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Research Article

Effects of Thermotherapy and Manual Therapy on Lengthening Contraction-Induced Damage in the Rat Gastrocnemius Muscle: A Histological Examination

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Abstract

Background: Delayed Onset Muscle Soreness (DOMS) develops 2-3 days after lengthening contraction (LC) or inexperienced vigorous exercise and disappears approximately 3-10 days later. This study histologically examined the effects of thermotherapy (Heat Therapy, HT) and Manual Therapy (MT) (muscle compression), which were shown to be effective for reducing mechanical hyperalgesia in an animal model of DOMS, on Exercise-Induced Muscle Damage (EIMD) and its recovery processes in the same animal model.

Methods: Rat gastrocnemius muscles were subjected to LC under anesthesia. These rats were allocated to three groups: Control-LC group that received only LC, LC+HT group that received HT after LC, and LC+MT group that received MT (muscle compression) after LC. After 3h, 12h, 27h, and 4days from LC, muscle specimens were collected and processed with hematoxylin and eosin protocol for histological investigation.

Results: Histological investigation of the muscle cross-sections indicated pathological findings including EIMD (opaque fibers, necrotic fibers, leukocyte infiltration) and regenerated muscle fibers in the three groups. Statistical comparisons indicated that the mean areal ratios of necrotic fibers were significantly lower in the LC+HT group than in the Control-LC group at 3h after LC. Furthermore, the areal ratios of regenerated muscle fibers to the pathological regions were significantly higher in the LC+HT and LC+MT groups than in the Control-LC group at 4 days after LC.

Conclusions: The results indicate that HT reduces EIMD, while HT and MT accelerate the muscle regeneration process after EIMD in an animal model of DOMS.

Keywords: Delayed Onset Muscle Soreness (DOMS); Exercise-Induced Muscle Damage (EIMD); Lengthening Contraction; Manual Therapy; Thermotherapy

Introduction

Vigorous sports and sudden movements lead to the development of musculoskeletal system damage (bone, joint, muscle, ligament, etc.). In particular, Delayed Onset Muscle Soreness (DOMS) is a muscular pain that develops due to Lengthening Contraction (LC) (i.e., muscle contraction during muscle stretch) 2-3 days after inexperienced vigorous exercise, and disappears approximately 3-10 days later. Conservative therapies for this muscle pain and damage include Manual Therapy (MT) and thermotherapy. MT, which includes stroking, rubbing, kneading, and compression, has been reported to ameliorate DOMS [1-3] and significantly reduce the creatine kinase level, a marker of cell membrane damage in the muscle fibers [4]. Thermotherapy such as Heat Therapy (HT) is applied for analgesia, metabolic acceleration, and reduction of muscle tonus, which might be mediated through an increased local blood flow in the affected part by thermal stimulation using a hot pack, microwave, paraffin bath, or ultrasound [5,6]. Furthermore, previous studies reported that HT accelerates the regeneration process of the muscle [7], while cold therapy for muscle crush in the acute phase delays muscle regeneration as well as function recovery [8]. However, review papers on physical interventions of DOMS in humans reported controversial results, and there is no enough evidence for the effectiveness of the interventions [6,9,10]. It might be difficult to avoid favorable preconceptions about physical interventions in human clinical studies [11].

LC that induces DOMS development causes Exercise-Induced Muscle Damage (EIMD) through sarcomere disruption and muscle cell membrane damage [12-14]. During the EIMD and subsequent muscle regeneration processes, necrotic fibers (pale muscle fibers) and/or opaque (hypercontracted) fibers appear in the initial phase, and leukocytes (neutrophils, macrophages, etc.) phagocytizing necrotic fibers accumulate in the inflammatory phase [13]. Thereafter, satellite cells (muscle stem cells) are activated to proliferate and differentiate into myoblasts to become mature muscle fibers via myotubes. However, no previous studies have histologically examined the effects of HT and MT on EIMD and muscle regeneration in muscles with

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DOMS. We developed a rat DOMS model using the gastrocnemius muscle and reported that both HT (heat therapy) and MT (muscle compression) significantly ameliorated mechanical hyperalgesia in this animal model [15-17]. However, DOMS intensity seems not to reflect the magnitude of EIMD in humans [18], suggesting that the HT and MT effective in ameliorating mechanical hyperalgesia are not necessarily to be effective in ameliorating EIMD in the muscle with DOMS.

