Dicle Üniversitesi Veteriner Fakültesi Dergisi
https://dergipark.org.tr/tr/pub/duvetfd
Araştırma Makalesi/Research Article
ISSN:1307-9972 Dicle Üniv Vet Fak Derg 2021;14(1):56-61 e-ISSN:1308-0679
DOI: 10.47027/duvetfd.886960

Evaluation of The Treatment Efficiency of Microfracture Technique and Intra-Articular Sodium Hyaluronate Injection on Osteoarthritis of Rats

Umut ARDA[1,a], Zeynep BOZKAN[1,b,✉]

[1]Department of Surgery, Faculty of Veterinary Medicine, University of Aydin Adnan Menderes, Aydin, TURKEY

[a]ORCID: 0000-0001-6692-0318; [b]ORCID: 0000-0003-4233-6496

Geliş Tarihi/Received	Kabul Tarihi/Accepted	Yayın Tarihi/Published
19.02.2021	13.04.2021	30.06.2021

Abstract

The animals with osteoarthritis suffer from clinical signs such as joint pain, reluctance to move, loss of performance and lameness. Many medical and interventional methods are used to manage osteoarthritis but no described treatment can completely repair damaged cartilage yet. In this study, 40 male Wistar Albino rats were randomly assigned as microfracture technique group, intra-articular hyaluronic acid group, microfracture technique + intra-articular hyaluronic acid group and control group (n=10). Clinically, knee circumference and body weight were measured and, leg posture was scored. Radiological examination findings were evaluated using a grading based on 3 parameter as joint space narrowing, subchondral bone sclerosis, osteophyte formation. Also, at the end of the experiment, following sacrification, the joint of the relevant leg was scored according to the severity of osteoarthritis. Considering the changes in the leg posture, the knee circumference measurement and the radiological findings, the development of osteoarthritis was successfully achieved with monosodium iodoacetate application in this study. In line with the findings obtained from the presented study, it was concluded that combining microfracture technique with hyaluronic acid application would not contribute additionally to the treatment process, and even using microfracture or hyaluronic acid application alone may produce more positive results.

Key Words: Osteoarthritis, rat, hyaluronic acid, microfracture, treatment

Ratlarda Osteoartrit Tedavisinde Mikrokırık Tekniği ve Eklem Içi Sodyum Hiyalüronat Enjeksiyonunun Etkinliğinin Değerlendirilmesi

Öz

Osteoartritisli hayvanlarda eklem ağrısı, hareket etmede isteksizlik, performans kaybı ve topallık gibi klinik belirtiler görülür. Osteoartriti tedavi etmek için birçok medikal ve girişimsel yöntem kullanılmaktadır, ancak tanımlanmış hiçbir tedavi ile henüz hasarlı kıkırdak tamamen onarılamamaktadır. Bu çalışmada, 40 adet erkek Wistar Albino rat; mikrokırık grubu, eklem içi hyaluronik asit grubu, mikro kırık + eklem içi hyaluronik asit grubu ve kontrol grubu olarak (n= 10) ayrılmıştır. Klinik bulgular olarak diz çevresi ile vücut ağırlığı ölçülmüş ve bacak duruşu skorlanmıştır. Radyolojik muayene bulguları eklem aralığı daralması, subkondral kemik sklerozu, osteofit oluşumu olmak üzere 3 veriye dayalı derecelendirme ile değerlendirilmiştir. Ayrıca, deney bitiminde sakrifikasyon sonrasında ratların ilgili eklemi osteoartritin şiddetine göre makroskopik olarak skorlanmıştır. Bacak duruşundaki değişiklikler, diz çevresi ölçümü ve radyolojik bulgular dikkate alındığında, bu çalışmada monosodyum iyodoasetat uygulaması ile osteoartrit gelişimi başarıyla sağlanmıştır. Sunulan çalışmadan elde edilen bulgular doğrultusunda, mikrokırık tekniğinin hyaluronik asit uygulaması ile birleştirilmesinin tedavi sürecine ek katkı sağlamadığı ve mikrokırık veya hyaluronik asit uygulamasının tek başına kullanılmasının daha olumlu sonuçlar verebileceği sonucuna varılmıştır.

