Effects of Swimming Exercise and Joint Mobilization on HSP 70 Levels in Osteoarthritic Rats

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Purpose: This study was performed to investigate the effect of joint mobilization on pain relief and cartilage repair in an induced osteoarthritis rat model by analyzing the expression of heat shock protein 70 in articular cartilage.

Methods: : MIA was injected into SD rats to induce osteoarthritis. These rats were divided into 4 groups: control group (n=30), no further treatment after the MIA injection ; experimental group I(n=30), performed swimming exercise after the MIA injection experimental group II (n=30), underwent joint mobilization after the MIA injection and experimental group III (n=30), performed swimming exercise and underwent joint mobilization after the MIA injection. For the histologic and pathophysiologic evaluation, safranin-O staining and for the immunohistochemical evaluation, the expression of HSP 70 in articular cartilage was analyzed 1, 7, 14, and 21 days after the MIA injection.

Results: The inflammatory response and loss of tissue declined in experimental groups I and II over time, whereas the greatest decreases were noted in experimental group III. In the articular cartilage, low expression of HSP 70 was observed in every group on day 1, whereas HSP 70 expression was elevated on days 7 and 14 in experimental groups II and III. After 21 days, experimental group II displayed the strongest positive reaction, whereas HSP 70 was higher in experimental group III at this time point compared to that after 14 days.

Conclusion: Our results showed that swimming exercise and joint mobilization had positive effects on pain relief and histologic and functional recovery in an induced osteoarthritis rat model.

Key Words: HSP 70, Joint mobilization, Osteoarthritis, Safranin-O

I. Introduction

Osteoarthritis is a disease caused by inflammation that develops when abrasion of articular cartilage and changes in subchondral bone occur because of metabolic changes in subchondral bone and articular cartilage. Mechanical damage to articular cartilage, which is involved in weight bearing, results in its destruction and subsequent tissue degeneration in the synovial membrane, cartilage, ligaments, and other structures, leading to other diseases.[1,2] Osteoarthritis occurs primarily in the knee joint, pelvis, ankles, toes, fingers, and cervical and lumbar spine, which participate in weight bearing. The knee joint, the largest joint in the body, displays the highest prevalence of osteoarthritis according to reports.[3]

Therapeutic approaches to osteoarthritis focus on reducing causal factors or relieving pain and on improving function. Drug treatment, exercise, and surgical treatment are used, but drug treatment has disadvantages such as side effects and high treatment costs.[4] Exercise treatment should be limited during the acute phase, during which joint stabilization is needed to reduce joint pain and edema. Exercise is recommended after the acute phase. Patients with

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osteoarthritis should perform muscle-strengthening exercise, which has a low impact, to prevent secondary soft tissue in the knee joint from being shortened and to maintain and increase the working ranges of joints.[5] For these reasons, walking and muscle-strengthening exercises are suggested as alternatives to avoid the side effects of drug treatment. However, these types of exercise also place stress on the knee joint due to weight bearing, making them difficult to apply to patients with osteoarthritis. In practice, underwater exercise is recommended for these patients.[6] So, Many studies have been reported effect of swimming exercise recently.[7-9]

Joint mobilization, a nonpharmacologic therapy, is commonly used to relieve pain and increase the working ranges of joints, and it is known that many pain-related problems in joints can be resolved by joint mobilization.[10] Joint mobilization can also alleviate soreness, muscle guarding, and spasm due to neurophysiologic and mechanical aspects, and it can be used as an effective treatment for joints exhibiting reversible hypomotility, gradual movement limitation, and functional fixation.[11] Heat shock proteins (HSPs), which are stress-inducible proteins, display increased expression in reaction to heat shock or any stress than can induce protein denaturation. HSPs maintain intracellular homeostasis and function as molecular chaperones a critical function for the maintenance of intracellular homeostasis for protein formation or restoration of denatured proteins and for maintenance of life.[12,13] It has been reported that HSP expression increases in diseases such as autoimmune diseases and osteoarthritis to protect cells against stress and increase their viability in response to excessive extrastimulation.[14,15]

This study was performed to investigate the effect of swimming exercise and joint mobilization on articular cartilage. In this aim, we subjected rats with induced osteoarthritis to swimming exercise and joint mobilization and compared the degree of safranin-O staining and HSP 70 expression in chondrocytes to investigate the restoration of damaged tissue and the levels of inflammation in the articular cartilage of the knee joint.

