Praca oryginalna *Original paper*

Treatment of osteoarthritis with sodium hyaluronate: an experimental study on rabbits

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Summary

The aim of this study was to evaluate the effects of intra-articular application of hyaluronic acid injection on the cessation of cartilage degeneration in an experimental rabbit's stifles osteoarthritis model induced by resection of the anterior cruciate ligament (ACL). Twenty clinically healthy rabbits weighing between 3150--4050 g, aged 7 months, were used in this study. Under general anesthesia osteoarthritis was induced on the right stifle joint of the rabbits. The ACL was resected and then the operation region was closed. Three months after the operation the animals were divided into two groups containing ten rabbits each. One group received a physiological saline injection, and the other received 1.0 ml of sodium hyaluronate (NaHa) dissolved in physiological saline that was injected into the right stifle joint given once a week three times. All subjects were sacrificed at the end of the postoperative 6th months. For the macroscopic examination, the cartilage surfaces of the loaded parts of the femur and tibia were grossly evaluated. The cartilage degeneration was increased in severity, and there was even the abrasion of the total cartilage layer in some cases. On the other hand, the degeneration in the NaHa-applied group was relatively minor, and stifles were smooth, white, and glistening. The joints subjected to NaHa treatment showed less granulation tissue than the other group did. According to light microscopic evaluation, cartilage exhibited an irregular surface and loss of normal cellularity in the saline group. Surface irregularity had nearly normal tidemark integrity and cellularity after treatment with NaHa. According to the electron microscopic evaluation, cartilage surfaces showed irregularities, loss of extracellular matrix metachromasia, and a decreased number of chondrocytes within their lacunae at several locations in saline-treated group. These disturbances were less prominent in the NaHa group compared to the saline group. Although degenerating chondroblasts and chondrocytes with their picnotic nuclei were apparent in both groups, the NaHa group had a normal chondral matrix area with healthy chondrocytes within their lacunae forming isogenous groups. As a result, it was concluded that using hyaluronic acid in the treatment of osteoarthritis is beneficial.

Keywords: osteoarthritis, rabbit, sodium hyaluronate, stifle joint

Osteoarthritis is a degenerative joint disease affecting primarily the weight bearing joints (5-7). It is characterized by a loss of matrix glycosaminoglycans, fibrillation in the cartilage joint surfaces and the eventual loss of the collagenous matrix leading to the exposure of underlying bone (1, 11). Hyaluronic acid is a type of glycosaminoglycan that is widespread in connective tissue. It constitutes the major ingredient of synovial fluid serving in the absorption of mechanical impact, joint lubrication and preservation of articular cartilage (2, 11).

Hyaluronic acid is a polysaccharide consisting of a long chain disaccharides (glycuronic acid plus N--acetyl glucosamine). It restores proteoglycan macromolecular complexes that constitute a dense network in the extracellular matrix of the cartilage thereby improving its elasticity (3, 4). It also plays an important role in keeping the chondroitin/keratin sulphate ratio in equilibrium (in osteoarthritis there is a relative increase in chondroitin sulphate) by blocking chondroitin sulfate (9). By reducing the viscosity of the synovial fluid, which is known to be increased in arthritis, hyaluronic acid is thought to improve translation and hence joint functioning (10).

The aim of this study is to investigate hyaluronic acid's benefits and changes after the administration in osteochondral tissues, periarticular tissues and articular synovium are evaluated following the osteoarthritis induced by splitting the anterior cruciate ligament in rabbits.

Material and methods

Based on this background, the effects of hyaluronic acid on the inhibition of cartilage degeneration were evaluated. An experimental rabbit osteoarthritis model induced by resection of anterior cruciate ligament (ACL) was utilized, as described by Pond and Nuki in dogs (14).

Materials. The sodium hyaluronate (NaHa) used in the experiment has an average molecular weight greater than one million. The NaHa was sterile and contained no endotoxins or antigenic substances. NaHa was dissolved in physiological saline (15 mg/ml). The kinematic viscosity of the solution was adjusted to 20,000-50,000 centistokes, and its osmolality was approximately 340 milliosmolese (Orthovisc, Anika Research Inc.). A physiological saline solution (Serum fizyolojik, Eczacibasi) was applied to the control group.