In the present study, to investigate the effects of HT (Heat Therapy) and MT (muscle compression) on pathological processes (EIMD and muscle regeneration) after LC, we histologically analyzed the gastrocnemius muscle after LC with and without the same HT and MT used in our previous studies that were effective for reducing mechanical hyperalgesia in a rat model of DOMS [16,17].

Materials and Methods

Subjects

A total of 60 male Sprague Dawley rats were used. The animals were allowed ad libitum access to water and pellets in a rearing room during the experimental period. All experiments including the present study were performed according to the Guidelines for Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (1996). Experimental procedures were approved by the Ethical Committee for Animal Experiments at the University of Toyama (Permit number; S-2010 MED-63 and A2013 MED-38). Muscle specimens were derived from the animals used in our previous studies on Capillary Electrophoresis Time-Of-Flight Mass Spectroscopy (CE-TOFMS) analyses of muscle metabolites [16,17] and histologically analyzed in the present study.

Experimental Protocol

The experimental treatments that the animals received were described in detail in our previous study [16,17]. Briefly, the rats that underwent LC under pentobarbital anesthesia (50 mg/kg, i.p.) were allocated to three groups: 1) intervention-free LC group (Control-LC group) [rats were sacrificed 3 h (LC/3h, n = 6), 12h (LC/12h, n = 5), 27h (LC/27h, n = 6), and 4 days (LC/4d, n = 5) after LC], 2) heat therapy group (LC+HT group) [rats with LC followed by HT were sacrificed 3 h (LC+HT/3h, n = 6), 12h (LC+HT/12h, n = 5), 27h (LC+HT/27h, n = 6), and 4days (LC+HT/4d, n = 5) after LC], and 3) manual therapy group (LC+MT group) [rats with LC followed by MT were sacrificed 27h (LC+MT/27h, n = 5) and 4 days (LC+MT/4d, n = 5) after LC], and were subjected to muscle sampling at each elapsed time. For control rats, animals only anesthetized with pentobarbital (50 mg/kg, i.p.) were used (n = 6), and sacrificed 3h after the anesthesia.

Lengthening Contraction (LC)

The experimental procedures for LC were described in detail in our previous study [17]. To induce DOMS, LC was applied as reported previously [15-17]. The left lower hind-leg muscle (i.e., gastrocnemius muscle) was used to evaluate the effects of MT and HT. Briefly, on day 0 (onset of LC), the animals were anesthetized with sodium pentobarbital (50 mg/kg, i.p.). LC was induced in the lateral head of the gastrocnemius muscle by electrical stimulation of the tibial nerve through a pair of needle electrodes inserted near the nerve. According to previous studies for the rat DOMS model [15-17], electrical stimulation was applied using a constant current stimulator for 1 s according to the following parameters: current strength of thrice the twitch threshold (< 150 μA) and a frequency of 50 Hz with a pulse duration of 1ms. The rat's paw and ankle joint were fixed to a foot plate. The foot plate was mechanically pulled to move the ankle joint from the plantar position (25° plantarflexion) to dorsiflexion (20°, total 45° range of motion) by using a linearized servomotor for lengthening the gastrocnemius muscle during a 1sec period autonomously synchronizing with electrical stimulation (resulting in LC), and then returning to the starting position during a 3 sec resting period. This cycle was repeated 500 times. After recovery from anesthesia with LC, the animals were allowed *ad libitum* access to water and pellets in the rearing room.

Heat Therapy (HT)

The experimental procedures for HT were described in detail in our previous study [17]. HT was performed immediately after LC in the LC+HT group. An insulated gel pack 6 x 5 cm (9 ml) was warmed to 42 ± 0.5°C in a thermostatic incubator, applied to the skin over the left gastrocnemius muscle, and replaced every 4 min (20 min total). This method of HT is previously reported to significantly reduce mechanical hyperalgesia in an animal model of DOMS [17]. Temperature of the gel in application was checked to be as shown above using thermography. The rats in the other groups were left for 20 min in the same way except this procedure.