II. METHODS

1. Subjects

We used 120 Sprague-Dawley rats (8 weeks old, male; Damul Science, Korea) weighing 250 ± 20 g; 30 rats were randomly allocated to each group. The breeding room featured a controlled temperature of 22 ± 11°C, humidity of 55% ± 10%, and a 12-h/12-h light/dark cycle, which were maintained constantly for the experiment period. Water and pellet feed (Samyang Corporation) were freely provided. The entire study was conducted in accordance with the procedures of the Experimental Animal Ethics Committee of Dongshin University.

2. Experimental groups

The experimental rats were divided into 4 groups as follows: control group (n = 30), no further treatment after the induction of osteoarthritis; experimental group I (n = 30), application of swimming exercise after the induction of osteoarthritis; experimental group II (n = 30) application of joint mobilization after the induction of osteoarthritis; experimental group III (n = 30), application of both swimming exercise and joint mobilization after the induction of osteoarthritis.

To make the induced osteoarthritis rat model similar to humans, we placed the rats, which were adapted to the breeding room, into an anesthetic chamber (Royal Medical, Korea), subjected them to insufflation general anesthesia with an anesthetic gas (70% N2O, 28.5% O_2, including 20% enflurane), fixed them on the operating table, and injected them intra-articularly with monosodium iodoacetate (MIA; I2512, Sigma, Poole, UK) in the left knee joint through the patellar ligament. MIA (0.9% sterile saline, dissolved to a concentration of 3 mg/rat) was injected (50 μ l per joint) with a 2-gauge needle. Three weeks after the MIA injection, the therapeutic intervention was conducted .[16]

3. Experimental Method

1) Swimming exercise

Swimming exercise was conducted in a cylindrical plastic water tank 50 cm in diameter. The water temperature was maintained at 30-35°C, and the water depth was 50 cm to permit continuous exercise without rest. The exercise, which

consisted of low-intensity free swimming in a zero load condition, lasted for 30 min. This exercise was conducted 5 times per week for 3 weeks.[17,18]

2) Joint mobilization

The rats were subjected to joint mobilization for 3 min followed by a 30-s rest period. This protocol was completed 3 times. Joint mobilization, that is, basically, extension mobilization, was conducted in the following manner: the femur of each animal was held gently with 1 hand, whereas the knee joint was bent and extended by moving the tibia of the animal backward and forward by 1 or 2 degrees with the other hand.[19] The exercise frequency was 5 times a week, and a professional manual therapist who completed a course in professional manual therapy at The Korean Society of Functional Manual Therapy (FMT) and who was approved by the German DGMSM performed joint mobilization for 3 weeks.

4. Measurement tools and Methods

To examine the destruction and degree of restoration of the knee joint cartilage, we induced osteoarthritis and harvested the knee joints postmortem from 5 rats in each group. For each animal, the harvested knee joint was fixed in 10% formalin for 24 h and then decalcified with 10% formic acid for 3 weeks before paraffin embedding. In addition, we removed the spine and enucleated spinal cord by a method that we amputates facet joints on thoracic vertebrae part. The tissue was subjected to dehydration, clearing, and infiltration for 14 h using automatic tissue processing equipment (4640B, Sakura, Japan).

We used automatic embedding equipment (Tissue-Tex, Japan) for the production and refrigeration of paraffin blocks. The paraffin block was sectioned to a thickness of 7 μ m, leaving a space of 250 μ m from the left articular surface of rats, by using a rotary microtome (Rotary Microtome 2040, Japan); then, it was exposed to a water bath and subjected to stretching and attached to a slide.

1) Safranin-O staining

We conducted safranin-O staining to measure the degree of inflammation on articular tissue and surrounding tissue and the degree of cartilage degeneration using the knee joint section obtained. We cleaned the safranin-O-stained slide for 5-10 min, which was sufficient to ensure clear coloration for the nucleus, performed the dehydration and clearing processes, and sealed the slide. The morphologic observation was performed with an optical microscope (Bx 50, Olympus, Japan). We observed in photo which structural changes in the damaged part of the knee joint after photographing the joint using the CCD camera installed in the microscope.

