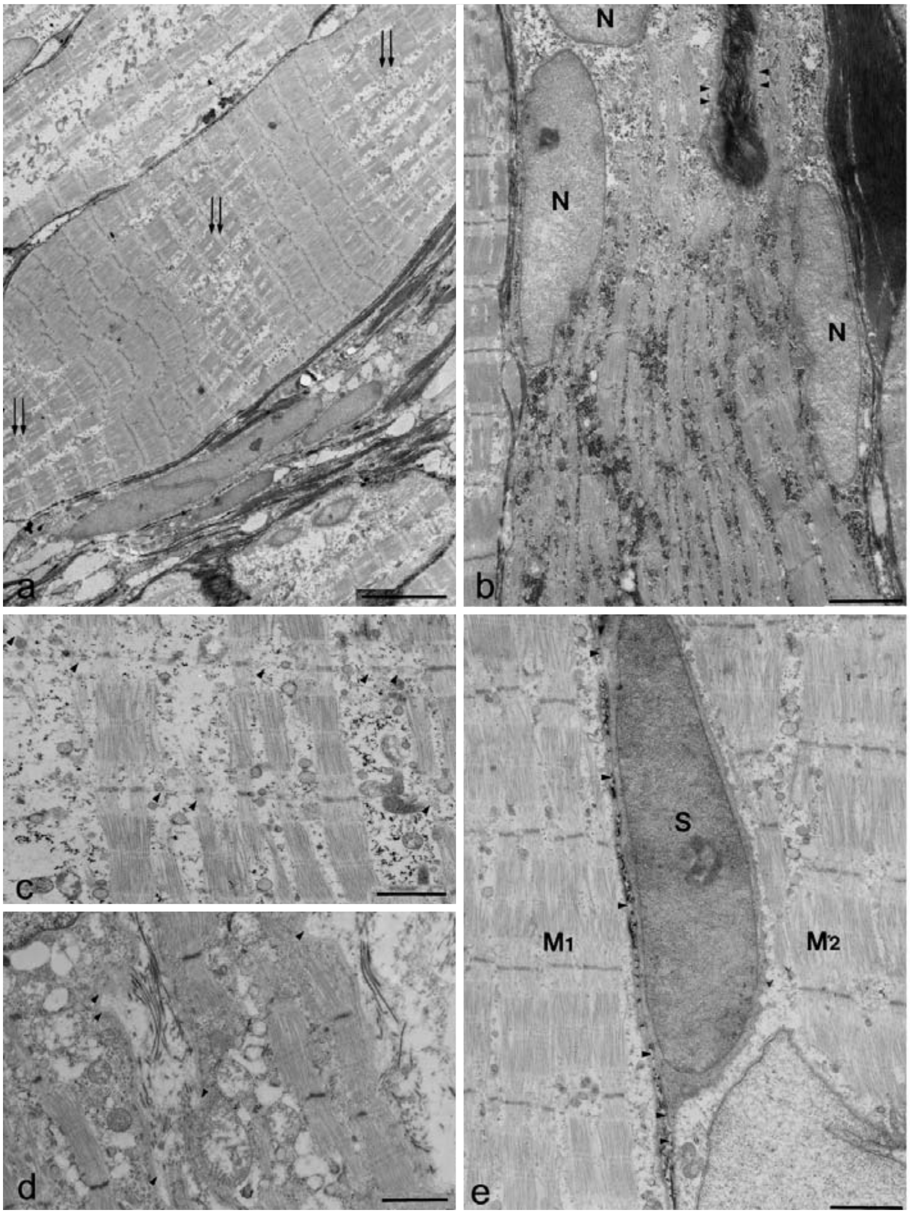

Fig. 7. Electron micrograph of 8 week denervated and immobilized red portion of gastrocnemius muscle in longitudinal sections. A narrow muscle fiber shows the intermittently severe loss of myofibrils and so different degrees of injury according to the longitudinal direction (double arrows). Another muscle fiber shows the longitudinally losing of myofibrils (a). Much glycogen granules are located among myofibrils. Three nuclei (N) are located at subsacolemmal area. A part of collagen fibers invaginated within muscle fibers (arrow heads) (b). Thin myofilaments and Z-band vanish more in plenty (arrow heads), compared with thick myofilaments (c and d). In spaces that myofibrils are lost, there are much the debris of cell organelles, damaged mitochondria, broken pieces of myofilaments and glycogen granules. Most of mitochondria are swollen or condensed (c and d)). Two damaged muscle fibers are shown (M1 and M2) (e). A satellite cell is located adjacent to a muscle fiber (M1) and covered with the common basal lamina (arrow heads) that cover the muscle fiber. bars = 1 μm (c, d, and e), 2 μm (b), and 5 μm (a).

