



The chondroprotective effect of intra-articular hyaluronic acid at early stages of osteoarthritis: An experimental study in rabbits

Eklem içine uygulanan hiyalüronik asidin erken evreli osteoartritte kıkırdak koruyucu etkisi: Tavşanda deneysel çalışma

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Amaç: Hiyalüronik asit (HA), osteoartritli eklemlerde özellikle ağrıyı gidermek için kullanılmaktadır. Bu deneysel çalışmada, eklem içine uygulanan HA'nın erken evredeki osteoartrit üzerine etkileri araştırıldı.

Çalışma planı: On adet tavşanın her iki dizinin ön çapraz bağı, ketamin ve ksilazin ile intramusküler anestezi altında kesilerek osteoartrit oluşturuldu. Sağ dizlere, haftada bir olmak üzere toplam üç kez 0.6 ml (15 mg/ml) HA, sol dizlere aynı şekilde 0.6 ml serum fizyolojik (SF) uygulandı. Çalışmanın değişik zamanlarında ölmeleri nedeniyle üç tavşan değerlendirmeden çıkarıldı. On ikinci haftada yüksek doz anestezi altında yaşamları sonlandırılan hayvanların dizlerinde kıkırdak alanı ölçümleri ve Mankin skalasına göre histopatolojik değerlendirmeler yapıldı.

Sonuçlar: Hiyalüronik asit ve SF uygulanan dizler arasında kıkırdak alanı ortalaması açısından anlamlı farklılık bulundu (sırasıyla 1.097 mm² ve 0.477 mm², p<0.05). Mankin skalası toplam puan ortalaması HA grubunda SF grubuna göre anlamlı farklılık gösterdi (sırasıyla 3.57 ve 11.14; p<0.05). Skalanın hücresel anormallik, matriks boyanması ve tidemark devamlılığı kriterlerinde gruplar arasında farklılık anlamlı değilken (p>0.05), kıkırdak yapısı açısından fark anlamlı idi (sırasıyla 0.86 ve 4.43; p<0.05). Tidemark devamlılığı açısından HA uygulanan dizlerin hiçbirinde bozukluk görülmezken, SF uygulanan dizlerin dördünde bozulma saptandı.

Çıkarımlar: Bulgularımız, HA uygulanan dizlerde erken evrede osteoartrit gelişiminin yavaşladığını, dolayısıyla kıkırdakın korunduğunu ortaya koymaktadır.

Anahtar sözcükler: Kartilaj, artiküler/yaralanma/ilaç etkisi; hastalık modeli, hayvan; hiyalüronik asit/terapötik kullanım; enjeksiyon, intraartiküler; osteoartrit, diz/patoloji/ilaç tedavisi; tavşan.

Objectives: Hyaluronic acid (HA) is used in osteoarthritis, especially for the control of pain. In this study, we investigated the effect of intra-articular HA on experimental osteoarthritis of early stage.

Methods: Osteoarthritis was induced in both knees of 10 rabbits by transecting the anterior cruciate ligament under intramuscular anesthesia with ketamine and xylazine. Intra-articular HA at a dose of 0.6 ml (15 mg/ml) and physiologic saline solution (0.6 ml) were injected into the right and left knees, respectively, three times with a week interval. Three rabbits died during the study period and were excluded. The remaining rabbits were sacrificed in the 12th week via high dose anesthesia to remove the distal femora for histological evaluation using the Mankin scale and for measurements of the cartilage area.

Results: The mean cartilage areas calculated in HA- and saline-injected knees differed significantly (1.097 mm² and 0.477 mm², respectively; p<0.05). The overall mean Mankin score was significantly lower in HA-injected knees (3.57 versus 11.14; p<0.05). Although, there were no significant differences between the two groups with respect to cellular abnormality, matrix staining, and tidemark continuity (p>0.05), the mean scores for the structure of the cartilage were significantly different (0.86 versus 4.43; p<0.05). The integrity of the tidemark was preserved in all the HA-administered knees, though a notable disruption was observed in four control knees.

Conclusion: Our results suggest that HA delays the development of osteoarthritis at early stages through exerting a chondroprotective effect.

Key words: Cartilage, articular/injuries/drug effects; disease models, animal; hyaluronic acid/therapeutic use; injections, intra-articular; osteoarthritis, knee/pathology/drug therapy; rabbits.