Anahtar Kelimeler: Osteoartrit, rat, hyaluronik asit, mikrokırık, tedavi

INTRODUCTION

Osteoartrit (OA) is a degenerative disease which charecterize with cartilage destruction, osteophyte formation, and subchondral sclerosis progressively occurring in synovial, diarthrodial and espescially load-bearing joints. Osteoarthritis that occurs idiopathically is classified as primary, and no known etiology can be detected. Secondary osteoarthritis occurs as a result of a previously developed joint damage (1).

First, the capsule and synovial membrane thickens, and fibrillation occurs in the of high-tension junctions following sclerosis in the bone. Cartilage debris pouring from the degenerated joint surfaces disrupts the normal synthesis of synovial fluid components such as hyaluronan and eventually the synovial membrane becomes inflamed. Viscoelasticity of synovial fluid synthesized in osteoarthritic joints and therefore its' lubricant efficiency for ligaments and articular cartilage reduces (2, 3).

Osteoarthritis is an important problem encountered in many animal species such as cat (4), dog (5), horse (6). The animals with osteoarthritis suffer from clinical signs such as joint pain, reluctance to move, loss of performance and lameness. (6, 7) Many medical and interventional methods are used to manage osteoarthritis. Medical treatment methods mainly include non-steroidal anti-inflammatory drugs, intravenous/intraarticular hyaluronic acid applications, glycosaminoglycans, intraarticular steroid applications (6). However, the fact that medical treatment is not a definitive solution for osteoarthritis has led to the search for more

permanent treatment options with various surgical interventions from the past to the present. Surgically, methods such as arthroscopic lavage and/or shaving, debridement, laser chondroplasty/abrasion, abrasion chondroplasty, pridie drilling, microfracture technique, spongialization osteotomy, joint distraction are used (8).

In this study, treatment efficiency of microfracture technique and intra-articular hyaluronic acid injection separately and in combination in the rats generalized osteoarthritis induced experimentally have been investigated.

MATERIAL AND METHODS

Animals

In this study, 40 adult male Wistar Albino rats at 300-350 grams weighing and 5-7 months old were used. During the experiment, the rats had free access to food and water and 12-hour shift of light and darkness. The experiment was started after the adaptation period of the rats for 3 weeks. The study was approved local ethics committee.

Experiment Groups

All rats were anesthetized by intraperitoneal injection of 8 mg / kg xylazine + 60 mg ketamine. 3 mg of monosodium iodoacetate (MIA) solution in 50 µl of saline was injected aseptically into the right genu joint of rats in the left lateral recumbency on the operating table. After 2 weeks to develop chronic OA, the rats were re-anesthetized. The genu joint was approached with a parapatellar medial incision (Figure 1a) and the patella was retracted laterally and the sulcus trochlea was exposed (Figure 1b). After this procedure, the rats were randomly divided into 4 groups containing 10 rats;

Control; The patella was returned to its position and the operation wound was closed without any intervention. Sterile saline of 0.15 ml was injected intra-articularly immediately after the operation and 5 times at 10 days intervals.

HA Group; The patella was returned to its position and the operation wound was closed without any intervention. Hyaluronic acid of 0.15 ml (Ostenil Plus 20 mg/ml, Bio-Gen, Ankara) was injected intra-articularly immediately after the operation and 5 times at 10 days intervals.

MF Group; Microfracture holes of 0.25 mm diameter and 1 mm depth were created on each trochlea using a Kirschner wire (Figure 1c). Sterile saline of 0.15 ml was injected intra-articularly immediately after the operation and 5 times at 10 days intervals (8, 9).

MF+HA Group; Microfracture holes of 0.25 mm diameter and 1 mm depth were created on each trochlea using a Kirschner wire (Figure 1c). Hyaluronic acid of 0.15 ml (Ostenil Plus 20 mg/ml, Bio-Gen, Ankara) was injected intra-articularly immediately after the operation and 5 times at 10 days intervals.

