Biochemical change in joint fluid after isometric quadriceps exercise for patients with osteoarthritis of the knee

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Summary

Objective: The purpose of this study was to analyze the biochemical changes in the joint fluid, and pain relief resulting from isometric quadriceps exercise in patients with osteoarthritis of the knee.

Design: Nineteen osteoarthritic knees in 17 patients with joint effusion were included. The patients performed isometric quadriceps exercise for 3 months. Isometric muscle torque at 30 and 60° flexion, pain as measured using the visual analog scale and biochemical markers in joint fluid were evaluated before and after the exercise.

Results: Pain score decreased from 3.9 to 2.3 after 12 weeks of exercise (P<0.001). Extension torque at 30 and 60° knee flexion significantly increased from 4.7 to 6.9 kgm (47% increase, P<0.001) and from 10.8 to 12.6 kgm (17% increase, P<0.005) after 12 weeks of exercise. The molecular weight of hyaluronan increased from 2.11 to 2.40×10⁶ (P<0.05) and the viscosity of joint fluid increased from 45.8 to 59.8 mPas after 12 weeks of exercise (P<0.05). Chondroitin 4-, 6-sulfate concentration in joint fluid decreased from 81.9 to 75.5 nmol/ml (P<0.05).

Conclusions: Isometric quadriceps exercise resulted in significant changes in joint fluid biochemical parameters, and these changes, at least in part, may explain the ameliorative effect of muscle exercise for osteoarthritis of the knee.

Key words: Osteoarthritis, Knee, Exercise therapy, Joint fluid, Hyaluronan.

Introduction

Osteoarthritis (OA) of the knee (knee OA) is a common cause of chronic lower limb disability in the elderly. Progression of knee OA produces joint effusion, pain, deformity, decreased range of motion and quadriceps muscle weakness, which limit activities of daily living. Although the pathogenesis of knee OA is still uncertain, several changes in biochemical markers in joint fluid have been reported.

Normal joint fluid contains high levels of hyaluronan (HA) synthesized by synovial lining cells, and HA influences joint lubrication and cartilage metabolism. It has been reported that HA concentration in joint fluid and its molecular weight are decreased in knee OA. High-molecular-weight HA (MWHA) has been injected intra-articularly, and although it is widely recognized to be an effective treatment option for knee OA, there has recently been an objection to its efficacy and it is still controversial. Other markers of cartilage degradation have also been measured in joint fluid. Keratan sulfate (KS) levels increased in various types of acute arthritis, such as gout, pseudogout and reactive arthritis, and chondroitin sulfate (CS) levels increased in the knees subjected to trauma.

Attempts have been made to predict the prognosis of OA by monitoring such markers in serum and/or in the joint fluid, but have not been practically successful.

Quadriceps muscle weakness is usually present in knee OA patients, although this weakness is not directly related to the degree of pain. Muscle exercise therapy has been a useful treatment option for the majority of knee OA patients. Although many studies have been published on the effect of muscle exercise on muscle strength and functional ability, the mechanism of its effect remains uncertain. The purpose of this study was to analyze the changes in biochemical markers in joint fluid caused by quadriceps exercise in knee OA patients.

Patients and methods

PATIENTS

The study group consisted of 28 OA knees in 20 patients (six men and 14 women) with joint effusion. All were outpatients in our hospital and joined this exercise program with informed consent. All patients had grade 2 or 3 knee OA according to the Kellgren and Lawrence criteria, based on weight-bearing radiographs. The rheumatoid factor and C-reactive protein were negative and the erythrocyte sedimentation rate was less than 40 mm/h in all of them. During the study period, the amount of joint fluid decreased and could not be aspirated in nine knees in six patients, who were excluded from the analysis. The remaining 19
knees in 17 patients (six men and 11 women) were evaluated. Five knees in five patients without any treatment for 12 weeks were evaluated as a control. The average durations of knee pain were 9.7±13.4 months in the study group and 13.0±19.6 months in control group (mean±standard deviation). No steroid or HA intra-articular injection had previously been given. No patients routinely used any drugs with known potential for altering cartilage metabolism, such as nonsteroidal anti-inflammatory drugs. In the study group, the average age was 64.9±6.6 years, and age ranged from 56 to 75 years. Average height, body weight and body mass index (BMI) were 157.5±10.6 cm, 61.9±8.4 kg and 25.1±3.5, respectively. In the control group, the average age, height, body weight and BMI were 64.0±8.7 years, 162.2±9.8 cm, 64.2±7.6 kg and 24.6±4.1, respectively. There was no significant difference in demographic data between the two groups. All the knees had varus OA, with an average femoro-tibial angle of 179.5±3.8°.