Although many surgical and conservative methods have been used for the treatment of osteoarthritis of synovial joints, a modality which can reverse the pathologic process in the joint, and also treat osteoarthritis completely has not been found yet. In patients with osteoarthritis in addition to relief of joint pain which is the main symptom of the disease, inhibition or slowing down the development of osteoarthritis also carries much importance. Therefore the cartilage must be protected. Nowadays hyaluronic acid (HA) is administered for osteoarthritic joints to alleviate pain and improve their functions.^[1,2] Studies have demonstrated analgesic, anti-inflammatory, anabolic, and chondroprotective effects of HA.^[3-5] Although some studies have shown its chondroprotective characteristics, these advantages have not been proved convincingly yet.^[3-5]

In this study, considering the progression of osteoarthritis from articular surface into subchondral bone,^[8] chondroprotective effects of HA on cartilage tissue in an early stage osteoarthritis model in rabbits were investigated. Anterior cruciate ligament was transected to create osteoarthritic changes. Instability resulting from the transection of anterior cruciate ligament induces deterioration of synovial cartilage and concomitant development of osteoarthritis. In rabbits, osteoarthritis developed as a result of instability after transection of anterior cruciate ligament.^[6,7,9-11]

Material and method

Study group

In the present study 10 mature male New Zealand rabbits weighing 3.2 kg (2.7-4.5 kg) were used. The approval of the Ethics Committee was obtained. To establish a model of osteoarthritis, anterior cruciate ligaments of two knees were transected. Beginning from the first postoperative weeks, hyaluronic acid was injected into knees of right legs of the rabbits (study group) once weekly 0.6 ml (15 mg/ml) for 3 weeks (Orthovisc, Biomeks; Woburn, MA, USA). Physiologic saline solution (0.6 ml) was injected intraarticularly into knees of left legs of the rabbits of the control group. All injections were administered by the same researcher. Three rabbits died at 5., 8. and 10. weeks and excluded from the analysis.

Surgical technique

Preoperatively the rabbits were anesthetized with 10 mg/kg ketamine(i.m.), and 8 mg/kg xylazine(i.m.). Preoperative prophylaxis was done with cephazoline sodium (50 mg/kg i.m.). Skin of the knees were shaved, sterilized, and draped, and then anterior midline vertical incision was used for operation. After dissection of subcutaneous tissues, knee was entered with medial parapatellar arthrotomy. Patella was dislocated laterally. Anterior cruciate ligament was transected with no:11 scalpel. Serum physiologic was used for intraarticular irrigation. For the closure of medial retinaculum and skin continuous 4/0 chromic catgut, and 4/0 silk sutures were used respectively. After the procedure, the rabbits were placed in 60x60x40 cm cages. For postoperative analgesia, 1-2 mg/kg acetaminophene was dissolved in 100 ml drinking water of rabbits. Rabbits were not immobilized. At 12. weeks postoperatively, the rabbits were sacrificed with high doses (200 mg/kg) of pentothal.

Histology and histomorphometry

At 12. weeks postoperatively, distal femur and condyles of the sacrificed animals were extirpated and immersed in buffered formaldehyde (10 %) solution. Then the specimens were left overnight in decalcification solution. After procedures of dehydration, achromatization and infiltration, pieces sagittally sectioned from medial condyles of the femora were embedded in paraffine blocks. Sections of 4 μ m thickness were prepared from each block. After de-paraffinization procedure, the sections were stained with hematoxyline-eosin ve safranin O FCF

In sections stained with hematoxyline-eosin morphology of the cartilage, cellular abnormality, degree of matrix staining and tidemark integrities were evaluated and scored using Mankin scale^[12](Table 1). In sections stained with safranin O, cartilaginous intermediate substance was evaluated. In these sections, cartilage areas were measured based on the amount of the intermediate substance stained. For measurements, four frontal section specimens prepared from each medial femoral condyle were used. Cartilage areas on specimens were measured as the number of smallest squares occupied within the field of ocular micrometer. The

Table 1. Mankin scale

1. Cartilage Anatomy	
Normal	0
Surface roughness, fissures extending into the radial layer	1
Pannus	2
Superficial layer of the cartilage is lost	3
Mild disorganization, loss of columnar alignment of cells, scarcity of cellular clusters	4
Fissures extending to the calcified cartilage layer	5
Complete loss of cellular organization, clusters of cells, osteoclastic activity	6
2. Cellular abnormalities	
Normal	0
Hipercellularity, including small clusters of superficial cells	1
Clusters of cells	2
Hypercellularity	3
3. Matrix Staining (Safranin O)	
Normal/slightly decreased staining	0
Decreased stained of radial layer	1
Decreased stained of interterritorial matrix	2
Only pericellular matrix is stained	3
Unstained	4
4. Tidemark integrity	
Intact	0
Destruction	1

average of measurements obtained for 4 separate frontal sections was estimated. The measurements were realized under x 40 magnification, and expressed in milimetre squares (mm²).