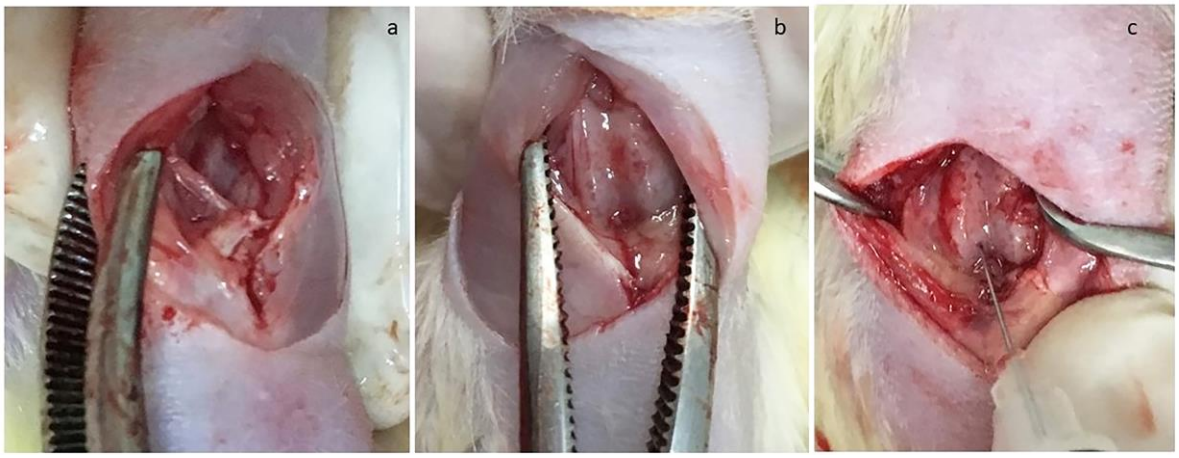

Figure 1. Reaching the genu joint with parapatellar incision (a), exposing the femoral condule and sulcus trochlea (b), creating holes with Kirschner wire to create the microfracture technique (c)

Clinical, Radiological and Macroscopic Evaluations

All clinical and radiological examinations in craniocaudal and mediolateral projection were performed before and after the operation on the 1st day and 10 days apart until the 50th day, just before intra-articular injections. Clinically, body weight and knee circumference were measured and, leg posture was scored. The scoring system (0= normal; 1= curling of toes; 2= eversion of foot; 3= partial weight bearing; 4= non-weight bearing and guarding; and 5= licking and/or avoiding contact with the limb) developed by Sluka et al. (10) was used to evaluate the leg posture. The degree of osteoarthritis based on the radiological examination findings, was evaluated using the scoring system developed by Evans et al. (11). There are 3 parameter as joint space narrowing, subchondral bone sclerosis, osteophyte formation that are graded as Normal, Mild, Moderate or, Severe).

At the end of the trial, all rats were euthanized with a high dose of anesthetic drug on the postop 50th day. Then the joint of the relevant leg was dissected and photographed and scored according to the severity of osteoarthritis (Normal, Mild, Moderate, Severe) using a scoring system developed by Bennet et al. (12).

Statistical Analysis

Statistical evaluation of the data was performed using the SPSS 22 statistical package program (Inc., Chicago, II, USA).

Since knee circumference and body weight values are measurable parametric tests, One Way – ANOVA test was used. If necessary, pairwise comparisons were made using the Post hoc (Duncan) test. Kruskal-Wallis test was used as it is nonparametric tests for Scoring of leg posture, joint space narrowing, subchondral bone sclerosis and osteophyte formation. If necessary, pairwise comparisons were made using the Mann-Whitney U test.

RESULTS

In the present study, body weight was not affected by inducing OA, it increased gradually and similiarly in all groups and no difference was found between the control group and the treatment groups. However, the development of osteoarthritis was successfully achieved with MIA application considering the changes in the knee circumference measurement, the leg posture score, and the radiological findings.

The knee circumference increased up to 0.5 cm in all groups on the 10th day after the operation, then gradually decreased in all groups similiarly. Leg posture score was increased on the 10th day after the operation, then gradually decreased compared to the other measurements, in all groups except control. The variation of leg posture score according to trial days and groups is presented in Table 1.

