Effects of Different Therapies on Articular Cartilage in Experimental Severe Osteoarthritis

Deneysel Şiddetli Osteoartritte Farklı Tedavilerin Eklem Kartilajına Etkisi

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Summary

Objective: To examine the effect of intraarticular injections of methylprednisolone, hyaluronic acid and therapeutic ultrasound on osteoarthritic lesions in experimental severe osteoarthritis (OA).

Materials and Methods: Thirty five adult white New Zealand rabbits were used in this study. The experimental OA was induced by the injection of papain (2 mg) to the knee joints bilaterally. Five weeks after the intraarticular injection of papain, rabbits were divided into 3 groups. Group 1: In 12 rabbits, 20 mg methylprednisolone was injected into the right knee once weekly for three weeks. Group 2: In 10 rabbits, 0.4 ml of HA (concentration 15 mg per ml) was injected into the right knee once weekly for three weeks. Group 3: In 10 rabbits 7 min. pulse sonication (US) was applied to the right knees with an intensity of 0.5 W/cm^2 once daily for a total of 10 times. The left knee joints were used as controls. Surface cartilage lesions on the condyles and plateaus was evaluated macroscobically, where as lesion severity was evaluated histologically.

Results: There were no significant differences between the groups for macroscopic and histologic grades of cartilage lesions on condyles and plateaus at the end of the treatment (p>0.05). Furthermore no statistically significant difference was observed between the treated and control knees of the rabbits in each group (p>0.05).

Conclusion: In this study, none of the treatments applied were found to be effective in cartilage lesions in severe OA. *Turk J Phys Med Rehab 2006;52:90-5*

Key Words: Experimental osteoarthritis, management

Özet

Amaç: Deneysel şiddetli osteoartritte (OA), intraartiküler metilprednizalon, hyaluronik asit (HA) ve terapötik ultrasonun, osteoartrit lezyonları üzerine etkisini incelemek.

Gereç ve Yöntem: Otuzbeş adet New Zealand tipi yetişkin tavşan çalışmaya alındı. Her iki dize, 2 mg papain enjeksiyonu ile deneysel OA oluşturuldu. İntraartiküler papain enjeksiyonundan 5 hafta sonra tavşanlar 3 gruba ayrıldı. Grup 1'deki 12 tavşanın sağ dizine, 1 hafta ara ile 3 hafta boyunca, 20 mg metilprednizolon enjekte edildi. Grup 2'deki 10 tavşanın sağ dizine, 1 hafta ara ile 3 hafta boyunca, 0,4 ml HA (konsantrasyon; 15 mg/ml) enjekte edildi. Grup 3'deki 10 tavşanın sağ dizine, yoğunluğu 0,5 W/cm^2 olmak üzere, 7 dakika puls US tedavisi 10 kez uygulandı. Sol dizler kontrol olarak alındı. Kondil ve platolardaki kartilaj lezyonlarının ölçümü makroskobik değerlendirme ile, lezyon şiddeti ise histolojik değerlendirme ile yapıldı.

Bulgular: Tedavi sonunda, kondil ve platodaki kartilaj lezyonlarının makroskobik ve histolojik evreleri arasında gruplar arasında istatistiksel anlamlı fark gözlenmedi (p>0,05). Her gruptaki tavşanların tedavi edilen ve kontrol dizleri arasında da anlamlı değişiklik saptanmadı (p>0,05).

Sonuç: Bu çalışmada, şiddetli osteoartritteki kartilaj lezyonlarında, uygulanan tedavilerden hiçbiri etkili bulunmamıştır. *Türk Fiz Tıp Rehab Derg 2006;52:90-5*

Anahtar Kelimeler: Deneysel osteoartrit, tedavi

Introduction

Osteoarthritis (OA) is a degenerative joint disease resulting in cartilage erosion, subcondral bone remodeling, osteophyte formation and synovial inflammation. Although OA might have multiple origins, current evidence suggests that both mechanical and biochemical factors play an important role in its progression (1).

