Laser Therapy in Knee Osteoarthritis: An Experimental Study

Diz Osteoartritinde Lazer Tedavisi: Deneysel Bir Çalışma

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ABSTRACT

Aim: In this study, it is aimed to examine the effects of Infrared Gallium-Arsenic (Ga-Ar) laser therapy in rat osteoarthritis (OA) model that is formed chemically on the knee joint cartilage.
Material and methods: Fifty-six female Wistar Albino rats having weights between 250 and 300 grams were used and the rats were divided in 3 groups. Group 1 and group 2 are the control (C) groups. In group 1 (C-1), 0.9% saline solution was aplied to the left knee joint of 32 rats and in group 2 (C-2), 1 mg monoiodoacetate (MIA) is applied to the right knee joint of the same 32 rats. Group 3 is the experimental (E) group and in this group, 1 mg MIA application to the right knees of 24 rats. Ga-Ar laser therapy was started after 24 hours for group 3 and it was applied for 15 days with 24-hour intervals. In all 3 groups, knee bending test was applied daily. Euthanasia was applied on 1st,7th and 15th days to 8 rats in C-1 and C-2 and on 7th, 15th and 30th days to 8 rats in group E. Their knee joints were removed, and they were histopathologically assessed under a light microscope.
Results: As a result of the statistical assessment, in the assessment between the total scores between C-1 and C-2 groups, scores of the C-2 group were found higher at all the time points (p=0.001, p=0.002). Also when the E and C-2 groups were compared, no statistically significant difference was observed (p>0.05). There was no correlation
between the pain assessment of knee bending test and the level of histopathological findings.
Conclusions: We found no effect of laser therapy in the treatment of early knee osteoarthritis.

Key words: Osteoarthritis; rats, wistar; laser therapy, knee joint

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ÖZET

Amaç: Bu çalışmada Infrared Gallium-Arsenic (Ga-Ar) laser tedavisinin, ratların dizlerinde kimyasal olarak oluşturulan osteoartrit modelindeki etkinliği araştırıldı.
Gereç ve yöntem: Çalışmada ağırlıkları 250 ile 300 gr arasında değişen 56 Wistar Albino rat kullanıldı. Ratlar 3 gruba ayrıldı. Grup 1 ve 2 kontrol grubu olarak belirlendi. Grup 1 ve grup 2 deki 32 ratın sol dizlerine %0.9 luk salin solüsyonu (C-1), sağ dizlerine 1 mg monoiodoacetate solüsyonu (MIA) (C-2) uygulandı. Grup 3 deneysel grup olup bu gruptaki 24 ratın dizine 1 mg MIA solusyonu uygulandı. Grup 3 deki ratlar için 24 saat sonra Ga-Ar laser tedavisi uygulaması başlandı ve 24 saat arayla 15 gün uygulandı. Her 3 gruptaki ratlara günlük diz bükme testi uygulandı. C-1 ve C-2 grubundaki 8 er rata 1,7,15 ve 30. günlerde, deney grubundaki 8 er rata 7,15,30. günlerde ötonazi uygulandı. Diz eklemleri çıkarılarak ışık mikroskobu ile histopatolojik olarak incelendi.
Bulgular: İstatiksel değerlendirmede C-2 grubunun skorları tüm zamansal noktalarda C-1 grubuna göre yüksek çıktı (p=0.001, p=0.002). Deney grubu ile C2 grubu karşılaştırıldığında ise, istatiksel olarak anlamlı fark bulunamadı (p>0.05). Diz bükme testi ile oluşan ağrı ile histopatoljik değişikliklerin seviyesi arasında korelasyon bulunamadı.
Sonuç: Laser tedavisinin erken dönem diz osteoartrit tedavisinde etkinliği bulunamadı.

Anahtar Sözcükler: Osteoartrit, rat, wistar, laser tedavisi, diz eklemi

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INTRODUCTION

Osteoarthritis (OA) is a degenerative joint disease characterized by erosion in joint cartilage, bone hypertrophy in joint ends, subchondral sclerosis, various biochemical and morphological changes in the synovial membrane and joint capsule that are frequently seen in elders (1). There is no specific and radical single treatment method that exactly stops or suspends the process of OA (1,2).

Physical treatment methods are especially used in osteoarthritis of large joints such as hips, knee and in vertebrae (3). Besides hot and cold compresses, deep heating modalities such as ultrasound, short-wave diathermia and analgesic modalities such as transcutaneous electrical neurostimulation, diadynamic current etc. and also other methods such as acupuncture, hydrotherapy and fluidotherapy, pulsatile electromagnetic area and laser are among the physical treatment methods that are used for this purpose.

Laser is a safe and easily applicable method and there are clinical and experimental studies showing that it is efficient in OA treatment (4,5,6,7). Laser beams increase the collagen production and they stimulate tissue regeneration. Due to its effects on microcirculation and local tryphic activity, it has an anti-inflammatory effect and a significant analgesic effect (4).

By this experimental study, we aim to assess the efficacy of the laser application on joint histopathology and pain in the treatment of early period OA on Wistar albino rats.

