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REVIEW

Effects of Physical Agents on Muscle Healing with a Focus on Animal Model Research

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ABSTRACT. Skeletal muscle injury is caused by a variety of events, such as muscle laceration, contusions, or strain. Muscle fibers respond to minor damage with immediate repair mechanisms that reseal the cell membrane. On the other hand, repair of irreversibly damaged fibers is achieved by activation of muscle precursor cells. Muscle repair is not always perfect, especially after severe damage, and can lead to excessive fibroblast proliferation that results in the formation of scar tissue within muscle fibers. Remaining scar tissue can impair joint movement, reduce muscular strength, and inhibit exercise ability; therefore, to restore muscle function, minimizing the extent of injury and promoting muscle regeneration are necessary. Various physical agents, such as cold, thermal, electrical stimulation, and low-intensity pulsed ultrasound therapy, have been reported as treatments for muscle healing. Although approaches based on the muscle regeneration process have been under development, the most efficacious physiological treatment for muscle injury remains unclear. In this review, the influence of these physical agents on muscle injury is described with a focus on research using animal models.

Key words: Muscle injury, Muscle regeneration, Physical agents

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Skeletal muscle injury is caused by a variety of events, such as muscle laceration, contusions, or strain[1]. Muscle injuries are one of the most common injuries in relation to sports[2]. Muscle fibers respond to minor damage by utilizing limited immediate repair mechanisms to reseal the cell membrane. On the other hand, repair and replacement of irreversibly damaged fibers is achieved by activating muscle precursor cells that are predetermined to differentiate into skeletal muscle[3,4]. Satellite cells are the major source of myogenic cells in adult skeletal muscle. They are located between the plasma and basement membranes of muscle fibers[5] and play a key role in muscle repair[6]. Satellite cells are normally quiescent in undamaged adult muscle, only being activated in response to injury. When activated, these cells can proliferate, move to the area of damage, and fuse with preexisting fibers and the surviving segments of damaged fibers. However, muscle repair does not always perfectly align the surviving fiber stump with the newly forming repair segment, and thus, many fibers can become branched after regeneration[4], especially after severe or extensive damage, such as deep wounds, leading to the excessive proliferation of fibroblasts and the formation of scar tissue within muscle fibers. Scar tissue exhibits no contractile function and has low extensibility; therefore, remaining scar tissue can impair joint movement, reduce muscular strength, and inhibit exercise ability[7]. For these reasons, minimizing the extent of injury and promoting muscle regeneration is necessary to restore muscle function.

Skeletal muscle regeneration is stimulated by muscle injury, after which, it undergoes sequential phases of degeneration, inflammation, regeneration, and the formation of new myofibers or fibrosis[1,8] (Table 1). The inflammatory response is essential to the effective repair of tissue, and the inhibition of these events inhibits the repair phases that follow[9,10]. Therefore, to apply optimal physical agents, it is necessary to understand the muscle regeneration process. In the case of pharmacological treatments, anti-inflammatory drugs, growth factors, and fibrosis inhibitors are prescribed as appropriate in accordance with the healing process[1,11]. Regarding physiotherapy interventions, various physical

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Table 1. Phases of skeletal muscle healing[8].

Healing phase	Changes within the muscle
Degeneration/inflammation phase	Characterized by rupture and necrosis of myofibers, formation of a hematoma, and important inflammatory reactions
Regeneration phase	Phagocytosis of damaged tissue, followed by myofiber regeneration, leading to satellite cell activation
Remodeling phase	Maturation of regenerated myofibers with recovery of muscle functional capacity, fibrosis, and scar tissue formation

agents, including cold, thermal, electrical stimulation, and low-intensity pulsed ultrasound therapy (LIPUS), have been used as treatments for muscle healing. The purposes of applying physical agents are to diminish pain following injury and to accelerate muscle healing. In this review, the influence of these physical agents on muscle injury is described with a focus on research using animal models.

Cryotherapy

Cryotherapy, defined as the use of cold modalities, such as icing, cold packs, and cold water immersion, is widely applied as a treatment in the acute phase of muscle injury. The physiological responses to cryotherapy include decreased blood flow, tissue metabolism, and nerve conduction velocity, which diminish bleeding and acute inflammation symptoms[12,13] such as swelling[14] and pain[15-17]. Ischemia due to bleeding and increased enzyme activity following the primary injury can cause additional damage to uninjured tissues around the injury site, which are referred to as secondary injuries. Cryotherapy suppresses secondary injuries because it reduces local metabolism and cellular energy demands for surviving tissues[18,19], and is therefore considered valuable in minimizing the extent of muscle damage.