Although there is no treatment method for which efficacy has been proven in preventing or reversing the structural changes caused by OA which is the most prevalent joint disease, an appropriate treatment may improve the quality of life of the

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patient considerably (2). For this purpose; simple analgesics, non-steroidal anti-inflammatory drugs, physical therapy agents, intraarticular injections, exercise and surgical procedures are applied (3). However, there has been no consensus with on the efficacy of most of the treatments on cartilage and synovial tissue.

Although objective evidence is limited, it has been stated in the treatment protocol of American College of Rheumatology (ACR) that intraarticular steroid applications may be used in the treatment of knee OA (4). There is data revealing that intraarticular steroid injections are successful in modification of the symptoms (5) and in restoration of the articular functions (6). While it was reported in some previous studies that it resulted in worsening of osteoarthritic lesions with cartilage injury (7,8), it has been demonstrated in experimental OA models that lower-dose steroid injection reduced osteophyte formation and the progression in cartilage erosions (6,9,10). These contradictory publications about steroids always leave suspicion on the physicians' mind regarding their usage (11).

It is has been stated that an alternative intraarticular treatment, hyaluronic acid (HA), increases the viscosity in synovial liquid and facilitates the slipperiness by constituting a layer on the surface of cartilage and also protects the soft tissue, reduces the pain and is effective on inflammatory cells as an immunomodulator by working as a shock absorbent in trauma on the joint (3,12). However, there are also opinions defending that HA injection is not superior to placebo and therefore it may not be involved in the routine treatment of OA (13). The characteristics of a suitable candidate for the application of intraarticular HA injection has not been fully determined. For applications; exact rules related to experienced trauma or deformity, age, radiographically diagnosed OA and grade of the symptoms or level of physical activity can not be stated. Usually, patients with severe OA are not included in the studies (3). In patients with serious grade of OA, although it is possible to delay the application of HA and total knee replacement, there is no data supporting this.

Therapeutic ultrasound (US) has been advocated by ACR in the non-pharmalogical treatments for the management of knee OA (2). It has been stated that US has effects on muscle relaxation, increase in membrane permeability and increase in tissue regeneration (2). Information about the efficacy of US in knee OA treatment is present and usually, especially about the useful effects on pain and functional recovery (14,15). Also, it is stated that US is effective in tendon recovery and in cartilage reparation by stimulating collagen synthesis (2,16). The data about US is obtained from a limited number of study results.

It is stated that these three treatment methods have useful effects different ways in knee OA. However, there is no evident data regarding their efficacy in severe knee OA. We planned to investigate the macroscopic and the histopathologic effects of these treatments on articular cartilage and synovium in severe experimental knee OA which are used in every stages of knee OA.

Materials and Methods

Thirty five mature New Zealand type of rabbits, 2-2.5 kg in weight were included in the study. The rabbits were supplied by the Experimental Surgery and Research Laboratory of Faculty of Medicine. All the applications were performed in the same set of laboratory by paying attention to standardization of laboratory conditions. Prior to study, approval of the local "Ethic Commitee of Experimental Surgery of Experiment Animals" was obtained and the guidelines for animal use and care were followed. All of the applications related to rabbits were performed by an investigator who had previously completed the "Course of Experimental Animals". During the injections and other applications to rabbits, sedation was achieved by using the combination of Xylazine 2 mg/kg and Ketamin 20 mg/kg. In injections and applications to knee, the application area was shaved and cleaned with Betadine solution.

To constitute a knee OA in rabbits, 100 mg of 2x crystalized suspensions of a preparation of papain (16,17) of which 1 mg shows 16-40 units of the activity, pH at 4.5, including 0.01% timol involved in 0.05 M sodium acetat was used (Sigma Chemical Co., St Louis, USA p3125).

A prestudy was performed to determine the effective papain dose which constitutes severe OA. Separate doses of 0.5 mg, 1 mg and 2 mg papain were injected by intraarticular way to both knees of each rabbit, using a 26 gauge needle. After 5 weeks of injection, 3 rabbits were sacrificed with an over-dose of anestethetic substance. Removing both knees of these rabbits, macroscopic and microscopic examinations were performed. It was observed that 2 mg of intraarticular papain injection led to severe knee OA. It was decided to continue the study with this dose and duration.