Table 1. Leg posture scoring

Day / Groups	1		10		20		30		40		50	
	n	$\overline{X} \pm S_{\overline{x}}$	n	$\overline{X} \pm S_{\overline{x}}$	n	$\overline{X} \pm S_{\overline{x}}$	n	$\overline{X} \pm S_{\overline{x}}$	n	$\overline{X} \pm S_{\overline{x}}$	n	$\overline{X} \pm S_{\overline{x}}$
MF	9	2.60 ± 0.18	8	3.75 ± 0.17	8	2.90 ± 0.12^{b}	8	2.50 ± 0.19^{b}	8	1.75 ± 0.16^{b}	7	1.60 ± 0.20^{b}
MF+HA	9	2.70 ± 0.17	8	3.90 ± 0.12	6	3.20 ± 0.17^{bc}	6	2.33 ± 0.21^{b}	6	2.20 ± 0.31^{b}	6	2.20 ± 0.31^{b}
HA	9	2.70 ± 0.17	8	3.63 ± 0.18	7	3.14 ± 0.14^{bc}	7	2.43 ± 0.20^{b}	7	2.00 ± 0.31^{b}	7	1.60 ± 0.20^{b}
Control	9	2.70 ± 0.17	7	3.71 ± 0.18	7	3.60 ± 0.20^{ac}	7	3.30 ± 0.18^{a}	7	3.30 ± 0.18^{a}	7	3.30 ± 0.18^{a}
P	-		-		*		*		**		***	

a,b,c: Differences between means with different letters in the same column are significant. (*: P < 0.05; **: P < 0.01; ***: P < 0.001).

Radiologically, the joint space narrowing score of all groups increased (Figure 2 and 3) on the 10th day after the operation in this study. At the end of the trial, the scores of all treatment groups were similarly significantly lower than the control group. However, the MF and HA group was showed significantly lower scores earlier than the other 2 groups (P <0.05) (Table 2).

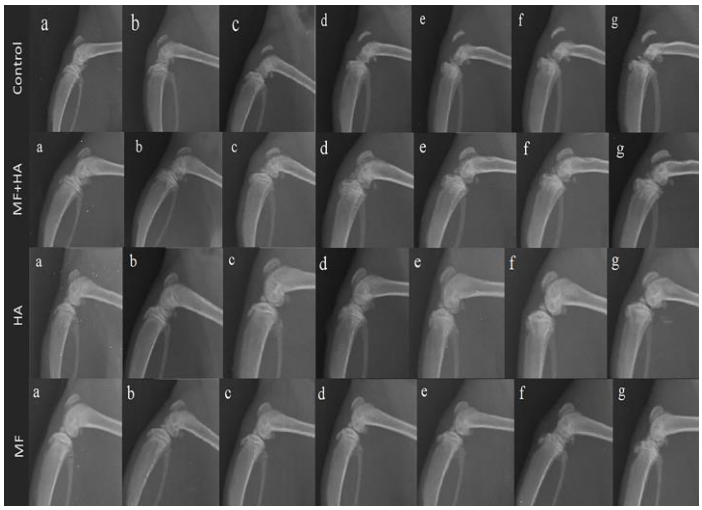

Figure 2. X-ray evaluation of a rat randomly selected from each group in medio-lateral position. (a) before MIA application; (b) 14th day after MIA; (c) postop 10th day; (d) postop 20th day; (e) postop 30th; day; (f) postop 40 day; (g) postop 50th day.

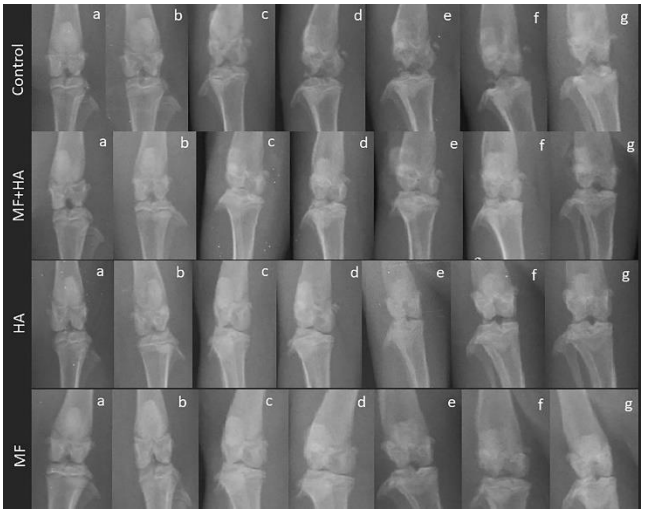

Figure 3. X-ray evaluation of a rat randomly selected from each group in antero-posterior position. (a) before MIA application; (b) 14th day after MIA; (c) postop 10th day; (d) postop 20th day; (e) postop 30th; day; (f) postop 40 day; (g) postop 50th day.