While cryotherapy has been reported to exert a beneficial effect for posttraumatic symptoms, it has also been shown to retard muscle regeneration (Table 2). Takagi et al.[20] showed that ice applied immediately after a muscle crush injury can retard muscle regeneration and induce collagen deposition. They also found that the expression of both transforming growth factor (TGF)-β1 and insulin-like growth factor-1 was retarded in the icing group, which suggested that these growth factors, which were produced by macrophages, regulated the proliferation and differentiation of satellite cells and collagen synthesis, thereby indicating that icing delayed muscle regeneration. Shibaguchi et al.[21] investigated the effects of icing after bupivacaine-induced muscle injury. Their results also showed an increasing collagen area, which might be related to the delay in the timing of the inhibited expression of TGF-β during regeneration. Ito et al.[22] also investigated the effects of cryotherapy using a cardiotoxin-induced muscle injury model. They found that the wet weight of regenerated muscle and the cross-sectional area of myofibers were decreased after cryotherapy. Cryotherapy applied immediately after injury inhibited the accumulation of macrophages in the inflammatory process[20,23]. As inflammatory cells play essential roles in regulating the muscle repair response and development of fibrosis after muscle injury[9,10,24]; if these inflammatory events are impaired by cryotherapy, muscle regeneration is also affected.

On the other hand, it has been reported that cryotherapy inhibits the inflammatory process without changing the expression of myogenic regulatory factors, such as desmin and myoD, or collagen deposition[13]. Singh et al.[25] demonstrated that icing attenuated or delayed the infiltration of inflammatory cells and the expression of proangiogenic factors in regenerating muscle. Despite these differences, no significant differences were observed in the capillary density or myofiber cross-sectional area between the icing and no icing groups. Ikezaki et al.[26] investigated the effects of icing on the muscle regeneration process, including molecules related to pain, and found that icing was effective for alleviating muscle soreness; however, icing had no influence on histological features, such as the cross-sectional area of regenerated muscle fibers or the ratio of central nucleated fibers. Cryotherapy is generally used in the acute phase of muscle injury to alleviate inflammatory symptoms and secondary injuries. However, the results of studies investigating the effectiveness of cryotherapy on muscle regeneration have been inconsistent, as described above, which suggests that cryotherapy should be administered after muscle injury only after careful consideration.

Thermal Therapy

Several thermal modalities are available for heat application to tissues. Thermal therapy is categorized into superficial heating, such as hot packs, warm whirlpool and paraffin, and deep heating, such as continued ultrasound and diathermy. The physiological effects of elevating tissue temperature results in an increase in blood flow to the area, attributable in part to the vasodilatory response in surface blood vessels. In addition, increasing tissue temperature is associated with an increasing metabolic rate[27]. Based on these responses, heat stress appears to play a beneficial role in wound healing after the acute phase as a result of in-

Table 2. Studies on cold therapy for muscle injury in animal models.

Reference	Injury type	Method of cryotherapy	Timing of cryotherapy	Effects in the cryotherapy compared with the injured without cryotherapy group
Ramos et al.[13]	Freezing injury	Ice pack (plastic bag filled with crushed ice) 3 sessions of 30 min, 2 h apart	Immediately after injury, 24 h and 48 h after injury	Decreased macrophage infiltration and accumulation of TNF-α, NF-κB, and TGF-β No influence injury area, expression of desmin and MyoD, and collagen I and III protein levels.
Takagi et al.[20]	Crush injury	Ice pack, 0.3~1.3 °C (plastic bag filled with crushed ice) 20 min	5 min after injury	Retarded number of macrophages and immunohistochemical expression of TGF-β1 and IGF-I Decreased muscle fiber cross-sectional area Increased collagen fiber area
Shibaguchi et al.[21]	Bupivacaine-induced muscle injury	Ice pack, 0 °C 20 min	Immediately after injury	Delayed the timing of disappearance of TGF-β Increased collagen deposition
Ito et al.[22]	Cardiotoxin-induced muscle injury	Ice-cold water 20 min	IE group: 1 h after injury ID group: 8 days after injury	IE group: decreased muscle wet weight and muscle fiber cross-sectional area ID group: no influence of muscle wet weight and muscle fiber cross-sectional area
Miyakawa et al.[23]	Crush injury	Ice pack, 0.3~1.3 °C (plastic bag filled with crushed ice) 20 min	5 min after injury	Inhibited accumulation of macrophages. Delayed neutrophil and monocyte chemoattractant protein-1 + cells.
Singh et al.[25]	Contusion injury	Icing 20 min	5 min after injury	Attenuated and/or delayed neutrophil and macrophage infiltration, expression of vWF, VEGF, and nestin No influence on capillary density or muscle fiber cross-sectional area
Ikezaki et al.[26]	Bupivacaine-induced muscle injury	Ice pack, 0 °C 20 min	Immediately or 3 days after injury	No influence on muscle fiber cross-sectional area Decreased expression of myoD and BKB2 receptor mRNA