Manual Therapy (MT)

The experimental procedures for MT were described in detail in our previous study [16]. The LC+MT group received MT 24h after LC, which significantly reduced mechanical hyperalgesia previously [16]. On day 1 (following the LC day), the left gastrocnemius muscle of the rats in the LC+MT group received MT without anesthesia. MT consisted of the following three steps: (1) 1-min handling of the animals by their trunks to reduce stress and enable relaxation, (2) 10 min application of intermittent and rhythmical compression (1-2 Hz) using the thumb, which was followed by (3) another 1min handling of the animals to promote relaxation. Mechanical pressure on the thumb was monitored online through an interface using a strain gauge [diameter = 5 mm (area of the sensor = 19.6 mm^2)]. The pressure was maintained at < 12 kPa/19.6 mm^2; total force over the whole area of the thumb was < 2.8 N to avoid tissue damage and behavioral excitation.

Histological Procedures

The all animals were decapitated under deep anesthesia (sodium pentobarbital, 70mg/kg i.p.) after each elapsed time, and the left lateral gastrocnemius muscle was removed. The muscle was divided into three parts: upper, central (length = 5mm), and lower parts of the gastrocnemius muscle. The upper and lower parts of the muscles were frozen in isopentane cooled in liquid nitrogen, and stored in a freezer at -80°C until section cutting, while the central part of the muscle was stored in a freezer at -80°C for a different study for metabolomic analyses of the muscle. The upper and lower parts of the muscle were cut from the stump sides into 10 μm cross-sections using a cryostat. The sections were stained with hematoxylin and eosin.

Histological Analysis

A total of 40 sections per animal (20 sections from the upper part of the muscle and 20 from the lower part of the muscle at the

stump sides) were analyzed by an all-in-one digital microscope (Bz-9000, Keyence Corporation, Osaka, Japan). The digital images were processed and analyzed using the BzII analyzer software (Keyence corporation, Osaka, Japan). Using this software, muscle cross-sectional area, areas of tissue damage (opaque fibers, necrotic fibers, and leukocytes), and area of regenerated muscle fibers were measured in each section in each animal.

Muscle pathological changes after acute muscle mechanical stress such as LC are caused largely by muscle cell membrane damage [14]. Previous histological examination of muscle damage reported changes in muscle fibers during the time course from EIMD to the muscle regeneration process (see below).

Opaque fibers: The opaque fiber is a densely stained hypertrophic muscle fiber due to hypercontraction, indicating a degenerative finding in the stage prior to the appearance of necrotic fibers [19]. These opaque fibers have been reported in various pathological conditions, including LC, muscular dystrophy, multiple myositis, and administration of myotoxic substances, such as bupivacaine hydrochloride injection [19]. Opaque fibers are suggested to have membrane damage, from which extracellular calcium (Ca^{2+}) and sodium (Na^{+}) ions enter the intracellular space to induce hypercontraction of muscle cells [20-22]. Furthermore, it has been reported that Ca^{2+} activates proteases, leading to autolysis [14].

Necrotic fibers: EIMD causes muscle degeneration. Histologically, necrotic fibers are classified into (1) muscle fiber that becomes swollen or round, (2) muscle fiber that stains lighter, and (3) muscle fiber with blurred outline [8,23]. After the degeneration, these fibers are phagocytized by leukocytes, including macrophages, prior to progression to the muscle regeneration process.

Leukocytes: Leukocytes infiltrate the sites of EIMD to phagocytize muscle fibers that have undergone necrosis. It has been reported that macrophages, more than any other leukocytes, not only phagocytize necrotized tissues but also produce Interleukin 6 (IL-6), a cytokine facilitating proliferation and differentiation of myoblasts in the initial stage of muscle regeneration, and suppress excessive collagen production, which results in scar tissue [24-26]. It has also been reported that macrophages act to directly facilitate myoblast proliferation [25]. To quantify the number of leukocytes, we measured nuclei area of leukocytes.