Animals. Twenty white New Zealand rabbits, weighing 3150-4050 g, within 7-months-of-age, were used in this study. All rabbits were housed in metal cages with a wire netting bottom at a temperature of 23°C (± 5°C). The animals were allowed free access to a solid diet and tap water. Motor load was provided by leaving the rabbits free in a small garden during the day-time. All procedures were in full compliance with Turkish Law 6343/2, Veterinary Medicine Deontology Regulation 6.7.26 and with the Helsinki Declaration of Animal Rights.

Resection of anterior cruciate ligament. All subjects were anaesthetized by a combination of intramuscular injection of xylazine hydrochloride (Rompun, Bayer, 2 mg/kg) and ketamine hydrochloride (Ketalar, Parke Davis, at a dose of 10 mg/kg). The skin over the stifle joint was shaved off and sterilized. The skin was then incised longitudinally for a length of approximately 1 cm within an area slightly inward from the patellar tendon. The articular capsule was also incised longitudinally, and the ACL was macroscopically resected. Following the resection, anterior instability was manually confirmed by an anterior drawer test. The joint cavity was washed with physiological saline solution. The articular capsule and the skin were sutured and sterile dressing was applied. The same surgical procedure was performed on the contra lateral stifle. Following the surgical operation, penicillin (Devapen, Deva) was injected intramuscularly at a dose of 40.000 IU/kg, twice daily for 1 week.

Three months following the operation, all subjects were divided into two groups of ten on a weight basis. One group received 1.0 ml of sodium hyaluronate dissolved in physiological saline, and the other received a physiological saline solution. Both solutions were injected into the right stifle joint once a week three times. The animals were sacrificed at the end of the 6 months.

Gross morphologic examination. The articular surfaces of the stifle joints were dissected immediately after sacrificing the animals. The articular surfaces were examined for macroscopic lesions with the unaided eye and then photographed. The lesions were classified according to the following scheme that is proposed by Mejersjö and Kopp (13): normal, fibrillation (superficial fraying, fissuring or flaking), erosions (losing of cartilaginous tissue), bone exposure and bone destruction filled with fibrous connective tissue or granulation tissue.

Light microscopic examination. For the light microscopic examination, the lateral and medial condyle of the femur and tibia were fixed in 10% neutral buffered formalin (pH 7.4) and decalcified with 20% EDTA (Sigma, Aldrich). The decalcified condyles were dehydrated in a graded series of ethanol and embedded in paraffin. The micro sections were stained with hematoxyline and eosin and evaluated for the following variables: surface irregularities, cartilage thickness, cell cluster formation and chondrocyte necrosis.

Electron microscopic examination. Immediately after the macroscopic evaluation, full-width articular cartilage specimens (approximately 2 × 2 mm) were obtained from the medio-central part of the femoral condyle and fixed in 2.5% glutaraldehyde in Sorenson's phosphate buffer (Fluka, Germany) (PBS). After washing in PBS, they were post-fixed in 1% osmium tetroxide in PBS at 4°C for 1 hour. The specimens were then dehydrated in a graded series of ethanol preparations for embedding in araldite Cy 212 (Agar, Germany). Semi-thin sections were stained with methylene blue-Azur II. Thin sections were stained with uranyl acetate and lead citrate; then they were examined and photographed.

Results and discussion

Macroscopic evaluation. The cartilage surfaces of the loaded parts of the femur and tibia were grossly evaluated. The femoral condyle showed the most severe changes. The cartilage degeneration was increased in severity, and there was even abrasion of the total cartilage layer in some cases. On the other hand, the degeneration in the NaHa-applied group was relatively minor, and stifles were smooth, white and glistening. The joints subjected to NaHa treatment showed less granulation tissue than the other group does.

Light microscopic evaluation. In the saline group, cartilage exhibited an irregular surface, loss of normal cellularity and tidemark integrity. Surface irregularity was nearly normal, as were tidemark integrity and cellularity after treatment with NaHa. Fibrillated cartilage surface and vertical clefts into the transitional zone were found in some specimens, but the tidemark was preserved with a normal appearance in all specimens in NaHa-injected stifles.