METHODS

After taking the approval of ethics committee (5 Feb 2010; number: 10/5-K), study was performed on 7-week-old 56 Wistar Albino rats having a weight between 250 and 300 g. They are placed in solid-surface plastic cages that are designed to have the rats reach the standard laboratory food and water as much as they like. Animals were kept in a 12:12-h light–dark cycle at a temperature-controlled room 22°C. Food intake of the rats was released 2 hours before the anesthesia application.

Intraperitoneal anaesthesia applied with 0.2 mg/kg %2 xylazine and 0.5 mg/kg %10 ketamine to 32 rats that were selected as the control group (C1, C2). Then, a single dose of 50 microliters 0.9% saline solution was applied to the left knees (C-1) and intrarticular MIA was applied to their right knees (C-2).

Like C-2 group, standard single dose of 1 mg MİA (Sigma) was dissolved in 50 microliters of 0.9% saline solution and it was applied to 24 rats that were selected as the experimental group (E) via a hamilton injector (E). After 24 hours, for experimental group, 240 sec/day laser application was performed transcutaneously at room temperature in the laboratory for 15 days with 24-hr intervals. For laser therapy; Elettronica Pagani branded (Roland series IR 27 Ga-As, wave length 904 nm, frequency interval wavelength 5-7.000 Hz, divergence 30°, beam area 0.1 cm^2 laser device was used. The device was adjusted at averagely 7.2 mW output power at 3000 Hz frequency in an uninterrupted mode in order to have the energy intensity per point as 4.3 J/cm2.

Knee Bending Test

Starting 24 hours after the MIA and SF injections of the rats; behaviours regarding pain were recorded via assessing with knee bending test as 1 times/day for 30 days. Assessments were performed every day before the laser application.

Knee bending test: It is a pain assessment test that includes 5 flexion movements and 5 extention movements and allows to make a scoring based on the type of animal response (exerting/audio response-squealing)

Grade 0: There is no response to the flexion or extension of the joint.

Grade 0.5: Exerting in the animal at maximal flexion/extension.

Grade 1: Exerting occurs via mild flexion/extension and audio response is taken with maximal flexion/extension.

Grade 2: Audio response (squealing) occurs at mild manipulation (flexion/extension).

Total of the responses obtained with 5 flexion and 5 extension movements (score of the knee bending test is maximum 20) shows the nociception level of the animal (8).

Histopathological Assessment

Euthanasia was applied to 8 rats in C-1 and C-2 on the 1st, 7th, 15th and 30th days and to 8 rats in E group on 7th, 15th and 30th days and their right knee joints were removed. Femorotibial joint was fixed in a 10% formal and then decalcified. Then it was exposed to routine histological processes and the samples were blocked in paraffin. Via a rotary microtome, the sections taken at a thickness of 5 µm were stained by hematoxylene eosine and toluidine blue.

Histopathological findings in the joint were assessed in a light microscope by using a scoring system (9) in terms of the below mentioned characteristics by a histopathologist who was blind to the experiment protocol.

Scoring scale for lesions of rat stifle (9)

Structure
0 = Normal
1 = Slight surface erosion or flaking of superficial zone
2 = Erosion no deeper than superficial zone
3 = Erosion into middle zone with or without fissuring
4 = Erosion into deep zone with or without fissuring
5 = Erosion into calcified zone
6 =Erosion into the subchondral bone (eburnation)
7 = Fibrous tissue on eburnated areas

Tidemark
0 = Normal
1 = Touched by blood vessels
2 = Crossed by blood vessels

Doubling of tidemark
0 = Normal (a basophilic line)
1 = Doubled

Metachromatic staining
0 = Normal
1 = Increased in all layers of articular cartilage
2 = Significantly decreased or no deeper than superficial zone
3 = Significantly decreased or absent, no deeper than middle zone
4 = Significantly decreased or absent no deeper than tidemark
5 = No staining at all

Chondrocyte morphology
0 = Normal
1 = Enlarged cells close to the surface of articular cartilage
2 = Hypercellular with or without small clones
3 = Noticeable hypocellularity with or without clones
4 = Significant hypocellularity with or without clones
5 = Severe hypocellularity

Osteophyte formation (0–2)
0 = None
1 = Extensive mix tissue formation and remodeling at joint margin
2 = Osteophyte

Synovitis (0–4)
0 = Norma1 (1- to 3-cell-thick synovium and few mononuclear cells in subintima)
1 = Slight increase in number of synoviocytes and mononuclear cells
2 = Mononuclear cell infiltration and hyperemic blood vessels
3 = Hyperplastic synovium
4 = Extensive hyperplasia with pannus formation

Statistical Evaluation

SPSS 16.0 statistical package programme was used for the statistical analysis. Firstly, normal distribution of the data was examined via the Shapiro-Wilk test and non-parametric methods were used in the analysis of data that did not comply with the nomal distribution. Median of double data that did not distribute normally were compared by “Mann-Whitney U Test” and the median of triple data was compared by Kruskal-Wallis. Because the data did not distribute normally, correlations were performed via Spearman correlation analysis. Findings were accepted as significant at $p \leq 0.05$ level.