All histological and histomorphometric evaluations were done by two independent pathologists who had no information about rabbit's group.

Mann-Whitney- U tests for cartilage areas, and two-sampled Kolmogorov-Smirnov test for Mankin scoring were used for statistical evaluations. $P < 0.05$ was considered statistically significant.

Results

During experiments, adverse effects due to injections were not observed in any rabbit, and any evidence of postoperative infection was not noted. Significant differences between mean cartilage areas of knees subjected to hyaluronic acid or saline injections were found (1.097 mm² and 0.477 mm² respectively, $p < 0.05$). The overall mean of Mankin

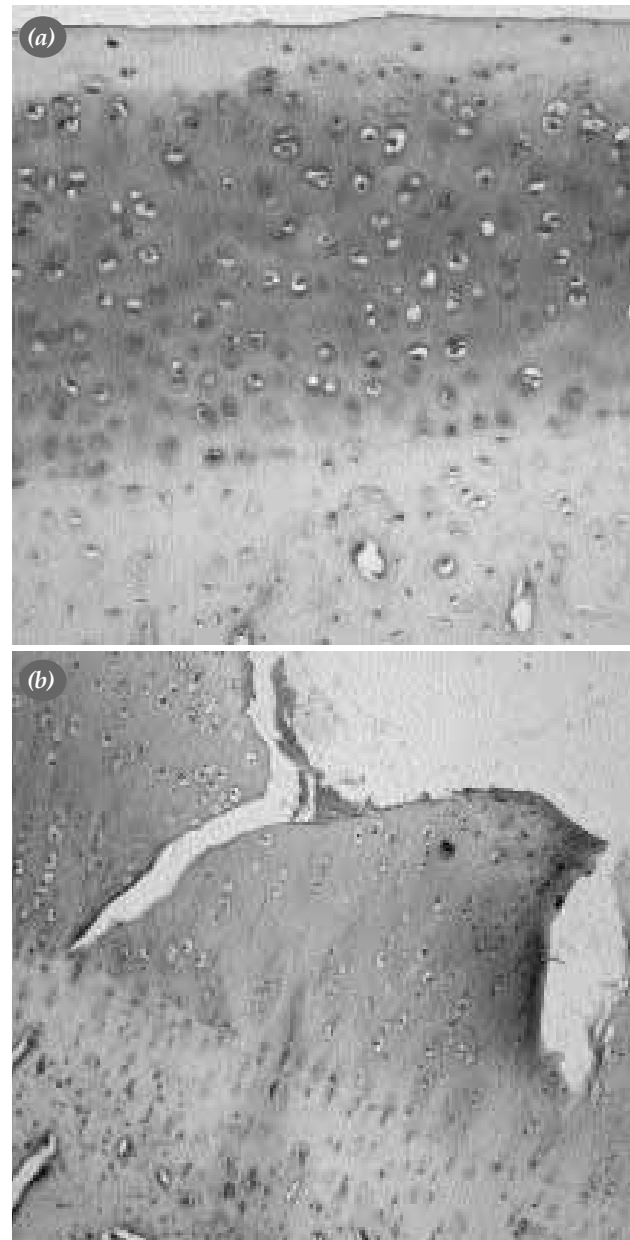


Figure 1. (a) Histological appearance of a rabbit knee in HA group. Loss of superficial chondrocytes and decrease in proteoglycan content (stained with safranin O, x20). (b) Histological appearance of a rabbit knee in SF group. Fissures extending into tidemark; marked decrease in proteoglycan content (stained with safranin O, x20)

scale scores in HA group was significantly different from that estimated in SF group (in HA group 3.57, in SF group 11.14) ($p < 0.05$). While the mean scores for criteria related to cellular abnormalities (in HA group 1.29, in SF group 2.57), matrix staining (in HA group 1.43, in SF group 3.57) and tidemark integrity (in HA group 0.0, in SF group 0.57) were

not significantly ($p>0.05$) different among groups, mean scores for cartilage morphology differed significantly (in HA group 0.86, in SF group 4.43) as favored for HA group ($p<0.05$). As for tidemark integrity, degenerative changes were not observed in any of HA injected knees, while 4 knees applied SF demonstrated signs of degeneration (Figure 1).

