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Chondroprotective effects of high-molecular-weight cross-linked hyaluronic acid in a rabbit knee osteoarthritis model

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SUMMARY

Objectives: We hypothesized that high-molecular-weight (MW) cross-linked (CL) hyaluronic acid (HA) improves joint lubrication and has an enhanced chondroprotective effect. We examined the histopathological changes and friction coefficients in osteoarthritic knee joints after injecting high-MW CL HA.
Design: A bilateral anterior cruciate ligament transection (ACLT) model in 20 Japanese white rabbits was used. From week 5 after transection, low-MW HA (0.8×10^6 Da; HA80) or high-MW CL HA (6×10^6 Da; HA600) was injected weekly into 10 right knee for 3 weeks; normal saline (NS) was injected into the 10 left knee. A sham operation was undertaken to exclude spontaneous osteoarthritis (OA) in five knees. Results were evaluated with macroscopy, histopathology (Kikuchi's score), biomechanical testing, and rheological assessment of the joint fluid viscoelasticity. Statistical analysis was performed using one-way analysis of variance with a 95% confidence interval (CI) ($P < 0.05$).
Results: The macroscopic findings showed severely damaged cartilage in 30% of the NS group and 20% of the HA80 and HA600 groups and intact cartilage in 100% of the sham group. The histological scores and friction coefficients of the HA600 group were significantly lower than those of the NS group ($P = 0.007$ and $P = 0.002$, respectively). Viscoelasticity measurements of the joint fluid showed no significant differences between the three treatment groups.
Conclusion: High-MW CL HA exerts potential chondroprotective effects and produces superior friction coefficients. Our results suggest that HA600 delays the progression of OA effectively and improves joint lubrication significantly.

Introduction

Current treatments for osteoarthritis (OA) range from minimal nonsurgical measures to invasive and surgical modalities. Nonsurgical nonpharmacological measures include exercise, weight loss, and bracing; surgical measures vary from arthroscopic debridement and osteotomy to arthroplasty. Pharmacological therapies, such as analgesics, intra-articular injections, and topical treatments, mainly target the palliation of pain[1,2]. Chondroprotective agents are defined as compounds that (1) stimulate the synthesis of collagen and proteoglycans by chondrocytes and production of hyaluronic acid (HA) by synoviocytes; (2) inhibit cartilage degradation; and (3) prevent fibrin formation in the subchondral and synovial vasculature[3]. Compounds that show some of these characteristics are endogenous molecules of the articular cartilage, including HA, glucosamine, and chondroitin sulfate.

Viscosupplementation is an intra-articular therapeutic modality based on the physiological importance of HA in the synovial joints[4,5]. HA is a heteropolysaccharide formed by a variable number of repeating units of D-glucuronic acid and N-acetylglucosamine. It is formed by synoviocytes, fibroblasts, and chondrocytes inside joints and is present in the synovial fluid and the extracellular matrix of the cartilage. HA is crucial to the viscoelastic properties that allow the efficient movement of articular joints[6–9].

Several HA preparations are being marketed with varying molecular weights (MWs), ranging from 0.5×10^6 Da to 6×10^6 Da. Clinical trials have not indicated a clear advantage of one product over another[10]. One of the goals of HA therapy is to restore the viscoelasticity of the synovial fluid and the natural

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protective functions of HA in the joint. In humans, the MW of HA in a healthy knee joint is 6×10^6 Da. It has been reported that the MW of HA in a human arthritic knee joint decreases to 0.5–3×10^6 Da[11]. HA can potentially improve viscoelasticity, and a higher MW of HA is thought to improve the viscoelastic properties of HA preparations. However, the effects of high-MW cross-linked (CL) HA on joint viscoelasticity and joint lubrication have not yet been reported.

We hypothesized that high-MW CL HA improves joint lubrication and has an enhanced chondroprotective effect compared with lower-MW HA. We examined the histopathological changes in osteoarthritic knee joints after HA injection and investigated the relationship between the friction coefficient and the MW of HA.

Materials and methods

OA model

Twenty-three female Japanese white rabbits aged 12 weeks and weighing 2.6–2.9 kg were purchased from a professional breeding company (Japan SLC Inc., Hamamatsu, Japan) and used for this experiment. Our institutional Animal Care Committee approved the study, which was conducted according to its ethical guidelines and regulations. All animals were anesthetized with an intravenous injection of 0.05 mg/kg phenobarbital sodium, followed by gas anesthesia with isoflurane. Both knees of each rabbit were shaved, prepared, and draped in a sterile environment. To generate osteoarthritic joints, anterior cruciate ligament transection (ACLT) was performed in both knees using a medial parapatellar approach. After the patella was dislocated laterally, the knee was flexed maximally so that the anterior cruciate ligament could be readily visualized and identified. It was then transected with a #11 blade. The knee joints were inspected for bleeding and washed thoroughly with sterile normal saline (NS). The joint capsule was closed with a running suture of 4-0 nylon, and the skin incision was closed with running mattress sutures of 3-0 nylon[12]. Instability was confirmed in all knees with positive anterior drawer and Lachman tests. After surgery, the animals were allowed unrestrained cage activities while they were monitored for infections and other complications. To rule out the development of spontaneous OA, a bilateral arthrotomy without the ACLT was performed as a sham non-OA model.