2) Immunohistochemical assessment

We performed deparaffinization on the probe-on slide, microwaved the slide in 30% Tris-EDTA, and cooled it for 20 min to eliminate nonspecific reactions during immunohistochemical staining. Subsequently, as preprocessing, we blocked the activation-intrinsic peroxidase using 3% hydrogen peroxidase. After this process, we used a PBS solution containing 15% blocking serum to obtain a primary antibody solution, which was absorbed by the inside of the tissue well. We cleaned the knee joint cartilage several times with 0.01 M PBS and exposed the tissue overnight at 4°C to the primary antibody, that is, HSP 70 antibody (Santa Cruz, USA), used at a concentration of 1:300. Then, we reacted the tissue with a universal antibody for 90 min after cleaning with PBS. Next, we cleaned the tissue 3 times with 0.01 M PBS for 10 min each and exposed it to streptavidin at room temperature for 30 min. After cleaning the tissue with PBS, we performed color development with 3,3′-diaminobenzidine (60382248, ZYMED Lab, Germany) for 10 min. We then cleaned the tissue 3 times with PBS and 3 times with water for 10 min each, placed the tissue on a slide, and performed hematoxylin counterstaining. Then, we washed the tissue with running water, dehydrated it in an ethanol series, that is, 80%, 90%, and 100% ethanol, for 10 min each, treated it with 100% xylene for 10 min twice as a clearing process, and sealed it with Canada balsam (Sigma, USA). The immunohistochemical reaction was evaluated in a semiquantitative manner as follows: a slight positive reaction (+), denoting slight staining; a positive reaction (++) and moderate positive reaction (+++), indicating semi-moderate

Table 1. Heat shock protein HSP 70 immunoreactivity in intra-articular tissue

	Heat shock protein 70			
	Day 1	Day 7	Day 14	Day 21
Control	±	+	+	+
Group I	±	+	++	+++
Group II	±	++	+++	++++
Group III	±	++	++++	+++

Control: monosodium iodoacetate-induced osteoarthritis
Group I: osteoarthritis + swimming exercise
Group II: osteoarthritis + joint mobilization
Group III: osteoarthritis + swimming exercise + joint mobilization
+: Mild expression, ++: Moderate expression, +++: Severe expression
++++: More severe expression

staining; and a strong positive reaction (++++), denoting strong staining.

III.RESULTS

We conducted safranin-O staining to examine histologic changes at the end of the experiment. On day 1, we observed articular cartilage damage and serious inflammation in every group. On the 7th day of the experiment, inflammatory symptoms were observed in the control group and experimental group I, but the extent of inflammation and damage was lower in experimental groups II and III compared to that on day 1. On day 14, inflammation and damage in articular cartilage were most severe in the control group, whereas the experimental groups exhibited decreased inflammation and tissue damage. These findings were particularly evident in experimental groups II and III, in which articular cartilage displayed restoration. On day 21, we observed that the articular cartilage damage had worsened in the control group. On the contrary, we noted that damaged articular cartilage was gradually restored in all of the experimental groups, with the greatest restoration noted in experimental group III (Figure 1).

Table 1 presents the results of the immunohistochemical assessment of HSP 70 expression on days 1, 7, 14, and 21 of the experiment. On day 1, weak HSP 70 expression was noted in articular cartilage in every group. On day 7, the control group and experimental group I displayed weak HSP 70 expression, whereas HSP 70 expression was higher in experimental groups II and III. On day 14, HSP 70 expression remained weak in the control group, whereas its expression was higher in the experimental groups, particularly in experimental group III. On day 21, compared to the findings in the control group, HSP 70 expression was upregulated in all of the experimental groups; however, the greatest expression was observed in experimental group II, whereas HSP 70 expression appeared to be lower in experimental group III compared to that on day 14 (Figure 2).

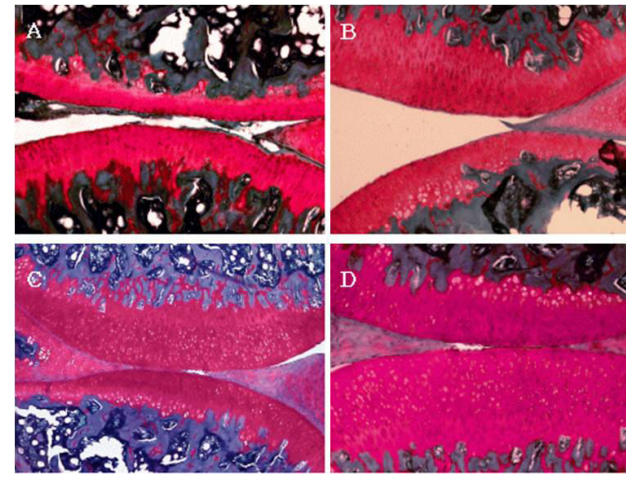

Figure1. Histologic findings for articular cartilage on day 21 (safranin-O stain, × 100, A (control): monosodium iodoacetate-induced osteoarthritis, B (group I): osteoarthritis + swimming exercise, C (group II): osteoarthritis + joint mobilization , D (group III): osteoarthritis + swimming exercise + joint mobilization)