EXERCISE PROGRAM

A straight leg raising (SLR) exercise was chosen as an isometric quadriceps exercise because it could be continued by elderly patients without difficulty at home. The patients were instructed to lie on their back with the affected leg to be kept straight and the opposite knee flexed. Then, the leg was raised straight, approximately 20 cm off the ground, and maintained for 10 s with attention paid to feeling quadriceps muscle contraction. This exercise was repeated 10 times for both legs (Fig. 1) and patients were instructed to exercise 90 times a day. The number of repetitions was counted and recorded daily in a form provided to the patients. Every 4 weeks, the patients were seen in the outpatient clinic, and whether they had performed the exercise correctly was checked. No special instructions were given to limit their daily activities. The values recorded before and after the exercise are discussed in the following sections.

PAIN

A 10-cm horizontal visual analog scale (VAS) was used for pain assessment. VAS as a measure of self-reported pain has high test–retest reliability and validity26. VAS was recorded at baseline and after 4, 8, and 12 weeks of exercise.

CIRCUMFERENCE OF THE THIGH

The circumference of the thigh was measured above 10 cm from the proximal edge of the patella.

MUSCLE STRENGTH

Maximum isometric quadriceps and hamstring forces were measured at 30 and 60° of knee flexion using a MYORET RZ-450 (Kawasaki Heavy Industries, Ltd., Japan) at baseline and after 4, 8, and 12 weeks of exercise. Briefly, the patient was positioned in sitting with 90° hip flexion. The ankle was attached to a support just above the malleoli. The axis of the dynamometer was positioned at the center of rotation of the affected knee. Maximum extension and flexion contraction were performed continuously for 5 s. Two trials in each position were performed at 1-min intervals after two practice contractions.

ANALYSIS OF JOINT FLUID

Joint fluid was aspirated from the knee at baseline and after 12 weeks of exercise. The pH of the joint fluid was immediately measured using a portable analyzer (i-STAT®; i-stat corp. USA). The remaining sample was centrifuged, and the supernatant was collected and stored at −80°C for subsequent analysis. The levels of total protein, albumin,
chondroitin 4-sulfate (C4S), chondroitin 6-sulfate (C6S), HA, and the ratio of C6S to C4S (C6S:C4S), and MWHA and joint fluid viscosity were measured. Total protein was measured by the Biuret method, albumin by nephelometric immunoassay, CS by high performance liquid chromatography after chondroitinase ABC digestion, and HA by sandwich binding protein assay using a Hyaluronate-Chugai test kit (Chugai Pharmaceutical Co., Tokyo, Japan). The MWHA was calculated from the intrinsic viscosity of HA in joint fluid, which was measured with a capillary viscometer. Apparent viscosity of joint fluid was measured using a rotating viscometer at a shear rate of 40/s at 37°C.

STATISTICAL ANALYSIS

Wilcoxon signed-rank test was used for comparisons between values obtained before and after exercise. Time-course of changes in VAS score and maximum torque were analyzed by one-way repeated-measures ANOVA and Bonferroni test. P values less than 0.05 were considered significant.

Results

Patients performed SLR exercise 65 times a day on average (mean, range: 13–117 times) throughout the period of investigation. The adequacy of exercise was confirmed at each examination, and there were no drop-outs due to complications.

PAIN

VAS score gradually decreased from 3.9±0.6 to 2.3±0.7 (mean±S.E.) after 12 weeks (Fig. 2). The decrease in VAS score reached significance after 8 weeks of exercise. Among the patients, the VAS score of one patient worsened and those of three patients remained unchanged. There was no significant correlation between the amount of exercise and VAS score.

CIRCUMFERENCE OF THE THIGH

The circumference of the thigh did not change before and after exercise (from 43.2±0.6 to 42.9±0.5 cm).

MUSCLE STRENGTH

Knee extension torque at 30 and 60° flexion significantly increased from 4.7±0.5 to 6.9±0.6 kgm and from 10.8±0.7 to 12.6±0.8 kgm (mean±S.E., 147%, P<0.001 and 117%, P<0.005, respectively), respectively, after 12 weeks of exercise. Similar to pain score, the effect on muscle strength reached significance after 8 weeks. The knee flexion torque at 30 and 60° flexion changed from 5.5±0.4 to 5.3±0.5 kgm (96%) and from 4.1±0.3 to 4.6±0.4 kgm (112%), respectively, but these differences were not significant (Fig. 3).