2 mg intraarticular papain injections were applied to both knees of 32 rabbits. After 5 weeks of papain injection, rabbits were randomly assigned to 3 different treatment goups. The left knees of all rabbits were evaluated as control group and no treatment were given.

20 mg of metilprednisolone (0.3 ml) (Prednol-L® flacon, Mustafa Nevzat, Istanbul, Turkey) was injected for 3 times with one week interval to the right knees of 12 rabbits which were included in the first group. In the second group, 0.4 ml of HA (15 mg per ml, Orthovisc®, Anika Therapeutics, Inc, Woburn, MA) was injected for 3 times with one week interval to the right knees of 10 rabbits. In the third group, 0.5 W/cm^2 dose of pulse ultrasound treatment (Mettler Electronics, Sonicator 730, USA) was performed for 7 minutes inside the water for 10 times to the right knees of 10 rabbits. In this procedure, an applicator with a frequency of 1 MHz was used directed over an effective area of 5 cm^2. The head of the therapeutic US probe was placed into the water at a distance of 1 cm to the anteromedial part of the right knee. In the third group, only 4 rabbits completed the study. The other rabbits in this group died due to problems in laboratory conditions. At the end of the treatment, all rabbits were sacrificed. The left and the right knees of rabbits in all three groups were resected carefully. The resected knees of the sacrificed rabbits were sent to pathology laboratory. By dissecting the knee joints, capsule of the joint, ligaments, synovial tissue, meniscus, cartilages of the joint and subchondral bone tissue were examined under dissection microscope. To facilitate distinguishing the eburnation and fibrillation areas, the surface of the femoral condyle and the surface of the tibia plateau were re-examined following the application of India ink. Prior to cross-section, the photographs of surfaces of the joint were taken. The dimensions of the lesions observed in femoral condyle and in tibial plateau were measured millimetrically. Sagittal cross-sections were performed throughout the longest axes of the lesions.

Since they were colored by Indian ink, fibrillation and fissure areas were observed as black-grey, while eburnation areas were observed as yellow-cream and the surrounding normal cartilage was observed as ivory white in dissected knee joints.

After being fixed in 10% buffered formalin for 3 days, each sample was decalcified for 4 days in 20% formic acid prepared with 10% buffered formalin. The tissues obtained were buried into paraffin blocks. 5 microns of cross-sections were taken by Leica RM 2145 microtome. Hematoxylene and eosin for histopathological examination, and toluidine-blue and safranin-O histochemical stains for qualitive evaluation of proteoglycan density were applied. The cross-sections were examined by two pathologists. A two-head Olympus BX-50 microscope was used. Since there were widespread eburna-

Table 1. Histologic Grading Scale.

Presence of Eburnation	Present	1
	Not Present	0
For Lesions with Eburnation		
Regenerated Tissue	100 percent of normal adjacent cartilage	0
	50-99 percent of normal cartilage	1
	10-50 percent of normal cartilage	2
	Regenerated tissue not present	3
Cell morphology of regenerated tissue	Hyaline cartilage	0
	Fibrohyaline cartilage	1
	Fibrocartilage	2
	Non-cartilage only	3
Staining of the matrix in regenerated tissue (Safranin-O, Toluidine blue)	Normal	0
	Slightly reduced	1
	No metachromatic stain	2
For Lesions without Eburnation		
Surface regularity of cartilage	Intact	0
	Superficial fibrillation	1
	Deep fibrillation	2
	Necrosis	3
Structural integrity of cartilage cells	Normal	0
	Cell disintegration	1
	Increase of cells (no cluster)	2
	Cluster of chondrocytes	3
	Lost of cells	4
Staining of the matrix in lesions without eburnation (Safranin-O, Toluidine blue)	Normal	0
	Slightly reduced	1
	No metachromatic stain	2
Features of Tissue Surrounding the Lesion		
Surface regularity	Intact	0
	Superficial fibrillation	1
	Deep fibrillation	2
	Necrosis	3
Structural integrity	Normal	0
	Cell disintegration	1
	Increase of cells (no cluster)	2
	Cluster of chondrocytes	3
	Lost of cells	4
Staining of the matrix (Safranin-O, Toluidine blue)	Normal	0
	Slightly reduced	1
	No metachromatic stain	2
Synovial Tissue		
Synovial hyperplasia	Not present (until 2 rows)	0
	Present (more than 2 rows)	1
Villous hyperplasia	Not present	0
	Present	1
Mononuclear inflammatory cells infiltration	A few scattered infiltration, no cluster	0
	Cluster including less than 15 cells	1
	Clusters including at least 15 cells	1