Electron microscopic evaluation. The cartilage surface and matrix were further evaluated at semi-thin and thin plastic sections for tissue degeneration. Cartilage surface showed irregularities at some locations both in saline- and NaHA-treated groups at semi-thin sections. Cartilage thickness also showed alterations in different specimens and at different locations of the same specimen. The cartilage became rough and fibrillated. The extracellular matrix lost its metachromasia, and the number of chondrocytes was decreased within their lacunae at several locations. These disturbances were less prominent in NaHA group compared to the saline group. The fibrillation was incomplete in this group. Degenerating chondroblasts and chondrocytes with their picnotic nuclei were apparent in both the NaHA and saline groups. However, the NaHA group had nearly normal chondral matrix areas with healthy chondrocytes within their lacunae forming isogenous groups. The proliferating chondrocytes exhibited intense pericellular metachromasia with methylene blue-Azur II indicating the repair of the cartilage matrix and the recovery of the

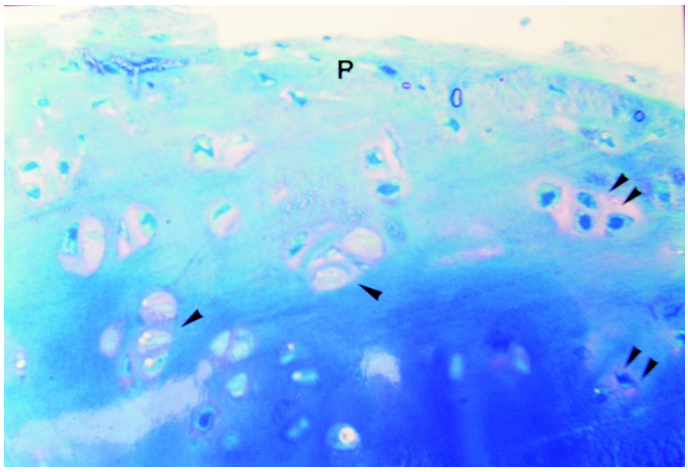

Fig. 1. A semi-thin section from the saline group. Chondrocytes in their lacunae appear to be degenerating in general with their picnotic nuclei (double arrowheads). P: Perichondrium, Arrowheads: Isogenous groups. Methylene blue Azur II, 40 ×

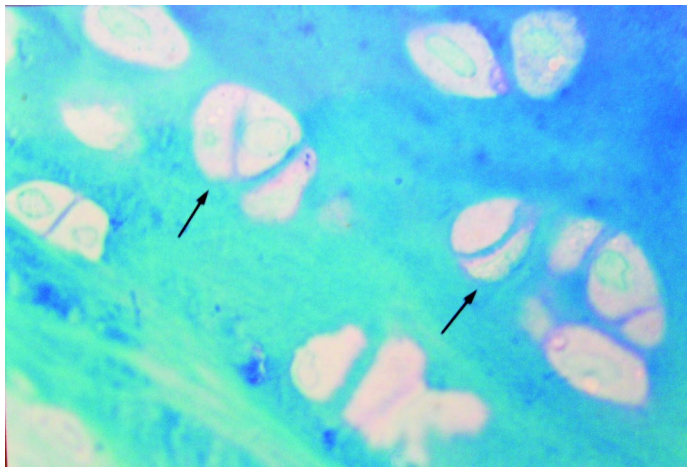

Fig. 2. A micrograph from the NaHA-treated group. Chondrocytes forming isogenous groups (arrows) in their lacunae appear to be healthy compared to the saline group. Pericellular metachromasia is evident. Methylene blue Azur II, 100 ×