ANALYSIS OF JOINT FLUID

The results of measurement are summarized in Table I. The amount of joint fluid significantly decreased from 10.7±1.7 to 6.8±2.0 ml after 12 weeks of exercise (mean±S.E., P<0.05). pH changed from 7.363±0.008 to 7.385±0.007 (P<0.05). Total protein level in joint fluid slightly decreased from 2.74±0.17 to 2.66±0.16, but this difference was not significant. Albumin level decreased from 1.80±0.08 to 1.66±0.06 (P<0.005). Although C6S level did not change significantly (from 61.9±6.5 to 57.1±7.5 nmol/ml), C4S level decreased from 20.0±1.4 to 18.3±1.5 nmol/ml (P<0.05). C4S+C6S level decreased from 81.9±7.7 to 75.5±8.7 nmol/ml (P<0.05). Ratio C6S:C4S did not change significantly (from 3.04±0.19 to 3.03±0.22). The MWHA increased from 2.11±0.13×10⁶ to 2.40±0.12×10⁶ (P<0.05), while HA concentration remained unchanged (from 1.35±0.13 to 1.34±0.12) (Fig. 4). Joint fluid viscosity increased from 45.8±7.6 to 59.8±8.6 mPas (P<0.05). In contrast, there were no significant changes between at baseline and after 12 weeks in the patients without any treatment. No correlation between these changes in joint fluid and the amount of exercise was found.

Discussion

The present study showed that isometric quadriceps exercises were clinically effective after 3 months. Pain relief was observed within 4 weeks and reached statistical significance after 8 weeks. This study also analyzed the corresponding biochemical changes in joint fluid. There have been a few previous studies of biochemical and metabolic changes in joint fluid after exercise. Roos et al. reported no significant changes in either cartilage proteoglycan concentration in joint fluid, or the HA or KS level in serum after high-intensity weight-bearing exercise in healthy athletes. Messier et al. investigated joint fluid also after dietary and weight training and walking exercise in obese older adults with knee OA and reported KS level did not change, but interleukin-1β decreased after 6 months. Bautch et al. also failed to demonstrate significant changes in KS and hydroxyproline level in joint fluid after 12 weeks of low-intensity walking exercise in knee OA patients.
this study, however, significant changes in MWHA, joint fluid viscosity and CS level were observed. To our knowledge, this is the first report demonstrating such changes in biochemical markers after quadriceps muscle training as home exercise in knee OA patients.

HA plays various roles in normal and pathologic joints, e.g., as a lubricating substance\(^{10}\), in cartilage proteoglycan metabolism\(^{8}\), in suppression of phagocytosis and chemotaxis by polymorphonuclear leukocytes\(^{31}\) and in analgesia\(^{32}\). It has been reported that intra-articular HA injection inhibits cartilage degeneration in experimental OA joints, and that its inhibitory effect is more potent with the higher MWHA\(^{33-35}\). This study showed that the MWHA in joint fluid increased from 2.11 to 2.40×10\(^6\) (\(P<0.05\)) after exercise and became closer to that reported for fluid in normal joints (2.70×10\(^6\)) measured by the same method as used in the present study\(^36\). The higher molecular weight HA was shown to have higher viscoelasticity and, thus, was advantageous in view of the joint lubrication\(^{37,38}\). This study has demonstrated that joint fluid viscosity increased from 45.8 to 59.8 mPas (\(P<0.05\)) after exercise. Thus, it is reasonable to assume that higher molecular weight HA and consequently higher viscoelasticity contributed to the pain relief through effects on joint lubrication.

KS and CS are the major glycosaminoglycans attached to a core protein of cartilage proteoglycan. More than 90% of the CS isomer in adult articular cartilage is C6S\(^{39}\), while C4S is the predominant isomer found in other adult joint tissues, such as meniscus, ligament, and synovial capsule. The ratio C6S:C4S in OA patients is lower than in normal individuals and patients with trauma and higher than in rheumatoid arthritis patients\(^5\). Therefore, these joint fluid parameters reflect alterations in cartilage metabolism. In our study, C4S level in joint fluid significantly decreased from 19.4 to 18.3 nmol/ml (\(P<0.05\)) and total concentrations of C4S and C6S decreased from 81.9 to 75.5 nmol/ml (\(P<0.05\)). This decrease in CS level may indicate inhibition of degradation of the articular cartilage and intra-articular components after exercise therapy.