TNF-α, tumor necrosis factor-α; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; TGF-β, transforming growth factor-β; IGF-1, insulin growth factor-1, factor; vWF, von Willebrand factor; VEGF, vascular endothelial growth factor; BKB2 receptor, Bradykinin B2 receptor; IE group, icing at early stage of muscle injury group; ID group, icing at delayed stage of muscle injury group.

creased blood flow, which improves wound and periwound tissue perfusion and increases both oxygen and wound oxygen tension[27]. In addition, thermal therapy is generally contraindicated in acute phase injuries because heat stimulation increases the inflammation response and metabolic rate excessively, and can aggravate secondary injuries. However, heat stress has also been reported to exert a beneficial effect in the acute stage of muscle damage (Table 3). Kojima et al.[28] showed that whole-body heat stress immediately after injury stimulated the proliferation of satellite cells and protein synthesis during the regeneration process in a cardiotoxin-induced muscle injury model. In addition, the application of hot water immersion has been shown to accelerate the recovery of fiber size and myonuclear number, and to increase the numbers of total and activated satellite cells[29]. Hatade et al.[30] applied a hot pack to a muscle crush injury and found that heat stress immediately after the injury could enhance the proliferation and differentiation of myogenic cells and the expression of muscle regulatory factors MyoD and myogenin. In addition, heat stress was shown to facilitate the migration of macrophages to the injury site, the proliferation and differentiation of satellite cells, the growth of muscle fiber, and the inhibition of collagen synthesis in the regenerating muscle in other thermal stimulation experiments using hot packs after a muscle crush injury[31]. Moreover, in a rat model, no painful behavior responses were exhibited after heat treating. Shibaguchi et al.[21] examined the effects of intermittent heat stress compared with icing on the regeneration process in a bupivacaine-induced muscle injury. The results indicated that heat stress suppressed the increasing fibrosis and partially promoted the recovery of muscle mass, protein content, and size of the muscle fibers in injured skeletal muscle. In addition, enhanced macrophage infiltration, the pro-

Table 3. Studies on thermal therapy for muscle injury in animal models.

Reference	Injury type	Method of thermotherapy	Timing of thermotherapy	Effects in the thermotherapy compared with the injured without thermotherapy groups
Shibaguchi et al.[21]	Bupivacaine-induced muscle injury	Hot water immersion 42 °C 30 min	Initiated 48 h after injury and continued every other day	Reduced the development of fibrosis Increased muscle mass, myofibrillar protein content, muscle fiber cross-sectional area, and expression of Pax-7-positive satellite cells and heat shock protein
Kojima et al.[28]	Cardiotoxin-induced muscle injury	Whole-body heat stress 41 °C 60 min	Immediately after injury	Increased protein content, Pax-7-positive satellite cells, and heat shock protein 72
Oishi et al.[29]	Bupivacaine-induced muscle injury	Hot water immersion 42±1 °C 30 min	Initiated 48 h after injury and continued every other day	Increased myonuclear number, numbers of Pax-7- and MyoD-positive satellite cells, and heat shock protein 72
Hatade et al.[30]	Crush injury	Hot pack 42 °C 20 min	5 min after injury	Earlier expression of MyoD and myogenin protein
Takeuchi et al.[31]	Crush injury	Hot pack 42 °C 20 min	5 min after injury	Increased muscle fiber cross sectional area Faster expression of ED1-positive macrophages Increased number of Pax-7-positive satellite cells Less collagen fiber area

liferation and differentiation of satellite cells, and the expression of heat shock protein (HSP) 72 were also observed under a heat stress condition. As a mechanism of these effects, the involvement of HSPs, some intracellular signaling pathways related to protein synthesis, and gene expression associated with muscle growth has been suggested. HSPs are proteins that respond to stress within the body and play important roles in preventing protein denaturation, the regulation of cell signaling, and the maintenance of cell homeostasis[32,33]. It has also been reported that heat stress attenuates skeletal muscle atrophy[34,35] and induces hypertrophy[29,36-38]. These previous studies also report that thermal stimulation is effective for muscle regeneration. However, thermal therapy has not been applied to acute phase injuries in the clinical setting because it may increase pain and bleeding. If thermal therapy is used in the acute phase of muscle injury in clinical settings, the therapeutic protocol might need to be modified to deal with acute phase symptoms such as pain.