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normal or contralateral at 8 weeks after operation. The shapes of muscle fibers in affected legs were round, triangular, quadrilateral and polygonal. Each muscle fiber was demarcated by the connective tissue (Fig. 6a). Myofibrils of the gastrocnemius muscle fibers in most affected legs were very small and shapeless, and some portions of myofibrils were lost. The arrangement of thick and thin myofilaments is generally not regular, and the intervals among the myofibrils were not regular (Fig. 6b-e). Many nuclei were oval and euchromatic or weak heterochromatic, and sometimes nucleolus was observed (Fig. 6c). Most of mitochondria were swollen and condensed. Tubular structures were rarely observed, and it was difficult to classify them into sarcolemmal reticulum or t-tubules. And amorphous and electrodense materials were observed in whole sarcoplasm (Fig. 6b, c). A satellite cell was observed adjacent to muscle fiber. Nucleus and cytoplasm of satellite cell were electrodense than those of muscle fibers. The satellite cell had relatively small cytoplasm that includes a few cell organelles. A common basal lamina covered the muscle fiber and its satellite cell (Fig. 6d). The connective tissue, endomyosium, among muscle fibers was generally thin, but some times thick (Fig. 6a-c). Endomyosium was electrodense than muscle fibers. The connective tissue included the fibroblasts, fibrocytes, collagen fibers and capillaries. Capillaries were usually located among the corners of polygonal muscle fibers and sometimes between the muscle fibers. In rare cases, there was a capillary that is located at the invaginated portion of the lateral wall of affected muscle fiber. Endothelial cells had their junctions with each other and much vesicles in their cytoplasm (Fig. 6e).

In the longitudinal sectional view, the diameter of affected muscle fibers was very narrow definitely than that of normal or contralateral (Fig. 7a, b). The arrangement and diameter of myofibrils were not relatively regular. The injuries of myofibrils showed some aspects; General losing of components of myofibrils, longitudinally losing of myofibrils, and different degrees of injury according to the longitudinal direction (Fig. 7a). Although most of sarcomeres were composed of Z line, I band and A band, height of Z line of myofibrils were narrow and many Z lines were lost (Fig. 7c, d). And thick and thin myofilaments were lost partially and scattered around. Some myofilaments were divided and appeared v- or y- shaped. Sometimes thin and thick myofilaments were observed without Z lines, and thick myofilaments were preserved only without thin myofilaments and Z line (Fig. 7c). Nuclei of affected muscle fibers were mainly long and sometimes multilobulated-like in shape (Fig. 7b). Most of nuclei were located at the subsracolemmal area, but some nuclei were somewhat far from sarcolemma. Most of sarcolemmal and intermyofibrilar mitochondria were damaged. Some mitochondria were swollen and lost their cristae partially and totally (Fig. 7d). Some mitochondria were condensed, and were fused completely or incompletely and closed with one another (Fig. 7d). Many perinuclear mitochondria were lost, too. I-band limited mitochondria were not preserved and lost or damaged (Fig. 7a-e). Generally glycogen granules were scattered in whole sarcoplasm. Rarely glycogen granules were accumulated among the severely damaged and thin myofibrils (Fig. 7b). Satellite cells were observed adjacent to muscle fiber, and a common basal lamina covered the muscle fiber and satellite cell (Fig. 7e). There were much collagen fibers running longitudinally in the connective tissue, endomyosium. The collagen fibrils contacted closely with the sarcolemma of muscle fiber. The collagen fibrils were located at the invaginated site of muscle fiber, and rarely some collagen fibrils contacted closely with sarcoplasmic structures through a portion of discontinuous basal lamina and sarcolemma (Fig. 7b).

Discussion

It is known that the denervation leads to muscle

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atrophy (Al-Amood and Lewis 1989, Carlson et al. 1996), and the muscle atrophy in adult muscle by the denervation leads to about 80% of its mass for several months (Gutmann and Zlelna 1962, Hnik 1962). This study presented the similar result. The weights, areas and diameter of the affected gastrocnemius muscles were reduced compared with those of contralateral, in this study.