The present study revealed a slight but significant increase in pH and decrease in albumin concentration in joint fluid after exercise. The pH has been reported to influence lubrication of countersurfaces in artificial joints. The variation in pH in periprosthetic fluids, which consist of
HA solution with γ-globulin, affected the friction coefficient of countersurfaces between ultra-high-molecular-weight polyethylene and stainless steel\(^4\). However, the changes in pH in the present study are within the normal range\(^4\), and further study is needed to determine whether these subtle changes influenced lubrication conditions. The reduction of albumin was probably due to decreased inflammatory reaction, since its level in joint fluid is determined by the permeability of synovial membrane.

A ‘reflex inhibition’ mechanism has been recently advocated as an important cause of quadriceps weakness in knee OA patients in addition to disuse atrophy\(^19,4\). The idea of reflex inhibition was that various proprioceptive receptors are present in ligaments, tendons, joint capsule, joint synovium and hairy skin\(^4\), and these receptors are stimulated by pain, ligament stretching, capsule pinching and effusion\(^4\), leading to neurogenic inhibition of the quadriceps muscle. The presence of this mechanism in knee OA patients was confirmed by the finding that they have quadriceps weakness even in the asymptomatic stage\(^19,5\). Our results showed that 3-month exercise resulted in a significant increase in quadriceps strength of up to 147% without an increase in thigh circumference. This finding indicated that relief of reflex inhibition played an important role for increase in muscle strength (Fig. 5). It has been reported that aspiration of joint effusion produces increase in quadriceps strength\(^5,5\). According to another finding, the increase was only seen in acute cases, but not in chronic effusion\(^5\). In this study, despite the presence of considerable effusion (65% of baseline level) after 3-month exercise, significant muscle strengthening was observed. This indicated that relief of reflex inhibition was not fully explained by the decrease in joint effusion.

In this study, pain relief was observed as early as 4 weeks after exercise and became significant after 8 weeks, accompanied by increase in muscle strength. One fundamental question to be addressed is whether the changes observed in biochemical markers were the cause of pain relief or simply the result of joint stabilization by improvement in muscle strength. It has been widely believed that stabilization of the knee by muscle strengthening is the cause of pain relief in knees with OA. Some researchers in Japan have claimed that the effect of quadriceps exercise is due to something other than knee stabilization, based on the observation that pain relief preceded increase in muscle strength (H. Kurosawa, personal communication). In fact, a significant effect of joint movement on HA metabolism has recently been reported by Pitsillides et al.\(^5\). They found that immobilization of the joint resulted in decrease in HA level in joint fluid and in synovial cell (type B) uridine diphosphoglucose dehydrogenase (UDPGD) activity, which is essential for HA synthesis. Thus, it is possible that the increase in MWHA and other biochemical changes in joint fluid were due to stimulation to synovial cells or intra-articular components by repeated muscle contraction (Fig. 5). Although metabolism in the joint is affected by complex regulatory mechanism, such as circulation, nervous system and humoral factors, the findings in this study demonstrated that a biomechanical stimulation to the synovium by muscle exercise had altered physiological condition of synovial cells, which

### Table I

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<th>Exercise group (n=19)</th>
<th>Control group (n=5)</th>
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N.A., not available.

\(^\dagger\) P<0.05. P determined by Wilcoxon signed-rank test for comparison before and after exercise.

\(^\dagger\dagger\) MWHA, molecular weight of HA.

![Fig. 4. Changes in MWHA after isometric quadriceps exercise in all the 19 knees. The MWHA increased in most of the patients. The means differ significantly before and after exercise (P<0.05 by Wilcoxon signed-rank test).](image-url)
resulted in the change in MWHMRA and subsequently led to the pain relief in knee OA. Further study is needed to determine as to how these biochemical changes in joint fluid lead to improvement in clinical symptoms in knee OA.

In conclusion, 8 weeks of isometric quadriceps exercise for patients with knee OA was very effective in improving clinical symptoms. In addition to significantly increasing in quadriceps muscle strength, the exercise had effects on HA metabolism in arthritic knee joints after 3 months. Such biochemical changes might be directly responsible for pain relief in OA patients.

References