Regenerated muscle fibers: Satellite cells localized between the basement membrane and the plasma membrane of muscle fibers play a central role in muscle fiber regeneration [27,28]. Satellite cells are activated to differentiate into myoblasts in the initial phase of the muscle regeneration process and to differentiate into myotubes and muscle fibers for muscle regeneration [29]. In the present study, we categorized muscle fibers with central nuclei as regenerated muscle fibers, which appear in the initial phase of muscle regeneration [30]. Along with maturation of the muscle fiber, the nucleus in the fiber moves to the margin (membrane).

Statistical analysis

The data derived from the control group were excluded from the statistical analysis, since no animals in this group showed any pathological changes (see Results). All data were expressed as mean ± standard error. First, mean muscle cross-sectional area, mean areas of tissue damage (opaque fibers, necrotic fibers, and leukocytes), and mean area of regenerated muscle fibers were estimated from the data of 40 sections in each animal. Second, mean areal ratios of each pathological region [EIMD (opaque fibers, necrotic fibers, leukocytes), and regenerated muscle fibers] to the muscle cross-sectional area (i.e., each pathological area/muscle cross-sectional area) were compared between the two groups with LC (Control-LC group *vs*. LC+HT or LC+MT group sacrificed at each elapsed time after LC) by t-test.

Third mean areal ratios of regenerated muscle fibers to the all pathological regions (i.e., area of regenerated muscle fibers/ pathological area) were compared among the three groups with LC (Control-LC group, LC+HT, and LC+MT groups sacrificed at 27h and 4days after LC) by one-way Analysis of Variance (ANOVA) with multiple comparison test (Tukey test). Statistical significance level was set at $p < 0.05$.

Results

Effects of HT on LC- Induced Pathological Processes

Figure 1 shows photomicrographs of muscle tissue specimens in the naive (A) and Control-LC groups (B-F). In the control group (3h after anesthesia), no animals showed any pathological changes (A). In the Control-LC group, compared to the control group, opaque fibers and necrotic fibers appeared 3h after LC (LC/3h) (B, C), and the number of leukocytes increased gradually over 12h (LC/12h) (D), 27h (LC/27h) (E), and 4 days (LC/4d) after LC (F). In addition, regenerated muscle fibers appeared 4 days after LC (LC/4d) (F).

Figure 2A shows photomicrographs of muscle tissue specimens from the LC+HT group 3h (LC+HT/3h) (Aa), 12h (LC+HT/12h) (Ab), 27h (LC+HT/27h) (Ac), and 4 days (LC+HT/4d) (Ad) after LC.