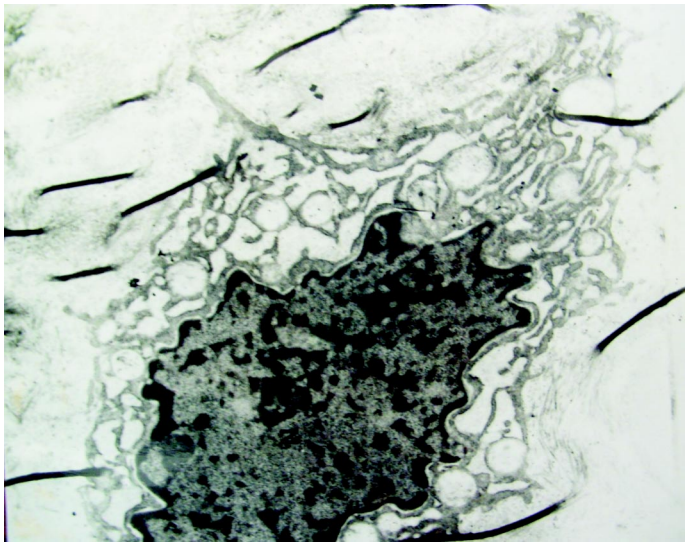

Fig. 3. A chondroblast exhibiting degenerative changes in saline group. The ultrastructural disturbance is more prominent in the rough endoplasmic reticulum cisternae. N: Nucleus. Uranyl acetate- lead citrate, 13 500 ×

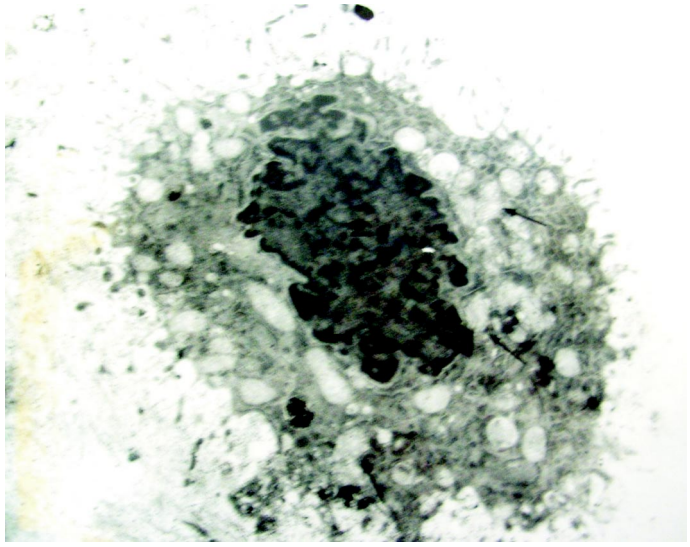

Fig. 4. A chondroblast is observed to reserve its active ultrastructural features. Extensive dilated endoplasmic reticulum cisternae contain a moderately electrondense material (arrows). N: Nucleus. Uranyl acetate- lead citrate, 13 500 ×

synthetic function of chondral cells in NaHA treated group (fig. 1 and 2).

At the ultrastructural level, degenerative changes in chondrol cells were observed in both of groups (fig. 3). The condrogenic cells appeared to be stimulated for the synthesis of matrix components in NaHA group. Chondroblasts had dilated endoplasmic reticulum cisternae with electrondense material in them reflecting the active synthetic capacity of these cells in this group (fig. 4).

The stifle joint is the most frequently affected joint by osteoarthritis (4, 5, 7). This occurrence is not a surprise due to the pressure arising from patella's sliding motion in intercondulary sulcus during flexion and extension. Fibrosis in synovial and capsular tissues, damage in cruciate ligaments, irregularities on the articular surface, loss of stability, pain, and inactivation atrophy are inevitable results of a stifle with osteoarthritis. The articular capsule and ligaments play a major role in joint stabilization. These structures are innervated by proprioseptive nerve ends. When the joint is overloaded, they report it to the high centers and assist in load compensation through muscular support (5). In this study, the cartilage surfaces of the loaded parts of the femur and tibia were grossly evaluated. The femoral condyle showed the most severe changes. In the saline group, the cartilage degeneration was increased in severity, and there was even abrasion of the total cartilage layer in some cases. The degeneration in the NaHa group, on the other hand, was relatively minor, and the stifles were smooth, white and glistening. The joints subjected to treatment with NaHa showed less granulation tissue than the joints treated with saline.

