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.¹² 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
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. 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. So, many studies have been reported effect of swimming exercise recently.

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. 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. 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.

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. 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 ± 1°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.

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 dehydation, 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 μm, leaving a space of 250 μm 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.
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. Heat shock protein HSP 70 immunoreactivity in intra-articular tissue

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<tr>
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<th>Day 1</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
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<tbody>
<tr>
<td>Control</td>
<td>±</td>
<td>+</td>
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<tr>
<td>Group I</td>
<td>±</td>
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<td>Group II</td>
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<tr>
<td>Group III</td>
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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

IV. DISCUSSION

Osteoarthritis is a degenerative disease that causes disorders
thus maintaining and increasing the working range of the
joint. Strong motion could lead to serious disorders and
structure deformation of the joint. 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
cased by decrease in the amount of sensory information.
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. 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
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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