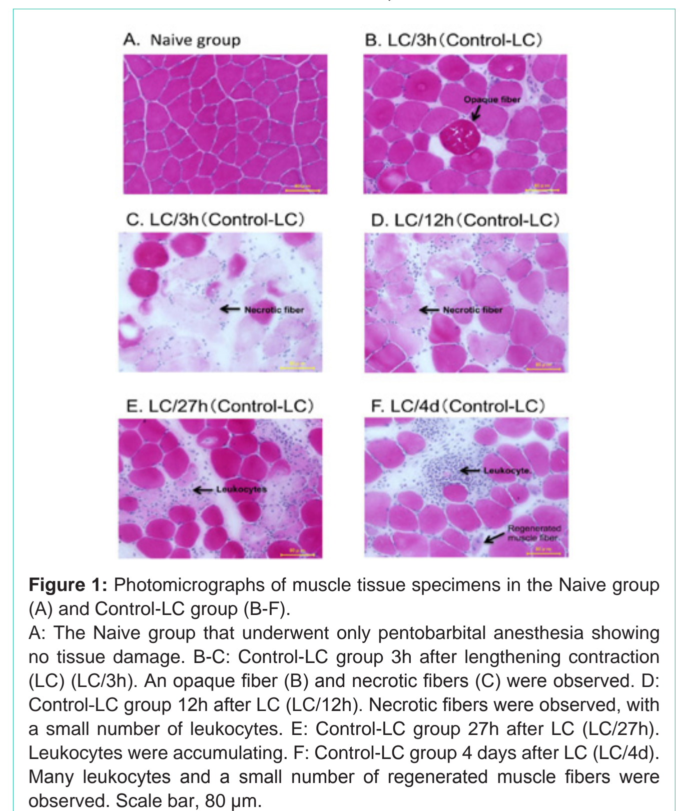

Figure 1: Photomicrographs of muscle tissue specimens in the Naive group (A) and Control-LC group (B-F).
A: The Naive group that underwent only pentobarbital anesthesia showing no tissue damage. B-C: Control-LC group 3h after lengthening contraction (LC) (LC/3h). An opaque fiber (B) and necrotic fibers (C) were observed. D: Control-LC group 12h after LC (LC/12h). Necrotic fibers were observed, with a small number of leukocytes. E: Control-LC group 27h after LC (LC/27h). Leukocytes were accumulating. F: Control-LC group 4 days after LC (LC/4d). Many leukocytes and a small number of regenerated muscle fibers were observed. Scale bar, 80 µm.

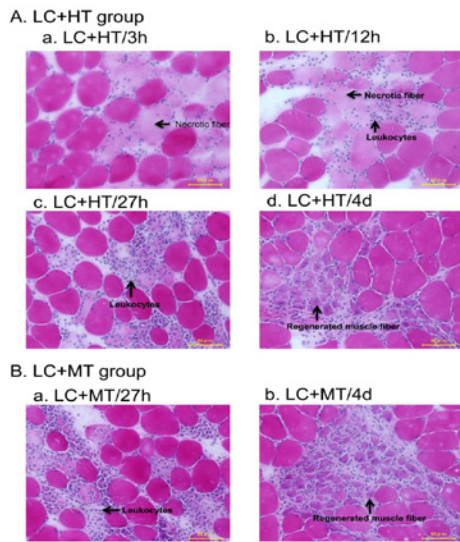

Figure 2: Photomicrographs of muscle tissue specimens in the LC+HT group (A) and the LC+MT group (B).
A: LC+HT group. a: 3h after LC (LC+HT/3h). Necrotic fibers were visible. b: 12h after LC (LC+HT/12h). Necrotic fibers and leukocyte infiltration were visible. c: 27h after LC (LC+HT/27h). Leukocyte accumulation was visible. d: 4 days after LC (LC+HT/4d). Abundant regenerated muscle fibers were visible. Scale bar, 80 µm.
B: LC+MT Group. a: 27h after LC (LC+MT/27h). Leukocyte accumulation was visible. b: 4 days after LC (LC+MT/4d). Many regenerated muscle fibers were visible. Scale bar, 80 µm.

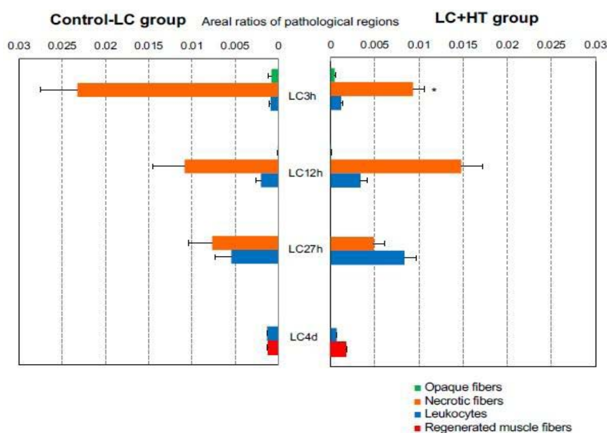

Figure 3: Comparison of pathological processes in the muscle after LC between the Control-LC (left graph) and LC+HT (right graph) groups.
The graph shows areal ratios of each pathological finding (opaque fibers, necrotic fibers, leukocytes, or regenerated muscle fibers) to the muscle cross-sectional area (i.e., each pathological area/muscle cross-sectional area) in the Control-LC and LC+HT groups. Compared with the Control-LC group, the areal ratio of necrotic fibers was significantly lower in the LC+HT group at 3 h after LC (LC3h) (*, $p < 0.05$). Green bar, opaque fibers; orange bar, necrotic fibers; blue bar, leukocytes; red bar, regenerated muscle fibers. LC3h, 3h after LC; LC12h, 12h after LC; LC27h, 27h after LC; LC4d, 4 days after LC. Vertical axis, time after LC; horizontal axis, ratio of each pathological area to the muscle cross-sectional area.

