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Quantification of papain-induced rat osteoarthritis in relation to time with the Mankin score

Sıçanlarda papainle oluşturulan osteoartritin Mankin skoru ile zamana bağlı derecelendirilmesi

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Amaç: Papainle deneysel olarak oluşturulan sıçan osteoartrit modelinde kıkırdak yapısındaki değişiklikler Mankin skorlama sistemiyle zamana bağlı olarak derecelendirildi.

Çalışma planı: Çalışmada 21 adet erkek Wistar sıçanda osteoartrit oluşturmak için %4'lük papain solüsyonu (10 μl) ve aktivatörü 0.03M sistein (10 μl) sağ diz eklemine 1, 4 ve 7. günlerde enjekte edildi. Kontrol olarak ise aynı miktarda steril serum fizyolojik solüsyonu sol dizlere enjekte edildi. Sıçanlardan rastgele seçimle yedişerli üç grup oluşturuldu. Her bir gruptaki sıçanların yaşamı son enjeksiyondan sırasıyla 1, 2 ve 4 hafta sonra yüksek doz eter anestezisi ile sonlandırıldı ve sağ ve sol diz eklemleri çıkarıldı. Eklem kıkırdağı yapısında oluşan değişikliklerin histolojik derecelendirmesi modifiye Mankin skoru kullanılarak yapıldı.

Sonuçlar: Kıkırdak yapısı değerlendirildiğinde, her üç grupta da yüzeysel tabakada düzensizlik ve fibrilasyonlar; geçiş ve radial alanlardaki hücre sayılarında azalma ve tabakalanma gözlenirken, pannus oluşmadığı izlendi. Her üç grupta da modifiye Mankin skoru papain uygulanan dizlerde kontrollere göre anlamlı derecede yüksek idi ($p<0.05$). Birinci, ikinci ve dördüncü hafta Mankin skorları papain grubunda sırasıyla 4.3±0.9, 6.9±1.3 ve 10.4±1.9; kontrol dizlerde ise 2.7±0.5, 4.0±0.8 ve 4.4±1.0 bulundu. Papain enjeksiyonundan sonra bir hafta ve dört hafta beklenen gruplar arasında Mankin skoru anlamlı farklılık gösterdi ($p=0.0471$).

Çıkarımlar: Enjeksiyondan dört hafta sonra elde ettiğimiz bulgular osteoartritin erken evre bulguları ile uyumlu görünmektedir. Bu araştırmanın sonuçları erken evre osteoartrit tedavisine yönelik çalışmalara ışık tutabilir.

Anahtar sözcükler: Kıkırdak, eklem/ilaç etkisi/patoloji; hastalık modeli, hayvan; diz eklemi/patoloji; osteoartrit/kimyasal yolla oluşan; papain; sıçan.

Objectives: The aim of this study was to evaluate structural changes in relation to time with the use of the Mankin scoring system in papain-induced rat osteoarthritis.

Methods: Osteoarthritis was induced in 21 male Wistar rats by injecting an admixture of 4% papain (10 μl) and its activator 0.03 M cycteine (10 μl) into the right knee joints on the first, fourth, and seventh days. The same volume of sterile saline solution was injected into the left knees as controls. The rats were assigned to three groups equal in number and were sacrificed under high-dose ether anesthesia after one, two, and four weeks of the last papain injection, respectively. The study and control knee joints were removed and histologic changes in cartilage structure were assessed and quantified with the modified Mankin scoring system.

Results: Histologically, all papain injected knees exhibited irregularity and fibrillation in the superficial layer, decreased cell count and multilayering in transitional and radial zones, and no pannus formation. The modified Mankin scores were significantly higher compared to the control knees in all the groups ($p<0.05$), being 4.3±0.9, 6.9±1.3, and 10.4±1.9 in diseased knees, and 2.7±0.5, 4.0±0.8, and 4.4±1.0 in the control knees after after one, two, and four weeks of the last papain injection, respectively. There was a significant difference between the Mankin scores of the rats sacrificed after one and four weeks of the last papain injection ($p=0.0471$).

Conclusion: Findings observed after four weeks of papain injection seem to be consistent with early osteoarthritic changes. Our results may provide insight into therapeutic strategies for early osteoarthritis.

Key words: Cartilage, articular/drug effects/pathology; disease models, animal; knee joint/pathology; osteoarthritis/chemically induced; papain; rats.