Subchondral sclerosis was scored significantly lower in both MF and HA group in all measurement then other 2 groups as seen in Table 3. MF group showed a significant difference to the control group in terms of osteophyte scoring throughout the whole trial (P <0.05). However, the values of the MF+HA group were similar to the control group in all measurements (Table 4).

Macroscopic findings, revealed that the MF and HA groups were similar and significantly lower than the other 2 groups (P <0.05). Although the mean scores of the MF+HA group were lower than the control (Figure 4).

Table 2. Scoring for narrowing of joint space

Day / Groups	1		10		20		30		40		50	
	n	$\bar{X} \pm S_{\bar{X}}$	n	$\bar{X} \pm S_{\bar{X}}$	n	$\bar{X} \pm S_{\bar{X}}$	n	$\bar{X} \pm S_{\bar{X}}$	n	$\bar{X} \pm S_{\bar{X}}$	n	$\bar{X} \pm S_{\bar{X}}$
MF	9	1.00 ± 0.00	8	1.40 ± 0.18^{b}	8	1.25 ± 0.16^{b}	8	1.40 ± 0.18^{b}	8	1.40 ± 0.26^{b}	7	1.30 ± 0.36^{b}
MF+HA	9	0.80 ± 0.15	8	1.90 ± 0.12^{a}	6	2.00 ± 0.00^{ac}	6	2.20 ± 0.17^{ac}	6	2.20 ± 0.31^{bc}	6	2.20 ± 0.31^{bc}
HA	9	1.00 ± 0.00	8	1.90 ± 0.12^{a}	7	1.71 ± 0.18^{bc}	7	1.71 ± 0.18^{bc}	7	1.30 ± 0.36^{b}	7	1.30 ± 0.36^{b}
Control	9	1.00 ± 0.00	7	2.00 ± 0.00^{a}	7	2.43 ± 0.20^{a}	7	2.60 ± 0.20^{a}	7	2.60 ± 0.20^{ac}	7	2.60 ± 0.20^{ac}
P		-		*		*		**		*		*

a,b,c: Differences between means with different letters in the same column are significant (*: P < 0.05; **: P < 0.01).

Table 3. Sclerosis scoring in subchondral bone

Day / Groups	1		10		20		30		40		50	
	n	$\bar{X} \pm S_{\bar{X}}$	n	$\bar{X} \pm S_{\bar{X}}$	n	$\bar{X} \pm S_{\bar{X}}$	n	$\bar{X} \pm S_{\bar{X}}$	n	$\bar{X} \pm S_{\bar{X}}$	n	$\bar{X} \pm S_{\bar{X}}$
MF	9	1.00 ± 0.00	8	1.00 ± 0.00^{b}	8	1.13 ± 0.12^{b}	8	1.00 ± 0.27^{b}	8	1.00 ± 0.33^{b}	7	1.30 ± 0.36^{b}
MF+HA	9	0.60 ± 0.18	8	1.90 ± 0.12^{ac}	6	2.00 ± 0.26^{ac}	6	2.20 ± 0.31^{ac}	6	2.33 ± 0.33^{ac}	6	2.33 ± 0.33^{a}
HA	9	0.33 ± 0.17	8	1.25 ± 0.16^{b}	7	1.43 ± 0.20^{bc}	7	1.30 ± 0.29^{bc}	7	1.30 ± 0.36^{bc}	7	1.30 ± 0.36^{b}
Control	9	0.90 ± 0.11	7	1.43 ± 0.20^{bc}	7	2.00 ± 0.22^{ac}	7	2.43 ± 0.20^{a}	7	2.60 ± 0.20^{a}	7	2.60 ± 0.20^{a}
P	-		**		*		**		**		*	

a,b,c: Differences between means with different letters in the same column are significant (*: P < 0.05; **: P < 0.01).