Similar time-dependent changes as those of the Control-LC group were observed 3h, 12h, and 27h after LC, and abundant regenerated muscle fibers were observed 4 days after LC.

Figure 3 shows mean areal ratio of each pathological region [EIMD

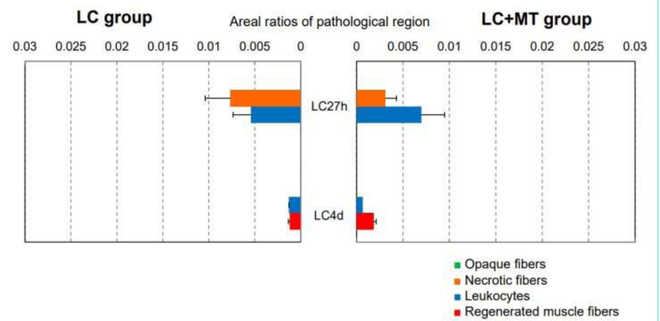

Figure 4: Comparison of pathological processes in the muscle after LC between the Control-LC (left graph) and LC+MT (right graph) groups.
The graph shows areal ratios of each pathological finding (opaque fibers, necrotic fibers, leukocytes, or regenerated muscle fibers) in the muscle cross-sectional area (i.e., each pathological area/muscle cross-sectional area) in the Control-LC and LC+MT groups. Comparison of ratios of each pathological area between the two groups indicated no significant inter-group difference ($p > 0.05$). Green bar, opaque fibers; orange bar, necrotic fibers; blue bar, leukocytes; red bar, regenerated muscle fibers. LC27h, 27 h after LC; LC4d, 4 days after LC. Vertical axis, time after LC; horizontal axis, ratio of each pathological area to the muscle cross-sectional area.

(opaque fibers, necrotic fibers, leukocytes), and regenerated muscle fibers] to the muscle cross-sectional area (i.e., each pathological area/ muscle cross-sectional area) in the Control-LC and LC+HT groups. Comparisons of the ratios of the opaque fibers, necrotic fibers, leukocytes, and regenerated muscle fibers between the two groups revealed that the areal ratios of necrotic fibers were significantly lower in the LC+HT group than the Control-LC group at 3h after LC ($p < 0.05$). However, there was no significant difference in the areal ratios of opaque fibers and leukocytes at 3h after LC ($p > 0.05$). In addition, there was no significant difference in the areal ratios between the two groups at 12h, 27h, and 4 days after LC ($p > 0.05$).

Effects of MT on LC - Induced Pathological Processes

Figure 2B shows photomicrographs of the muscle tissue specimens in the LC+MT group at 27h (LC+MT/27h) (Ba) and 4days (LC+MT/4d) (Bb) after LC. Nearly the same time-dependent changes as those in the Control-LC group were observed and abundant regenerated muscle fibers were observed 27h and 4days after LC (LC+MT/4d).

Figure 4 shows mean areal ratios of each pathological region [EIMD (opaque fibers, necrotic fibers, leukocytes), and regenerated muscle fibers] to the muscle cross-sectional area (i.e., each pathological area/muscle cross-sectional area) in the Control-LC and LC+MT groups. Statistical comparisons of the ratios of each pathological area between the two groups indicated no significant inter-group differences at 27h or 4days after LC ($p > 0.05$).