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Osteoarthritis (OA) is a dynamic phenomenon that reflects the inbalance between destruction and repair mechanism of not only cartilage tissue but also bone, synovium, joint capsul, ligaments, tendons and muscles.[1,2]

In Clinical studies there are severe limitations for accessibility of tissue sampling for standardization and grading.[3] Experimental osteoarthritic models are used for better understanding of disesase. They are necessary to evaluate structural changes in joint, enviromental or biologic risk factors which effects the sturucture of the joints and new therapeutic strategies to prevent OA.[4]

There are several experimental models induced in several species of animals for osteoarthritis studies .[5] Generally these models are induced by trauma, surgery, enzymes and chemicals that creating joint instability and destroy cartilage metabolism. Papain that is one of the proteolytic enzyme is used for induction of experimental OA. It effect on collagen integration and has no direct influence on chondrocytes. Its affect on cartilage can be revolve at low dose. These are the advantages to other chemical models. There are several histopathologic studies reporting that injection of papain induce OA that mimics human OA. In these studies, evaluation has been done histopathologicaly but the results were not quantified for comparision.Thus the aim of our study was to evaluate structural changes in papain-induced experimental OA model by quantifying according to time

Materıal and methods

21 male Wistar rats, weighting (200 - 300 g) were used in this study. It was evaluated and approved by Dokuz Eylul University Medical Faculty local ethic committee. Rats were kept under standard laboratory conditions(temperature 24°, 12h light-dark cycle). they were fed standard diet and drank tap water ad libitum. Rats were anesthetized with ether before every injection. The formation of osteoathritis was evaluated by histopatologically.

Induction of experimental osteoarthritis

Osteoarthritis was induced in the right knees of the first group and both knees of the others by injecting 0.2 ml of 4% papain solution with 0.1 ml of 0.03 M cystein as activator. Same amount of saline was injected into the left knees of the first group. Injection was repeated on the fourth and seventh days.[9] The rats were divided into 3 groups in equal number randomly(n=7) and were sacrificed under high-dose ether anesthesia after one,two, and four weeks of the last papain injection, respectively. The study and control knee joints were removed

Histopathological studies

Knee samples were washed in saline and fixed in 10% neutral buffered formaline and decalcified with 10% aqueous formic acid and embedded in paraffin to allow for 4 μm sections. They were then stained with hemotoxylin and eosin and with toluidin blue for microscopic examination. All examinations were done under light microscope and evaluated by using modified Mankin scoring system.

In modified Mankin scoring system; the structure of the cartilage, cell appearance, staining of the cartilage matrix by toluidine blue, tidemark and pannus formation are evaluated. Each sample take points in five different categories separetly. By this system,total score changes between 0 and 32. Low scores show slight changes in articuler cartilage where as high ones indicate severe osteoarthritis.

Statistical analysis

Data for each group are presented as the mean±SEM. Study and control knees in each group were compared by Mann Whitney U test. For comparision of three groups, Kruskal-Wallis test was used.A difference was taken to be statistically significant at $p<0.05$.

Results

Group I: One week after the last injection

When contralateral control knees compared with papain injected knees according to Modified Mankin score hypercellularity including superficial clusters and reduced staining was seen, tidemark was multilayered . According to Modified Mankin scores evaluation, there was significant differences between papain and control side in group I. (C: 2.71±0.52, P: 4.29±0.9 $p<0.05$) (Fig 1)

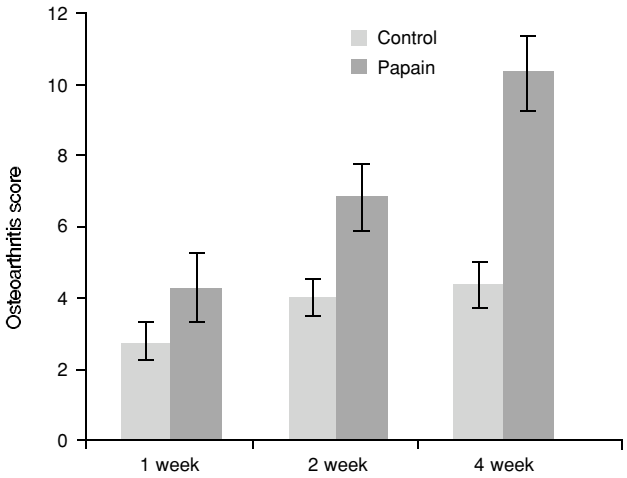

Figure 1. Modified Mankin scores of the group 1, 2 and 3

Group II: Two weeks after the last injection

In papain injected knees , irregular surface, fibrilation, roughing, swelling of the cells in tangential zone, hypocellularite in transitional and radial zones, reduction in staining and multilayered tidemark zone were seen. (Fig 2) According to Modified Mankin scores evaluation, there was significant differences between papain and control side in group II.(C: 4±0.75, P: 6.86±1.3, $p<0.05$) (Fig 1).

Group III: four weeks after the last injection
Speciements from papain injected group had fibrilation, roughing, swelling of the cells in tangential zone, hypocellularite in transitional and radial zones, cleft until tide mark, reduction in staining and multilayered tidemark zone.(Fig 3). Papain group's score was significantly different from control group (C 4.42 ± 0.99 , P: 10.4 ± 1.9, $p<0.05$) (Fig 1).

When we compared three groups,there was no significant difference between control groups. In papain groups only significance between group I and III was seen ($p=0.0471$).