IV. DISCUSSION

Osteoarthritis is a degenerative disease that causes disorders

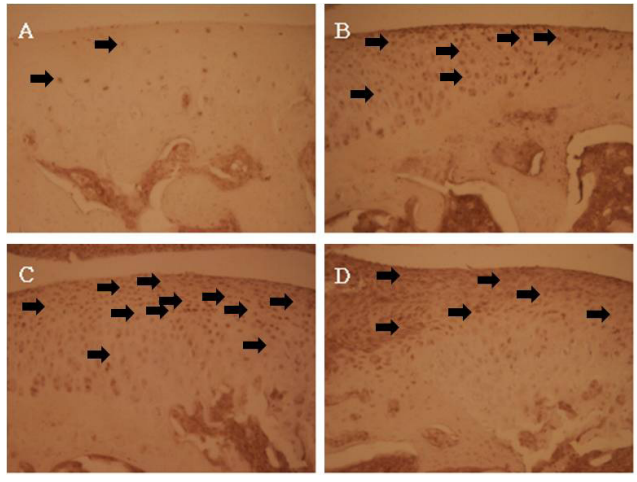

Figure2. Immunohistochemical analysis of heat shock protein 70 expression in intra-articular tissue on day 21 (immunohistochemical stain, × 200, A (control): monosodium iodoacetate-induced osteoarthritis, B (group I): osteoarthritis + swimming exercise, C (group II): osteoarthritis + joint mobilization , D (group III): osteoarthritis + swimming exercise + joint mobilization)

of joint motion, joint deterioration, and muscular weakness, as well as clinical symptoms such as arthrogryposis, resulting in harmful effects on physiologic functions directly related to the individual's quality of life.[20] Osteoarthritis is caused by the degeneration and deterioration of articular cartilage, resulting primarily from aging, overuse, and trauma. When ligaments and muscles, which participate in joint motion, weaken and the abrasion of cartilage worsens, osteoarthritis and pain occur.[21] Although the primary goal of osteoarthritis treatment is pain relief, relieving pain without improving joint function does not effectively facilitate spreading of the loads arising from daily physical activity because the lack of a protective mechanism.[22] It has been reported that therapies providing only pain relief result in increased dynamic loads on the knee joint, leading to the acceleration of regressive changes.[23]

For these reasons, exercise is utilized as a nonoperative and nonpharmacologic treatment that strengthens muscles and ligaments while relieving pain. In particular, underwater exercise, which simultaneously reduces the direct loads on joints and strengthens muscles, is being employed. It is also known that patients with osteoarthritis require underwater exercise, which has a low impact on the knee joint, to prevent secondary soft tissue in the knee joint from being shortened, thus maintaining and increasing the working range of the joint. Strong motion could load brings about serious disorder and structure deformation of joint.[24,25] In addition, joint mobilization produces some movement in joints, making this strategy effective for stimulating the production of synovial fluid, which nourishes articular cartilage, eventually helping to improve osteoarthritis. Joint mobilization is an efficient method for restoring or maintaining joint function and is also effective for relieving pain and increasing the working range of the joint. Consequently, reversible joint hypomotility, gradual movement limitation, and functional fixation are indications for joint mobilization.

We performed safranin-O staining to investigate the histology and pathophysiology of the joint. On day 1, there was no difference in the degree of tissue damage among the groups, but on day 7, the progression of tissue damage halted in the 3 experimental groups. On day 14, experimental groups II and III displayed restoration of damaged articular tissue, whereas on day 21, experimental group I also exhibited restoration of articular tissue damage. Patients with osteoarthritis of the knee joint exhibit insufficient sensory information compared to those with normal knee joints, and the reconstitution of the somatosensory cortex is caused by decrease in the amount of sensory information.[26,27] In this study, we observed cartilage tissue destruction after the induction of osteoarthritis, and we found that underwater exercise and joint mobilization effectively reversed inflammation and tissue damage.

When HSP 70 is stimulated, it reacts strongly and sensitively and thus its amount increases so much and in the recovery period it decreases first of all.[28]

Therefore, we performed immunohistochemical assessment of HSP 70 expression in articular cartilage, observing that the expression of the protein was low in all groups on day 1. However, on day 7, HSP 70 expression in experimental groups II and III, in which the animals were subjected to joint mobilization, was higher than that in the control group. On day 14, HSP 70 expression had markedly increased, especially in experimental group III. These findings were similar to results for safranin-O staining, and we believe that the upregulation of HSP 70 expression promotes tissue

restoration.

In this study, we found that when osteoarthritis was induced by MIA, cell metabolic activity was repressed, resulting in tissue damage and inflammation, and observed that swimming exercise and joint mobilization reversed inflammation and promoted tissue repair. However, more studies are necessary to explain the mechanism by which joint mobilization repairs tissue damage and reduces inflammation in articular cartilage and how the reversal of inflammation and damaged tissue affect the functional recovery of the knee joint.

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