Microcurrent Electrical Neuromuscular Stimulation (MENS)

Microcurrent electrical neuromuscular stimulation (MENS) was developed as a physical therapy modality capable of delivering a current with an amplitude less than 1 mA. The human epidermis exhibits a natural endogenous battery that generates a small electric current when wounded[39,40]. Applying electrical stimulation produces current flow in the tissues that mimics the natural skin battery, and thereby, promotes tissue healing. MENS has been shown to have beneficial effects in terms of wound healing[40,41], pressure ulcer healing[42], tendon or ligament repair[43,44], the alleviation of muscle soreness[45,46], and muscle regrowth[47]. The therapeutic effects of MENS on muscle injury have been evaluated in regard to muscle weight, muscle protein content, mean muscle fiber cross-sectional area, and number of muscle satellite cells[48] (Table 4). The results showed that MENS may facilitate the regeneration of injured skeletal muscle by activating its regenerative potential[48]. Yoshida et al.[49] investigated the effects of MENS with or without icing on the injured muscle regeneration process and found that both treatments had similar beneficial effects on the recovery of muscle protein content and muscle fiber cross-sectional area. However, judging from the fiber morphology and expression level of phosphorylated Akt, MENS combined icing stimulated regeneration of the injured muscle more effectively than did MENS alone. Another treatment attempt combined MENS and hyperbaric oxygen (HBO) therapy in a cardiotoxin-induced muscle damage model[50]. Although MENS or HBO alone was not effective, MENS combined with HBO increased the muscle fiber cross-sectional area. With regard to the mechanisms underlying the effects of MENS, an increase in the generation of adenosine triphosphate has been reported[51]. Another study demonstrated that MENS increased the protein content in C2C12 myotubes. MENS has also been found to upregulate the expression of caveolin-3, tripartite motif-containing 72, and creatine kinase isoenzyme MM. It has also been suggested that MENS stimulates not

Table 4. Studies on microcurrent electrical neuromuscular stimulation or therapeutic ultrasound for muscle injury using animal models.

Reference	Injury type	MENS or TPU treatment condition	Timing of MENS or TPU treatment	Effects in the treated compared with the injured without treatment groups
Fujiya et al.[48]	Cardiotoxin-induced muscle injury	MENS: intensity 10 μA frequency 0.3 Hz pulse width 250 ms stimulation time 60 min	Initiated 48 h after injury and 3 days a week	Increased muscle dry weight, protein content, muscle fiber cross-sectional areas, and number of Pax7-positive muscle satellite cells
Nagata et al.[58]	Cardiotoxin-induced muscle injury	TPU: intensity 30 mW/cm^2 frequency 1 MHz duty cycle 20% stimulation time 15 min	Initiated 24 h after injury and continued daily exposure	Increased muscle fiber cross-sectional. Downregulated expression of COX-2 protein Decreased number of inflammatory infiltrated cells Increased expression of myogenin and myosin heavy-chain protein Increased number of Pax-7-positive cells
Shu et al.[59]	Contusion injury	TPU: intensity 0.25, 0.5, or 0.75 W/cm^2 frequency 3 MHz duty cycle 20% stimulation time 5 min	Initiated 24 h after injury and continued daily exposure	Increased numbers of muscle satellite cells and myotubes, increased desmin expression Greater muscle protein, maximum load, and tensile strength
Chongsatientam et al.[60]	Contusion injury	TPU: intensity 0.3 W/cm^2 frequency 1 MHz duty cycle 20% stimulation time 5 min	Initiated 24 h after injury and continued daily exposure	Increased muscle fiber cross-sectional area and muscle contraction force Increased VEGF mRNA expression and capillary density No influence on mRNA expression of nitric oxide synthase
Chan et al.[61]	Laceration injury	TPU: intensity 30 mW/cm^2 frequency 1.5 MHz duty cycle 20% stimulation time 20 min	Initiated 24 h after injury and continued daily exposure	Increased muscle contraction force (fast-twitch and tetanic strength)
Rantanen et al.[62]	Laceration injury	TPU: intensity 1.5 W/cm^2 frequency 3 MHz duty cycle 20% stimulation time 6 min	Initiated 6 h or 3 days after injury and 2 consecutive days of treatment, followed by 1 day of "rest".	Enhanced myogenic precursor cell and fibroblast proliferation No influence on myotube production
Piedade et al.[63]	Contusion injury	TPU: intensity 0.57 W/cm^2 frequency 1 MHz duty cycle 50% stimulation time 5 min	Initiated 48 h after injury and continued daily exposure	Increased the differentiation of muscular lineage cells Larger deposition of collagenous fibers
Wilkin et al.[64]	Contusion injury	TPU: intensity 1 W/cm^2 frequency 3.3 MHz duty cycle 20% stimulation time 5 min	Initiated 6 h after injury and continued daily exposure	No influence on myonuclear number or cross-sectional area
Markert et al.[65]	Contusion injury	TPU: intensity 0.1 W/cm^2 frequency 3 MHz duty cycle 100% (continuous) stimulation time 5 min	Initiated 24 h after injury and continued daily exposure	No influence on muscle mass, protein content, or muscle fiber cross-sectional area