Tablo 4. Osteophyte scoring

Day / Groups	1		10		20		30		40		50	
	n	$\bar{X} \pm S_{\bar{X}}$	n	$\bar{X} \pm S_{\bar{X}}$	n	$\bar{X} \pm S_{\bar{X}}$	n	$\bar{X} \pm S_{\bar{X}}$	n	$\bar{X} \pm S_{\bar{X}}$	n	$\bar{X} \pm S_{\bar{X}}$
MF	9	1.00 ± 0.00	8	1.00 ± 0.00^{b}	8	1.13 ± 0.12^{b}	8	1.40 ± 0.18^{b}	8	1.50 ± 0.19^{b}	7	1.60 ± 0.20^{b}
MF+HA	9	1.00 ± 0.00	8	1.63 ± 0.18^{ac}	6	2.00 ± 0.36^{ac}	6	2.20 ± 0.40^{bc}	6	2.33 ± 0.33^{ac}	6	2.33 ± 0.33^{bc}
HA	9	1.00 ± 0.00	8	1.25 ± 0.17^{bc}	7	1.60 ± 0.20^{bc}	7	1.60 ± 0.20^{b}	7	1.60 ± 0.20^{bc}	7	1.60 ± 0.20^{b}
Control	9	1.00 ± 0.00	7	1.60 ± 0.20^{ac}	7	2.14 ± 0.26^{ac}	7	2.43 ± 0.20^{ac}	7	2.60 ± 0.20^{a}	7	2.60 ± 0.20^{ac}
P	Ö.D		*		*		*		*		*	

a,b,c: Differences between means with different letters in the same column are significant (*: P < 0.05).

Figure 4. Macroscopic photographs of a randomly selected rat from each group. (a) Control; (b) MF+HA group; (c) HA group; (d) MF group.

DISCUSSION AND CONCLUSION

Present study aimed to investigate the treatment efficiency of microfracture technique and intra-articular sodium hyaluronate injection separately and in combination in the rats generalized osteoarthritis induced experimentally. Effectiveness of intra-articular MIA solution application in inducing osteoarthritis in rats has been shown by previously studies (13, 14, 15). Therefore, MIA-induced OA was preferred as an experimental model. Considering the changes in the leg posture, the knee circumference measurements and the radiological findings, the development of osteoarthritis was successfully achieved with MIA application in this study.

While osteoarthritis is generally seen in young animals due to trauma, in geriatric animals it usually occurs due to old age. Arthroscopic methods are one of the most preferred applications among today's treatment options for osteoarthritis treatment. Arthroscopic micro-fracture technique is more preferred than other methods in terms of being single-stage, cost-effective and working with minimal surgical equipment. Intraarticular hyaluronic acid injections are also

used alone or in addition to osteoarthritis treatment. However, there is still no permanent treatment option for osteoarthritis. (7). In the literature review, it was determined that positive results were obtained from microfracture and HA applications individually and it was hypothesized that higher success could be achieved by combining the two (16, 17, 18).

The stimulating effect of body weight gain on osteoarthritis development is known (7). However, the effect of developing osteoarthritis on body weight gain is not fully known. In this study, body weight increased gradually and similiarly in all groups and no difference was found between the control group and the treatment groups. Similar to our results, it has been previously shown that osteoarthritis induced by MIA injection or meniscectomy does not affect body weight gain (19). However, there are also studies showing the opposite (20, 21). MIA-induced OA was also used in these studies, but different treatments were applied and this opposition may be attributed to differences in treatment.

In the present study, the leg posture score was increased on the 10th day after the operation, then gradually decreased compared to the other measurements, in all groups except the control. The microfracture group was significantly lower than the other 3 groups on the 20th day, and the control group was significantly higher than the other 3 groups on the 30th day (*: $P < 0.05$). This significant difference continued on the 40^{th} and 50^{th} days. ($P < 0.01$; $P < 0.001$, respectively). In addition, although it was not statistically significant, it was observed that HA and MF group scores were lower than the other 2 groups at the end of the experiment. In a study investigated that when the severity of osteoarthritis depending on dosage of bradykinin and treatment efficiency of various hyaluronic acid formulations by the scoring in gait pattern, it was seen that gait scoring values increased with increasing bradykinin dose and decreased with hyaluronic acid application comparing the control groups (22). In one study, in various gait analyzes performed on days 14, 28, and 56, no significant difference was found between the left knee receiving saline and the right knee receiving MIA. However, in macroscopic evaluations performed on the same days, ulcers on the tibial plateau, severe damage to the cartilage and fibrillation were observed on the 28^{th} day in the right knee with osteoarthritis. On the 56^{th} day, these lesions were observed to reach the subchondral bone, while no pathology was found in the saline group (13). Accordingly, it can be interpreted that postural scoring is not reliable enough to predict the actual damage caused by OA. Even so, the macroscopic findings at the end of the experiment were consistent with clinical and radiological findings in this study.