Discussion

Experimental OA models are useful for evaluating structural changes in joint, risk factors which effects the sturucture of the joints and the effect of therapeutic strategies to prevent OA.According to formation mechanism these models can be classified in two major groups as mechanical and structural.[3] In mechanical models OA is produced by creating joint instability or alteration of load distribution. Anterior cruciate ligament section is the sample of this group that is used frequently in the literatur.[11-15] In structural models there is a physicial, chemical or enzymatic agent which induce OA by effecting

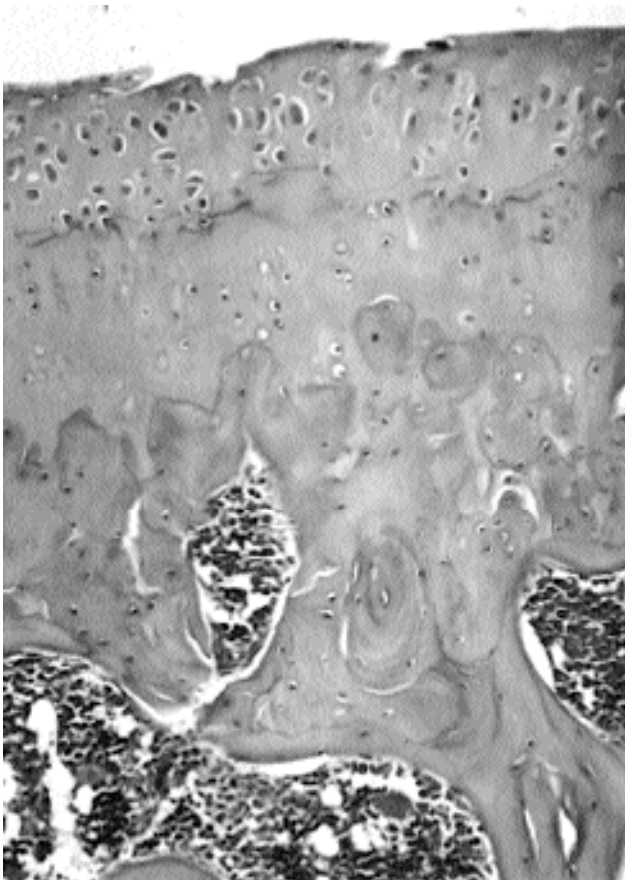

Figure 2. Irregular surface, swelling of the cells and multilayered tidemark (HEx40)

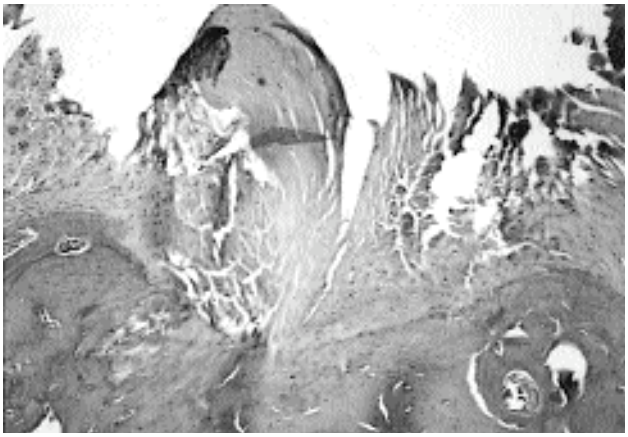

Figure 3. Hypocellularite in transitional and radial zones, cleft until tide mark, and multilayered tidemark zone.(HEx1)

directly cartilage tissue composition. It takes shorter time than mechanical ones.[16]

Papain is the proteolytic enzyme, causes osteoarthritis by releasing of chondroitin sulphate from the protein polysaccharide complex of the matrix of articular cartilage.[9] But it has no direct effect on condrocytes and collagen[17] it is determined that papain do not disturb repairing mechanism of cartilage tissue totally and repairing capacity.has inverse proportion with dosage of papain.[3,18,19] this condition will provide staging of OA and evaluating of alternatif treatment modelities in different stage of the disease. In some studies it was reported that intraarticular injection of papain in animal induce OA similiar to human OA histopathalogically.[4,7-10,17]

In these studies papain was used at different dosage and interval. Results were evaluated histopathologically but the results were not quantified for comparision

Mankin scoring system is a sensitive method and provide examining even slight degenerative changes in articular cartilage. Features of cell, change in matrix structure, tidemark zone, formation of pannus, the characteristic of articuler surface, in degeneratif cartilage can be evaluated by this scoring system.[11] It is an adequate system for comprising of all morphological changes seen in OA and having litlle difference between intra- and interobserver reliability.[20] It was found that The histopathological gradation of the severity of OAaccording to mankin appeared to be directly correlated with the metabolic state of the chondrocytes in the different stages of OA.[21]

Papain studies in the literatur introduced this relation histopathologicaly. But it was not studied statistically.In our study we found that there was a direct proportion between degree of degeneration and time in papain induced OA model and introduced by scoring system at the first time.

Findings observed after four weeks of papain injection seem to be consistent with early osteoarthritic changes. Our results may provide insight into therapeutic strategies for early osteoarthritis.

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