Effects of HT and MT On Muscle Regeneration

The above results indicate that HT and MT did not affect areal ratios of regenerated muscle fibers to the muscle cross-sectional area, while HT and MT tended to reduce areal ratios of the pathological regions in the LC+HT and LC+MT groups compared with the Control-LC group. The finding with no effects of HT and MT might be ascribed to less EIMDs in the LC+HT and LC+MT groups; less EIMDs might induce less muscle regeneration in the LC+HT and LC+MT groups. Therefore, we analyzed the areal ratios of regenerated

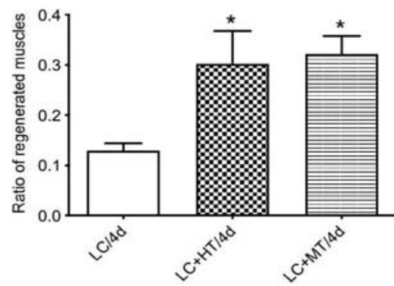

Figure 5: Effects of HT and MT on muscle regeneration after LC.
The graph shows areal ratios of regenerated muscle fibers to the pathological area (area of regenerated muscle fibers/pathological area). The areal ratio of the regenerated muscle fibers increased significantly in the LC+HT group (LC+HT/4d) and LC+MT group (LC+MT/4d) compared to the Control-LC group (Control-LC/4d) 4 days after LC (*, $p < 0.05$).

muscle fibers to the all pathological regions (i.e., area of regenerated muscle fibers/pathological area). Figure 5 shows comparison of the areal ratios of regenerated muscle fibers to the pathological area among the three groups 4 days after LC. A statistical analysis by one-way ANOVA indicated a significant difference among the three groups [$F(2, 13) = 6.872$, $p < 0.01$]. Post-hoc tests revealed that the areal ratios of regenerated muscle fibers were significantly higher in the LC+HT and LC+MT groups than in the Control-LC group (Tukey's multiple comparison test, $p < 0.05$). However, there was no significant difference in the areal ratios of regenerated muscle fibers to the pathological area among the three groups 27h after LC [$F(2, 13) = 2.525$, $p > 0.05$].

Discussion

Effects of HT

There is not sufficient evidence for the treatment of DOMS with HT in human clinical studies that reported conflicting findings: HT has been reported to be effective for the reduction of pain and muscle spasm, improvement of circulation, and facilitation of recovery from muscle damage [5,6], while HT is regarded critically, especially in an acute phase [31]. In our previous study, the same HT for the rat gastrocnemius muscle significantly ameliorated mechanical hyperalgesia after LC [17]. This study histologically examined the effects of the same HT on the rat gastrocnemius muscle that was subjected to the same LC. The present results indicated that the HT significantly decreased the areal ratio of necrotic fibers 3h after LC. Our previous study reported that the HT after LC increased muscle blood flow as well as metabolites involved in the improvement of blood flow and oxidative metabolisms in the same DOMS model [17]. Such improvement effects of HT on metabolic stress may contribute to reduction of necrotic fibers in the present study. Furthermore, it is reported that microwave HT induces Heat Shock Protein (HSP) in human skeletal muscle [32]. HSP, which appears when cells are stressed, has been indicated to prevent contraction-induced muscle damage [33,34]. The present results, along with these findings, suggest that HT is effective for reducing muscle damage through the improvement of muscle oxidative metabolism due to increases in blood flow and metabolite alteration, and through HSP induction.

In the EIMD and subsequent regeneration processes, emergence of hypercontracted muscle fibers (opaque fibers), necrotic fibers, inflammatory reaction (leukocytes), and muscle regeneration occur sequentially. Leukocytes accumulate to phagocytize necrotic fibers. Among them, macrophages have been shown to enhance proliferation of satellite cells involved in the regeneration of muscle fibers [35]. Macrophages (especially, M2 macrophages) secrete growth factors for proliferation of the satellite cells, including Insulin-Like Growth Factor-1 (IGF-1) and transforming growth factor beta-1 (TGF- ß1); these factors have been shown to induce muscle regeneration by facilitating differentiation and proliferation of satellite cells [36-39]. In this study, the areal ratio of regenerated muscle fibers to the pathological area increased significantly 4 days after LC in the LC+HT group, which might be attributed to these growth factors. A previous study in which crush injury was induced in the rat extensor digitorum longus muscle revealed that 20min heat application increased migration of macrophages important for muscle regeneration, activated satellite cells in the initial stage of muscle regeneration, and accelerated muscle regeneration in the thermal group compared to the control group (non-thermal group) [40]. These findings suggest that in our LC+HT group, IGF-1 and/or TGF-ß1 released from macrophages activated the differentiation and proliferation of satellite cells, as shown in the previous studies (see above). Further studies are required to determine whether these macrophage-associated chemical factors facilitate muscle regeneration in the muscle with DOMS.