Injection materials

In this study, we used two HA preparations of different MWs: HA80 (Artz Dispo®, Seikagaku Corp., Tokyo, Japan), with an average MW of 0.8×10^6 Da; and HA600 (Hylan G-F 20, Synvisc®, Genzyme, a Sanofi company, Cambridge, MA), with an average MW of 6×10^6 Da. Both are available for patient use as a medical device loaded with sterile injection material. We used NS (0.9%) as the control.

Treatment regimens

In postoperative week 5, 46 knees from 23 rabbits were injected with NS or HA (HA80 or HA600). NS was injected into the left knees and HA (HA80 or HA600) was injected into the right knees. The sham group (six knees from three rabbits) received bilateral NS injections. The intra-articular injection of 0.3 mL of each material was performed under intravenous anesthesia induced with 0.05 mg/kg phenobarbital sodium using a 26-gauge syringe under sterile conditions. Three weekly injections were administered, and all animals were killed 8 weeks after surgery (1 week after the third injection) with an intravenous lethal dose of phenobarbital sodium. From the OA model, 30 knees were randomly assigned to one of the three treatment groups (10 per group) with NS, HA80, or HA600. From the sham group, five knees were collected randomly from a total of six knees for evaluation.

Macroscopic evaluation

Macroscopic assessment of the knees (five knees per group) was performed in all four compartments of every knee: medial femoral condyle, lateral femoral condyle, medial tibial plateau, and lateral tibial plateau. A solution of India ink diluted with phosphate-buffered saline (1:5) was used to stain the articular cartilage, and the stained specimens were photographed using a high-resolution digital camera. The findings were evaluated according to the following classification: Grade 1 (intact surface), surface was normal in appearance and did not retain India ink; Grade 2 (minimal fibrillation), surface retained India ink as elongated specks or light-gray patches; Grade 3 (overt fibrillation), areas were velvety in appearance and retained India ink as intense black patches; and Grade 4 (erosion), loss of cartilage exposing the underlying bone. Grade 4 was divided further into the following three subgrades: Grade 4a, erosion <2 mm; Grade 4b, erosion ≥ 2 mm and <5 mm; and Grade 4c, erosion ≥ 5 mm[13].

Histopathological evaluation

The distal femur and proximal tibia from each knee (five knees per group) were fixed with 4% phosphate-buffered paraformaldehyde for 24 h, decalcified with 10% ethylenediaminetetraacetic acid (pH 7.4) for 10 days, and then embedded in paraffin. A 5 μm thick sagittal section was cut from the center of each medial femoral condyle. The sections were stained with Safranin-O. The slides were coded before microscopic examination and evaluated by three observers. Degenerative changes to the articular cartilage were assessed quantitatively using the scoring system described by Kikuchi *et al.* (Table I)[14].

Friction test

Fifteen knees from the OA model were used for this test (five per group), and these specimens were used exclusively for this test. The effects of the intra-articular injection of the test substances on joint lubrication were assessed using a pendulum friction tester designed by our laboratory for small samples [Fig. 1]. The knees were resected at the proximal end of the femoral shaft and at the distal end of the tibia and then secured to polyethylene tubes with bone cement. All soft tissues were removed from the joint except the joint capsule and the tendons and ligaments around the knee. The distal end of the tibia of each sample joint was attached to the base plate, and the femoral shaft was attached to the pendulum. The pendulum motion was calculated from two translational displacements by laser displacement sensors (LK-G30, Keyence, Tokyo, Japan), and the angular displacement was calculated by an accelerometer that detected the direction of gravity (100 Hz) (WAA-006, Wireless Technologies, Inc., Tokyo, Japan). The total weight of the pendulum, including its frame, was 40 N in normal sea-level gravity. During the experiments, the joints were kept moist with injections of NS. Based on the linear damping oscillation curve of the pendulum, the frictional coefficient μ was calculated using the following equation: $\mu = L\Delta\theta/4r$, where r is the radius of the rabbit femoral condyle, L is the distance between the pendulum's center of gravity and center of rotation, and $\Delta\theta$ is the average decrease in the amplitude of the pendulum swing per cycle.