There have been many trials to create an experimental osteoarthritis model in articular cartilage through many local, mechanical, physical, or chemical interventions to investigate mechanisms of the drugs and for biochemical research purposes. Among these, trauma, heat, freezing, acids, local cartilage defect, multiple

superficial incisions on the cartilage, joint instability, joint immobilization, and chondrolytic enzyme injection were reported (6-8, 12). Superficial lacerations on articular cartilage generally do not result in osteoarthritis, but defects destroying joint edges end up with this lesion (6, 13, 15). Because of that, interventions that would result in fibrillation, which is the earliest development in the cartilage, are preferred when creating rapid experimental osteoarthritis (in a month) (12). In this study, an environment for osteoarthritis formation was created through the splitting of the anterior cruciate ligament in rabbit's stifle model, and it was detected that osteoarthritis reached its maximum severity in the post-operative third month.

Metachromasia is among the most important characteristics of normal cartilage (5). Loss of metachromasia in cartilaginous tissue without other changes is the primary indicator of osteoarthritis. Metachromasia in articular cartilage is a characteristic of the basal substance, especially mucoploysaccharide acid (glucoseaminoglycan). Loss of this substance indicates damage in the basal substance. Lysosomal enzymes are involved and impair the biomechanical property of articular cartilage by breaking down the mucopolysaccharides (7). Normal „creep modulus" of the articular cartilage is proportional to the amount of mucopolysaccharides. Destruction of mucopolysaccharides on the weight bearing parts of the joint results in the loss of articular stiffness and progress of osteoarthritis. Meachim (12) reported that trauma leads to metachromasia loss in experimental osteoarthritis, and this picture has regressed in 15 days and with complete healing by the end of the 6th week. In this study, the extracellular matrix lost its metachromasia, and the chondrocytes decreased in number within their lacunae at several locations. These disturbances were less prominent in the NaHA group compared to the saline group shown by electron microscopy.

Superficial fragmentation in cartilage is called fibrillation. Early losses in proteoglycan structure (mucopolysaccharides) of the cartilage occur, and this is prominent on the superficial layers of the cartilage. Losses in cartilaginous matrix are accompanied by mechanical defects. As a result, the cartilage softens and its superficial layers begin to wear out under normal mechanical stress. According to that, the earliest lesion is the shedding of articular cartilage from the surface. Fibrillation proceeds centrifugally, and the cartilage is affected all over. The centrally localized bone underneath is destroyed (5). In this study, degenerating chondroblasts and chondrocytes with their picnotic nuclei were apparent in both groups. However, rabbits in NaHA group had nearly normal chondral matrix areas with healthy chondrocytes within their lacunae forming isogenous groups. The proliferating chondrocytes exhibited intense pericellular metachromasia with methylene blue-Azur II revealing the repair of the cartilage matrix and the recovery of the synthetic function of chondral cells in NaHA.

While superficial cartilage is damaged, and calcification in the deep layers of cartilage was increased leading to a new bone formation at the subchondral region. Cartilage layers were damaged. If the bone becomes the surface that bears the pressure, the thickness of the subchondral bone increases significantly. In order to compensate the different rates of stresses during the reformation of the bone, new bone formation occurs on the edges of articular cartilage which is known as osteophytes. These are the characteristic radiological findings of osteoarthritis. These characteristic alterations progress to an advanced stage leading to chronic cell infiltration inside the bone's medulla (5). In the present study, the cartilage in the control showed an irregular surface, abnormal cellularity, and tidemark integrity. Surface irregularity was nearly normal as in tidemark integrity and cellularity, after treatment with NaHa. Histologically, fibrillated cartilage surfaces and vertical clefts into the transitional zone were observed in some specimens, but the tidemark was preserved with normal appearance in all specimens in NaHa-injected stifles.

In conclusion, this study demonstrated that experimental anterior cruciate ligament incision is capable of inducing osteoarthritis in rabbit stifles. Changes in the stifle joint and related tissues were followed for three months by histopathological and radiological examinations. It was shown that intra-articular administration of hyaluronic acid into the rabbit's stifles as an anti-inflammatory, antioxidant and highly viscous agent blocked the inflammation, destruction and degeneration of synovial tissues.

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