Effects of MT

In our previous study, the same MT for rat gastrocnemius muscle subjected to LC significantly ameliorated mechanical hyperalgesia [16]. In the present study, although there was no significant difference in the areal ratios of EIMD regions (opaque fibers, necrotic fibers, and leukocytes) between the LC and LC+MT groups, the areal ratio of regenerated muscle fibers to the pathological area increased in the LC+MT group. This is the first report demonstrating that the same MT is effective not only for analgesia but also for regeneration process from EIMD in the muscle with DOMS.

It has been reported that pressure stimulation of normal muscle activates the immune system, including M2 macrophages that have an anti-inflammatory function and secrete TGF-ß1 [41,42]. These results suggest that MT, similar to HT, increased IGF-1 and/or TGF-ß1 production through macrophages to facilitate muscle regeneration, as shown in the previous study (see above). It is also probable that MT enhanced blood flow [43], leading to amelioration of DOMS-related pathology, which facilitates muscle regeneration. The ineffectiveness of MT to reduce EIMD in the present study might be ascribed to the timing of MT; MT was applied to the muscle on the next day after LC in the present study. However, the histological data in the Control-LC group (Figure 3) suggest that the acute phase of EIMD almost terminates within 24h after LC. MT application just after LC would be more effective to reduce EIMD.

It has been suggested using rats and humans that induction of muscle LC leads to the formation of hyperirritable taut-bands of the skeletal muscle, which are known as trigger points and strongly associated with muscle pain [44-46]. Various tissue damages were reported in the taut-band in experimental models of trigger points [46,47]. Various algesic substances are released from trigger points to induce muscle pain [44,45,48]. Our preceding study revealed that

muscle compression (MT) applied to trigger points of patients with acute low back pain lowered subjective low back pain, increased pressure pain threshold, and increased range of joint motion one week and one month after manual compression, respectively [49]. The findings suggest that MT ameliorates muscle pain, in part, by facilitating tissue repair.

Limitations

In the present study, the LC+MT group received MT 24h after LC but not immediately after LC, since our previous study indicated that this schedule (24h after LC) significantly reduced mechanical hyperalgesia [16]. However, it is possible that MT applied immediately after LC would be more effective. Further studies are required to investigate efficacy of MT applied immediately after LC.

Conclusions

Several studies including review articles on non-pharmacological interventions of DOMS in humans reported conflicting results and lack of sufficient evidence for the effectiveness of the physical interventions [6,9-11]. The present study investigated effects of HT and MT, which were previously reported to be effective to reduce mechanical hyperalgesia in the rat model of DOMS due to LC [16,17], on pathological processes in the muscle of the same rat DOMS model. The results indicated that HT decreased the mean areal ratios of necrotic fibers at 3h after LC, while both HT and MT increased the mean areal ratios of regenerated muscle fibers at 4 days after LC. The results suggest that the same interventions effective to reduce muscle mechanical hyperalgesia in DOMS also facilitate recovery processes from EIMD in humans. Previous studies reported that both mechanical (massage) and heat stimuli enhanced macrophage accumulation in the rat muscle [41,50], suggesting that facilitated recovery from EIMD in the LC+HT and LC+MT groups might be attributed, in part, to enhanced accumulation of macrophages that are critical for muscle repair after muscle damage [14,38]. The present results along with our previous studies on the animal model of DOMS [16,17] provide scientific basis for use of heat and manual therapies for treatment of DOMS.

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