tion areas in pathological cross-sections, not only the regions of lesion, but also the areas surrounding the lesion were evaluated. In histological evaluation of all these areas, the evaluations of previously defined different grading scales were used together (Table 1) (18-20).

Statistical Method

In statistical analyses, SPSS for Windows 10.0 pocket programme was used. Chi-square test was used in comparison of ratios between the treatment groups and between the knees treated in each treatment group and knees in control group. Mann Whitney-U test was utilized in evaluation of dimensions of the lesions in treatment groups and in comparison of the means between the groups. $p<0.05$ was taken as statistical significance level.

Results

Gross pathological findings: The left and the right knees of rabbits in each treatment group were evaluated. The regions of lateral femoral condyles (LF), medial femoral condyles (MF), lateral tibial plateau (LT) and medial tibial plateau (MT) lesions were separately measured millimetrically, including the most extensive area. For the evaluation of macroscopic lesions, no statistically significant difference was determined between the knees of treatment (right=R) and control (left=L) groups ($p>0.05$). In the same way, no statistically significant difference was observed between three treatment groups ($p>0.05$) (Table 2).

Histology of articular cartilage and synovium: Microscopic analyses of medial and lateral regions of tibial plateau and femoral condyle of both treatment and control knees of the rabbits were done separately. In microscopic samples, both treatment and control knees showed the features of severe OA.

Lesions in all areas and features of cells and tissues were evaluated according to the scorings defined previously.

For features of cartilage in lesions with and without eburnation, no significant difference was observed between the right and the left knees in each treatment group ($p>0.05$). No statistically significant difference was determined between three treatment groups ($p>0.05$).

As for the features of cartilage tissue surrounding the lesion, no significant difference was observed between the right and the left knees in each treatment group ($p>0.05$). No statistically significant difference was determined between three treatment groups ($p>0.05$).

In evaluation of synovial tissue surrounding the joint, no statistically significant difference was observed between the right and the left knees in each treatment group and between three treatment groups ($p>0.05$).

No complication as acute arthritis was observed due to OA in three treatment groups of the study.

Discussion

As the result of our study, we observed that each of three treatment methods showed no difference with regard to macroscopic and histologic evaluations and did not create any effect resulting in regeneration or prevention of progression of the cartilage lesions.

Although there is some data which reveals that intraarticular HA application has a disease modifying feature (1,21,22), this effect may not be supported by present limited evidence. Furthermore, there is no exact evidence regarding its effects in such cases with severe stage of OA. For symptomatic knee OA, the role of viscosupplementation is just in progress. Although results of the studies are not categorical in defining the most suitable candidate for injection treatment, the present data documents the long-term efficacy (3,23). Contrary to studies revealing that no benefit was obtained or minimal degrees of benefits were gained from intrarticular HA (13,24), randomized, controlled clinical studies (25,26) have suggested that HA compared with placebo has chondroprotective effects and inhibits apoptosis (1,22,23,27,28).

Although there are different results about the efficacy of steroids in OA treatment, they are included in intraarticular treatments of knee OA as a symptomatic treatment (29-31) and as a disease modifying treatment (6,9,10) according to some study results. In some studies, it has been seen that triamcinolone and metilprednisolone cause a reduction in formation and size of osteophytes, as well as in size and histologic severity of cartilage erosion and in stromelysin which is important in the activity of disease (6,9,10).