Although the volume of muscle fibers in affected legs decreased, many muscle fibers presented their sarcomeres similar to that of normal in this study. But generally the width of sarcomeres decreased compared with that of normal or contralateral. And the damage and loss of Z line were observed well than those of myofilaments. Riley et al. (1998, 2005) reported that the density of thin myofilaments decreased compared with thick myofilaments on soleus muscle, slow fiber. The same results, the thin myofilament were lost earlier than thick myofilaments in this study. But there is no clear evidence why the thin myofilaments were lost earlier. This may be related to the earlier losing of Z line or the earlier degradation of some components of thin myofilament, for example, troponin (Kedar et al. 2004).

Although most of nuclei in affected muscle fibers were located at subsarcolemmal area, and some nuclei were far from subsarcolemma in this study. This might be originated from the losing of perinuclear mitochondria and myofibrils. Viguie et al. (1997) reported that the number of myonuclei per muscle fiber decrease on the study about the effects of long-term denervation on the rat extensor digitorum longus muscle. Some muscle fibers had a crowd nuclei in this study. This finding multiple nuclei in the affected muscle fiber in this study may be derived from the extreme atrophy after denervaton, and not from their multiplications.

The greater part of mitochondria in muscle fibers is mainly clustered under the sarcolemma. This is explainable that mitochondria are the major sites of cellular oxygen consumption and capillaries are located adjacent to the basal lamina of muscle fibers (Hoppeler et al. 1997, Kreuzer et al. 1997). In this study, the damages of mitochondria were mainly the swelling and condensation, and sometimes fused with one another.

One of damages on gastrocnemius muscle fibers by neurectomy was the severe loss of t-tubules and sarcoplasmic reticula. There was only a few scattered tubular structures in the affected muscle fiber.

Lu et al. (1997) reported in a study about long-term denervated rat skeletal muscle that the satellite cells were activated in condition of denervation. And Murry and Robbins (1982a, b) reported that the mitotic activity of satellite cell after denervation is a peak, and McGeachie and Allbrook (1978) reported that the activity of satellite cells after denervation increase during the week following and then decrease late in the first and second months. Lu et al. (1997) mentioned that the activation of satellite cells is the elongation of cytoplasmic processes and increasing the amount of cytoplasm. But the satellite cells at 8 weeks after neurectomy in this study showed the inactivated or resting states. Although satellite cells in affected muscle were inactive, they were found easier than that of normal. The further study is needed to clarify whether the number of satellite cells increase or not.

There is rarely a recent report that the mechanism of atrophy in denervated muslce fibers may be derived from apoptosis (Borisov and Carlson 2000). They explained that the apoptotic nuclei were observed by fluorescent cytochemical staining of DNA and the apoptotic appearances of nuclei in leg muscles are found by electron micrography from 2 to 7 months after denervation. But, there is no nuclei that undergoes the apoptotic cell death in this study.

The capillaries were observed well among the affected muscle fibers in this study, although sometimes the lumens of capillaries became narrow. Borisov et al. (2000) reported that the degeneration and loss of capillaries after denervation occurs more rapidly than the

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loss of muscle fibers. Oki et al. (1996) reported that the occurrences of capillaries with both diaphragmed fenestrations and pores represent the degenerative changes in the long-term immobilized muscle of rats. But in this study, there is no capillary with fenestrations in the affected muscles and capillaries have their endothelial junctions and much cytoplasmic vesicles in this study. On the other hand, Oki et al. (1999) reported that the capillary changes with fenestrations in the contralateral soleus muscle of the rat after unilateral limb immobilization. This is not clear why these results are different.

The endomyosium presents thin in the normal or contralateral muscles, but it is partially thick in the affected muscles in this study. Especially collagen fibers were found well in the connective tissue of the affected muscles, and some fat cells and mast cells were found, too. This is a similar result to the report of some researchers (Borisov et al. 1996, 2000). The accumulation of connective tissue cells may be derived from the compensatory regeneration.