Discussion

Hyaluronic acid is a modified polysaccharide chain composed of multiple disaccharide units of N-acetyl glycosamine and glycuronic acid.^[1,3] It is synthesised by type B synoviocytes and synovial fibroblasts, and released into intraarticular spaces.^[3] HA covers cartilage surfaces as an amorphous layer of 0.6- 2 μ m thickness, and partially protects cartilage against cartilaginolytic enzymes, and penetration of inflammatory cells.^[1,13] The main function of hyaluronic acid is to maintain the elasticity and viscosity of the knee joint.^[3] Its effects are closely dependent on stressful impact directed against knee. The elasticity of HA increases, and its viscosity decreases when the knee is exposed to the high stress. During slow movements, the contrary is true.^[1,3] In osteoarthritic knees, HA content decreases 50 % or even 70 %, and its half-life shortens also.^[10] As a result, physical characteristics and protective functions of synovial fluid deteriorate, and collagen fibers which maintain the integrity of the cartilage detach from their normal insertions leading to loss of cartilage tissue.^[13]

HA administration into osteoarthritic knees is called viscosupplementation, and the main mechanism of action of HA is conceivably mediated through viscosupplementation.^[3] Hyaluronic acid has reportedly anti-inflammatory, anabolic, analgesic and chondroprotective and physical effects as well.^[3-5] It is contemplated that these effects are realized by suppressing the production of nitric oxide which also accounts for HA's therapeutic efficacy.^[14] A study is claimed that chondroprotective effects of hyaluronic acid have not been adequately established.^[3] It has been demonstrated that administered HA increases lubrication of synovial joints and decreases of friction on experimentally induced osteoarthritic knees.^[15,16] It has been reported that it decreases fibrinolytic factors which increase in number during the development of osteoarthritis,

and also increases glycosaminoglycan content of cartilaginous matrix.^[17,18] Some authors stated that it reinforces regeneration of menisci by accelerating collagen remodelling, and preventing the formation of edema in injured menisci.^[19,20]

Although some authors reported that they didn't get anticipated benefits from intraarticular HA administrations in osteoarthritic patients, in many studies intraarticular HA application decreased patients' complaints, the number of anti-inflammatory/analgesic drugs used, and also improved patients' general well-being as demonstrated by the improvement in evaluation scales.^[4,21,22] HA treatment administered after arthroscopic debridement of osteoarthritic knees, has been shown to be effective at most 6 months.^[23]

Osteoarthritis starts from the surface of the cartilage tissue and progresses deep into subchondral bone.^[1] It was reported that hyaluronic acid protects against osteoarthritis in the long-term^[6] and this effect increases with recurrent injections.^[7] Our results which showed the presence of intact tidemark integrity suggested that especially in HA treated knees the progress of osteoarthritis was slowed down protecting the cartilage tissue.

In our study, the effects of HA treatment on newly onset osteoarthritis were investigated. Since the first response of chondrocytes to osteoarthritic changes is cellular proliferation, and programmed cell death (apoptosis), the number of chondrocytes decrease in the early stages of the disease.^[24] In our study, the non-significant difference between two groups with respect to Mankin scale criteria for cellular abnormalities might be due to early stages of osteoarthritis investigated in this model. Although significant difference was not found as for tidemark integrity criteria between groups, none of the knees in group HA showed disruption in tidemark integrity, and in contrast deterioration in four knees in Group SF was noted. These findings suggest that HA decelerates the invasion of osteoarthritis from cartilage surface into subchondral bone. The reason for insignificant differences found between groups might be due to limited number of rabbits investigated in groups.

A study demonstrated improvements in chondrocyte content and matrix morphology in osteoarthrit-

ic knees used HA.^[25] In contrast, it was stated that intraarticular HA administration does not ensure improvement in radiological abnormalities in late stages of osteoarthritis,^[26] and its beneficial effects decrease with increasing age.^[27] However, HA apparently prevents and slows down the progress of osteoarthritis in the early stages of the disease. Reinforcement of this finding with experimental and clinical studies performed in late stages of osteoarthritis will emphasize the clinical significance of HA formulations.

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