MENS, microcurrent electrical neuromuscular stimulation; TPU, therapeutic ultrasound; COX-2, Cyclooxygenase-2; VEGF, vascular endothelial growth factor.

only protein synthesis, but also membrane repair in regenerated skeletal muscle[52]. These studies suggest that MENS has a beneficial effect on regeneration after muscle injury. In future research, it will be necessary to examine the most effective treatment parameters of MENS and to elucidate its underlying mechanism in terms of muscle regeneration.

Therapeutic Ultrasound

Ultrasound is a form of acoustic energy that involves mechanical pressure waves. Sound energy at frequencies > 20 kHz is defined as ultrasound. Ultrasound can be used for therapeutic purposes in both the low- and high-intensity ranges. Therapeutic ultrasound for muscle damage is applied at a low intensity or by pulsing[53]. Therapeutic ultrasound has been shown to induce biological activities related to tissue recovery, such as the stimulation of protein and collagen synthesis[54,55], and to increase the production of vascular endothelial growth factor (VEGF), basic fibroblast growth factor, and interleukin-8[56]. Therapeutic ultrasound has been shown to increase the production of nitric oxide[57]. These mechanisms may help promote tissue healing. The reported effects of therapeutic ultrasound on injured muscle include the modulation of the inflammatory response and the upregulation of myogenic differentiation in inflammatory conditions both in *in vitro* and *in vivo*[58]. In addition, it may increase the number of activated satellite cells, upregulate myogenic regulatory factors and skeletal muscle structural proteins, and increase the muscle fiber cross-sectional area in injured skeletal muscle[58-60]. Moreover, therapeutic ultrasound promotes VEGF mRNA expression and revascularization in injured muscle[60]. Regarding physiologic performance, therapeutic ultrasound may improve contractive properties after laceration injury[60,61], as well as maximum load and tensile strength[59]. By contrast, it has also been found to enhance myogenic precursor cell and fibroblast proliferation without affecting myotube production[62]. Piedade et al.[63] reported that therapeutic ultrasound after laceration injury increases the differentiation of muscular lineage cells and the deposition of collagenous fibers. Those results[62,63] suggest that ultrasound treatment also prolongs the proliferation phase of fibroblasts during muscle regeneration, which can increase the amount of permanent scar tissue production, leading to worse muscle function. Moreover, the results of other studies[64,65] suggest that ultrasound does not improve muscle regeneration. The cause of such discrepancies is related to a variety of factors, such as differences in muscle injury models and the irradiation conditions of therapeutic ultrasound, including frequency and intensity (Table 4). As mentioned above, evidence for the therapeutic effects of LIPUS on muscle injury is insufficient, and the mechanisms underlying ultrasound therapies remain unclear. Using C2C12 cells, Salgarella et al.[66] reported the effects of LIPUS at different frequencies and intensities. Their results indicated the most effective parameters for maximizing proliferation and differentiation, and could therefore be useful for conducting in vivo experiments in a future study.

Conclusion

Muscle healing is sometimes delayed, and scar tissue may remain within muscle fibers. As a result, the residual muscle injury itself might inhibit the progress of rehabilitation and delay discharge from hospital. For these reasons, muscle function needs to be restored as soon as possible. This review described the effects of physical agents on muscle healing with a focus on research using animal models. The application of physical agents for the treatment of muscle injury aims to diminish pain, promote muscle regeneration, and prevent sequela such as the formation of scar tissue in muscle. Although appropriate physical approaches have been attempted to be developed according to the muscle healing process, the most efficacious treatment for muscle injury remains unclear. Therefore, further research, including clinical studies, is needed to identify the most efficacious therapeutic conditions.

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