The effects of hyaluronan on the meniscus in the anterior cruciate ligament-deficient knee

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Abstract: Anterior cruciate ligament (ACL) deficiency often induces meniscal tears and, ultimately, degenerative joint disease. The hypothesis of this study was that hyaluronan (HA; MW = 8 x 10⁶) may have a protective effect on the medial meniscus following a period of ACL deficiency. The animal model consisted of creating an ACL deficiency by ACL transection (ACLT) in 51 mature New Zealand white rabbits. Postoperative injections started 4 weeks after ACLT to allow the ACL deficiency to create a degenerative change in the meniscus. The first group (n = 26) was injected with HA and the second group (n = 25) was injected with vehicle (phosphate-buffered saline) in their ACL-deficient knees once a week for 5 weeks, in a protocol similar to that used clinically. At the end of the injections, the HA-treated meniscus showed a reduced meniscus area histomorphometrically (P < 0.01), as well as a decrease in water content (P < 0.01) when compared with the vehicle-treated menisci. The matrix composition of the menisci was assessed by the total glycosaminoglycans (GAGs) content, which decreased in the vehicle-treated meniscus (P < 0.05) but did not decrease in the HA-treated meniscus. In our model, a positive effect of HA was observed biochemically on the preservation of the meniscus matrix composition in the ACL-deficient knee.

Key words: ACL deficiency, meniscus, hyaluronan

Introduction

Untreated anterior cruciate ligament (ACL) rupture has often been shown to contribute to secondary meniscal tears and, ultimately, degenerative joint disease. The incidence of meniscal tears in patients with chronic ACL deficiency has been reported to be higher than that in patients with acute ACL injury. The possibility for surgical repair of a torn meniscus exists as a treatment option at the time of ligament surgery, ie, ACL reconstruction. Although not all meniscal tears in the ACL-deficient knees are good candidates for surgical repair, we prefer to repair and save a torn meniscus as much as possible because the eventual effect of meniscectomy is characterized as an articular cartilage degeneration. Successful meniscal repair depends on the location, ie, vascularity, and the type of meniscal tears. Furthermore, the menisci with degenerative or deformed changes lead to unsuccessful results. It would be clinically meritorious if we could preserve the meniscus in the ACL-deficient knee and prevent its degeneration.

Hyaluronan (HA) is a component of proteoglycan aggregate. The intraarticular injection of HA decreases clinical symptoms in the early stage of osteoarthritis. It also has the effect of increasing proteoglycan and of preventing the release of glycosaminoglycans (GAGs). In a rabbit model of ACL transection (ACLT) we have demonstrated that HA inhibits articular cartilage degeneration. The hypothesis of the present study was that HA may have the effect of protecting the medial meniscus by preventing degeneration of its matrix after a period of ACL deficiency. Thus, the purpose of this study was to assess the effects of HA on the medial meniscus in ACL-deficient knees histomorphometrically and biochemically.

Materials and methods

Fifty-one New Zealand white rabbits, 7–8 months old and weighing 3.5–4.0 kg, were operated for unilateral ACL transection (ACLT) to create an ACL-deficient knee. These rabbits were skeletally mature, with closed...
epitrochlearis by roentgenogram. Each animal was anesthetized with intramuscular injections of ketamine (80–100 mg/kg) and xylazine (7–10 mg/kg). The left hind limb was shaved and disinfected with povidone-iodine solution. A medial parapatellar incision and arthrotomy were performed. The patella was dislocated laterally and the knee placed in full flexion. The ACL was visualized and transected with a no. 15 surgical blade. After ACLT, no bleeding and full range of motion of the joint were observed, and the joint was washed with sterile saline. The capsule was closed with 4–0 monofilament polypropylene sutures, and the skin was closed with 3–0 nylon sutures. After surgery, the rabbits were returned to cage activity (cage, 60 cm × 60 cm × 40 cm) and the limbs were not immobilized. The rabbits were given intramuscular injections of analgesic (buprenorphine, 0.01–0.02 mg/kg) for 3 days and antibiotic (enrofloxacin, 1.0–1.3 mg/kg) for 7 days after surgery.

Postoperative treatment started 4 weeks after ACLT to allow the ACL deficiency to create a degenerative change in the meniscus, as previously demonstrated in our laboratories. The rabbits were divided into two postsurgical treatment groups. The first group (n = 16) had their ACL-deficient knees (left knees) injected intra-articularly with highly purified sodium hyaluronan (HA; molecular weight, 8 × 10^5 Da; obtained from rooster combs and produced by Seikagaku, Tokyo, Japan). The injections of 0.3 ml HA were administered once a week for 5 weeks in a protocol similar to that used clinically. The right knees had no surgery and no injection, as contralateral controls. The second group (n = 25) had their ACL-deficient knees (left knees) injected intra-articularly with phosphate-buffered saline (carrier of HA) for 5 weeks and right knees as controls. During intraarticular injection the animals were anesthetized with small intramuscular doses of ketamine and xylazine. All rabbits were killed 9 weeks post-surgery with an intracardiac injection of a mixed solution of pentobarbital sodium, phenytoin sodium, ethyl alcohol, and propylene glycol.

**Gross morphological study**

Medial and lateral menisci from both knees of all animals were evaluated gross morphologically and pictures were taken using a close-up micro lens. The evaluated details included surface appearance, swelling, and meniscal tears.

**Histomorphometrical study**

Medial menisci from seven rabbits in both treatment groups were fixed in 10% buffered formalin with 1% cetylpyridinium chloride for 72h and embedded in paraffin. Six-micron-thick coronal sections of the central region in the medial meniscus were cut and stained with safranin O without fast green. The sections were evaluated histomorphometrically, using an image analysis system reported previously. Histological sections of the central region in the medial meniscus were digitally captured via a high-resolution video camera (HV-C10; Hitachi, Tokyo, Japan) attached to a light microscope (Microphot; Nikon, Tokyo, Japan), and defined on the monitor with the gray scale range from 1 to 256. After the outline of the total meniscus section was digitized, the area was calculated by a computer system (Macintosh Quadra 950) equipped with specialized software (NIH Image 1.55). A boundary between the stained and non-stained areas was determined using the density slice function. The parameters assessed were total area of the central region in the medial meniscus, percentage of area stained with safranin O, and mean intensity of safranin O staining. The percentage of area stained with safranin O was calculated by dividing the stained area by the total meniscus area. The mean gray scale was accurately determined, using the gray scale range as a mean intensity of safranin O staining.

**Biochemical study**

Biochemical characterization of menisci included measurements of hydration, total GAGs concentration, ^35^S-sulfate incorporation (GAGs synthesis), and ^3^H-thymidine incorporation (DNA synthesis). Samples were harvested from the whole medial meniscus without synovial tissues and ligament tissues. Joint inflammatory response was evaluated by measuring DNA concentration in the synovial tissues.

**Hydration.** Hydration levels in the medial meniscus from eight rabbits in both treatment groups were determined by measuring wet and dry weights in the menisci and calculating percent water content.

**Total glycosaminoglycans.** Total GAGs concentrations were determined by measuring the concentrations of hexosamine contained in the medial meniscus from eight rabbits in both groups. Two to four mg of dry tissue was hydrolyzed in 6N HCl at 100°C for 5h. The amount of hexosamine was quantified as described previously. Results were expressed as micrograms hexosamine per mg of dry tissue.

**^35^S-sulfate incorporation.** The relative rates of GAGs synthesis in medial menisci were determined by measuring the amounts of radioactive ^35^S-sodium sulfate incorporated over a specified time period. Freshly dissected medial menisci from four rabbits in both groups were taken steriley and incubated in