MyoD and myogenin of myogenic regulatory factors are thoght to be the markers of skeletal muscle growth and hypertrophy, participating in modulating the division of satellite cells, and they play a major role in coordinating the muscle developmental program and the process of adaptation in adult skeletal muscles (Eftimie et al. 1991, Buonanno et al. 1992, Sakuma et al. 1999, Hyatt et al. 2003, 2006). MyoD and myogenin decreased significantly with the severely atrophied muscle fibers in this study. This result may be related to the absence of activation or proliferation of satellite cells. Recently Russo et al. (2007) reported that the electrical stimulation reduced the expression of MyoD and atrogin-1 are decreased in the denervated rat muscle and suggested the possibility to apply to human.

In hypertrophy signaling, IGF-1 activates mTOR (mammalian target of rapamycin) through some pathway, and then mTOR activates p70S6K for protein synthesis. And mTOR suppresses MAFbx atrogin-1 to lead the protein degradation (Burnett et al. 1998, Hara et al. 1998, Rommel et al. 1999, 2001, Pallafacchina et al. 2002). FOXO activates MuRF1 and MAFbx atrogin-1 that are the signaling factors in atrophy signaling, and so FOXO1 leads to the protein degradation (Kamei et al. 2004, Lee et al. 2004, Sandri et al. 2004, Stitt et al. 2004). mTOR and FOXO1 decreased in this study. This result accords with the above reports.

Bodine et al. (2001) reported that MuRF1 is originally identified by searching for genes upregulated in models of skeletal muscle atrophy. MuRF1 of the affected gastrocnemius muscle increased 80.7% compared with contralateral ones in this study. This suggest that the muscle atrophy following the denervation may be affected by MuRF1 pathway.

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Korean J Phys Anthropol
21(2): 149 ~ 165, 2008

궁둥신경절단이 흰쥐 장딴지근에 미치는 영향

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간추림 : 근육위축은 신경절단이나 부동, 고정, 비운동, 비부하 등에 의해 유발될 수 있다. 본 연구는 신경절단 후 일어나는 근육 손상의 기전을 이해하고자 시행되었다.

흰쥐 왼궁둥신경을 절단한 후 8주에 양쪽 장딴지근의 형태학적 변화를 광학 및 전자현미경으로 관찰하였고, Western blot 분석을 통해 근육발육과 관련된 단백질의 발현정도를 측정하였다.

궁둥신경이 절단된 왼쪽 다리의 장딴지근의 무게는 유의하게 감소되었다. 광학현미경상에서 신경절단에 의해 근육섬유의 직경과 가로면의 면적이 정상대조군에 비하여 현저하게 감소하였다. 장딴지근에서 아교섬유가 신경절단 후 증가되었다. 전자현미경상에서 신경절단에 따른 근육섬유의 변화는 주로 근원섬유의 손상과 소실, 사립체의 손상과 소실, 가로세관 및 근육형질세망의 소실 등이었다. 근육원섬유의 직경이 작아지고, 근육원섬유의 구성성분인 굵은 및 가는 근육미세섬유의 수가 현저하게 감소하였으며 이들의 배열이 매우 불규칙하였다. 근육원섬유마디에서 끝가로막의 소실이 근육미세섬유의 소실보다 더 많았고, 가는근육미세섬유의 소실이 굵은근육미세섬유보다 더 심했다. 근육섬유의 핵은 퍼진염색질과 약한 뭉친염색질을 보였으며, 간혹 핵소체도 관찰되었다. 사립체의 손상은 주로 농축과 팽대 및 사립체능선의 소실 등이었다. 가로세관이나 근육형질세망은 그 수가 감소하였으며, 근육형질내에 무정형의 물질들과 함께 불규칙하게 흩어져 있었다. 결합조직내에서 간혹 근육위성세포가 근육섬유와 인접하여 관찰되었으며, 형태학적으로 비활성상태였다. 근육섬유의 발육관련 인자인 MyoD와 myogenin은 현저하게 감소하였고, 세포의 성장과 증식에 관련된 인자인 인산화 mTOR와 FOXO1도 감소하였다. 반면에 세포위축관련인자인 MuRF1은 현저하게 증가하였다.

이상의 결과는 신경절단에 의한 장딴지근의 형태학적 변화는 근육섬유의 발육을 유지하는 단백형성의 감소와 단백파괴의 증가에 의한 근육미세섬유와 세포소기관의 소실에 따른 세포위축에 기인하는 것으로 추측된다.

찾아보기 낱말 : 궁둥신경, 신경절단, 위축, 근육세포, 장딴지근

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