Studies in which steroid and HA applications are compared with each other are present in literature (12,32-34). Ronchetti et al. (12,35) have examined the samples of synovial membrane and cartilage by arthroscopy in their clinical studies. As a potential disease modifying drug, HA has been found to be superior to steroid in morphological analyses. Metilprednisolone treatment has caused a decrease in mast cells and HA caused a decrease in synoviocyte aggregation. Both of these treatments; have decreased macrophages, lymphocytes, mast cells, adipocytes and oedema, and increased fibroblast-like cells and formation of fibroblasts and collagen. In these studies, HA and steroid have been found to be effective clinically. But it has been seen that the effect of HA lasts longer. In the other comparative clinical studies, for pain and stiffness, HA has been found to be more effective than metilprednisolone (34), to act longer than triamcinolone hexacetonide (33) and to have similar effects as betamethasone (32).

In treatment, there are beneficial effects of US such as increasing the mucopolysaccharidation, affecting the formation

Table 2. The means of macroscopic lesion measurements of three treatment groups.

Macroscopic lesions (mm)	Steroid			HA			US		
	Right	Left	p	Right	Left	p>	Right	Left	p
FL	4.25±2.53	2.67±2.42	NS	3.33±1.80	3.44±2.13	NS	4±1.41	3±1.41	NS
FM	2±1.95	2.58±2.07	NS	1.80±2.10	1.67±1.73	NS	1.75±1.26	2.25±2.06	NS
TL	2.58±2.50	1.55±2.38	NS	3.50±1.27	3.20±1.69	NS	3.50±0.58	2.75±0.50	NS
TM	1.83±2.04	2±2.32	NS	2.50±2.27	2.10±1.97	NS	2.75±2.06	2.25±1.71	NS

FL: femur lateral, FM: femur medial, HA: hyaluronic acid, NS: non-significant, TL: tibia lateral, TM: tibia medial, US: ultrasound. Values are the mean±SD

of free radicals, ensuring the removal of inflammatory mediators, suppressing the cellular inflammation and increasing the tissue reparation (2). Non-thermal effect is thought to be much more important in tissue reparation (2,16). Sound waves cause an increase in diffusion and membrane permeability, thus protein synthesis increases and reparation process is influenced. However, since this reparation mechanism is a feature which depends on the cell membrane, it has been stated that if target cells are damaged, then the tissue reparation becomes limited and US is effective in only prevention of injury (16). The biological and histological effects of US in chronic arthritis have not been completely investigated. In an experimental study performed in rats (16), it has been stated that it may be effective on cartilage reparation in early stages and on prevention of progression in later stages. Although US is considered to be included in non-pharmacological treatments, there is no sufficient amount of experimental or clinical study that proves its efficacy in OA treatment in literature. Therefore, we believe that our study results might be useful.

Furthermore, features of the tissues which were surrounding the lesion and were outside of the overweight loaded areas have also been evaluated in our study. It has been revealed that treatments applied in this way not only have useful effects, but also they don't have any harmful effects on the lesion and its surroundings.

The present study has certain limitations. In the third group, only 4 rabbits completed the study. The other rabbits in this group died due to the problems in laboratory conditions. Therefore sample size is small.

Although there are studies comparing applications of steroid with that of HA, there is no study like this study in which these practical three treatments are compared with each other. Similarly, no study has been found in which these three treatments are compared with each other only in severe OA. Whereas, all of these treatments are used distinctly in clinical practice. These treatments have been found to be ineffective histologically in severe knee OA, while their efficacy have been revealed clinically and histologically in early stage of knee OA. We consider that this result should be supported by clinical studies. Taking into consideration the ratios of benefits and harms of these treatments to patient, application of effective treatment to a suitable patient at appropriate time is gaining importance. This becomes an important and conspicuous issue, especially in societies where the population of elderly is increasing gradually.

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