Osteoarthritis can cause the knee circumference to increase up to 1 cm due to inflammation in rats. (23, 24). Likewise, it is stated that there is an increase in the measurement of knee circumference due to OA in humans (25). In this study, the knee circumference increased up to 0.5 cm in all groups on the 10th day after the operation, then gradually decreased compared to the other measurements, but there was no difference between the groups. Similiarly, using platelet-rich plasma and hyaluronic acid in humans, similar findings were obtained at the 2nd, 6th and 12th months in the evaluations made in terms of joint mobility and knee circumference in middle-aged and moderate OA patients (25). In another study in rats reported that the knee joint swelling circumference reduced similarly by individual treatments with methotrexate and bee venom (23). Considering these results, it may be attributed that the treatment methods used in these studies affect the inflammation process similarly.

Radiological examinations of the present study showed that treatment with MF or HA revealed lower joint space narrowing scores earlier the other 2 groups and better subchondral sclerosis scores. MF group showed a significant difference to the control group in terms of osteophyte scoring throughout the whole experiment. It was interpreted that these findings indicated that the treatment efficiency was better. In a study on MIA induced osteoarthritis in rats, the degenerative changes in the bone started on the 21st day after the application was observed and, a significant degenerative change occurred at the end of the 28th day, macroscopically (26). Scoring macroscopic findings, it was determined that the MF and HA groups were similar and significantly lower than the other 2 groups ($P < 0.05$). Although the mean scores of the MF+HA group were lower than the control, the difference was statistically insignificant.

There are various studies evaluating the effectiveness of hyaluronic acid in the treatment of osteoarthritis created by different methods. A study working on MIA-induced OA in rats compared HA, daidzein and their combination and found the daidzein treatment promising for OA, especially in combination with HA (16). In another study, the lubricin and lubricin+ hyaluronic acid groups were found superior than the hyaluronic acid and control groups, and the hyaluronic acid group was better than the control group following anterior cruciate ligament rupture in rats (18). It was determined that when the treatment groups consisted of microfracture method alone and, then one-time hyaluronic acid or hyaluronic acid containing growth factor (TGF-3), the microfracture group alone filled the defect area less than the other 2 groups in another study (27). However, in the presented study, the healing results obtained from microfracture alone or HA application alone were similar and that the combination of microfracture and HA application gave no better results. On the other hand, there have been other studies that did not get the expected results from hyaluronic acid application. In a study performed by creating defects in rabbits, it was observed that some groups treated with HA had better results in the evaluation during three months, but there was no difference between the groups at the end of 6 months (28). In another study where rabbits were followed for six months, it was observed that the microfracture group and the microfracture + HA group were significantly better than the control group, but there was no significant difference between them (17).

As a result, considering the various studies conducted, it was seen that the results obtained by combining only microfracture technique application and microfracture technique with hyaluronic acid or other alternative applications may be variable. In line with the findings obtained from the

presented study, it was concluded that combining microfracture technique with HA application would not contribute additionally to the treatment process, and even using microfracture or HA application alone may produce more positive results.

ACKNOWLODGMENT

This paper summarized from the first author's MSc thesis**.** This study supported by Adnan Menderes University, Scientific Research Foundation. The study was approved with the session of ADÜ-HADYEK dated February 25, 2016 and numbered 64583101/2016/63.

CONFLICT OF INTEREST

The authors declare no financial or other conflicts related to this report.

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✉ **Corresponding Author:**
Zeynep BOZKAN
Department of Surgery, Faculty of Veterinary Medicine, University of Aydin Adnan Menderes, Aydin, TURKEY
E-posta: zbozkan@adu.edu.tr