RESULTS

In C-1 group joint histopathology; calcified and noncalcified sections of distal femur and proximal tibia joint cartilages are separated by basophilic tidemark (wavy hematoxylie - stained line demarcating junction of calcified and noncalcified layers of cartilage). In the distal femur condyle, thickness of calcified and noncalcified cartilages was similar but in some areas, especially in the non-bearing areas of the joint, calcified cartilage was slightly thicker. Noncalcified joint cartilage was morphologically divided in a superficial tengential layer, mid-layer and a deep layer. Distal femur and proximal tibia joint cartilage surface has a thin eosinophil line on which superficial fibrillations can be seen rarely. Cartilage matrix was stained denser in the mid and deep layers. Blood vessels did not pass either from the tidemark on the distal part of the femur or from the tidemark on the proximal side of tibia. Superficial condrocytes in the joint cartilage were generally in the form of a shuttle. This was more in the foreground of proximal side of tibia. Remaining part of noncalcified cartilage consisted of circular chondrocytes that were organized in columns adjacent to the tidemark. Pillarization on the distal side of the femur was less significant than the pillarization on the proximal side of tibia excluding the thick joint cartilage of the condyle. Chondrocytic clones were rarely obserbed on the femur and tibia mid layer. This morphology did not change from the 1st day till the 30th day.

In C-2 group joint; when compared to C-1, statistically insignificant limited grade of synovitis was observed on the first day. Cartilage fibrillations, cartilage erosion, synovitis and metachromatic staining loss had become evident as of the 7th day and reached the peak level on the 30th day (Figure 1). This change was found statistically significant. The presence of a fibrous tissue instead of a joint cartilage had started to be seen on the 15th day and it became clear on the 30th day. Sclerotic changes such as the formation of a myxomatous tissue in the subchondral bone were started to be rarely seen on the 15th day and they were observed more significantly on the 30th day. As the same, the invasion of the vessels derived from the subchondral bone to the noncalcified cartilage tissue by passing through the tidemark had started to be seen on the 15th day and became significant on the 30th day. The limited synovitis seen on the 1st day emerged as a picture in which the inflammatory cells were concentrated on the 7th day, the density increased on the 15th day and hyperplastic on the 30th day. In some sections, pannus formation, common hyperplasia were observed (Figure 2). Osteophitis formation was observed on some of the joint sections on the 15th day and the ratio on the 15th day did not change on the 30th day. Metachromatic staining loss that was started to be seen as of the seventh day was observed as a complete loss around the cartilage lesions on the 30th day (Figure 3).

In the experimental group, similar changes as in the C-2 group were observed (Figure 4). Despite the strength of the above mentioned pathological changes were less in the experimental group than the C-2 group on 7th, 15th and 30th days, no statistically significant difference was found.

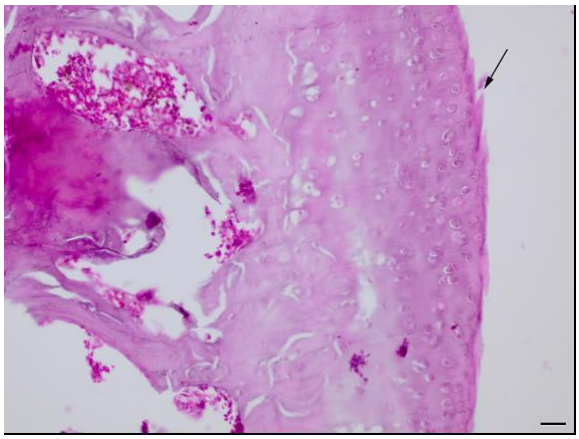

Figure 1. Erosion of the superficial layer on the joint cartilage. Limited superficial erosion (shown with an arrow) is shown in the joint cartilages of the rats in both group C-2 and group E. Group C-2, 7th day; proximal tibia; Hematoxylene&eosine staining; bar= 80 µm.

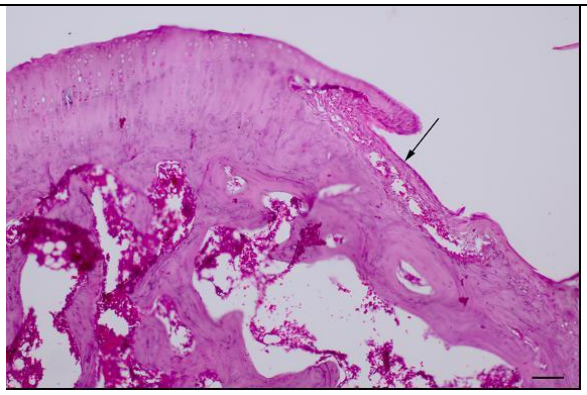

Figure 2. Pannus formation. Seen in both group C-2 and group E. Group C-2 30th day; proximal tibia; Hematoxylene&eosine staining; bar=140 µm.

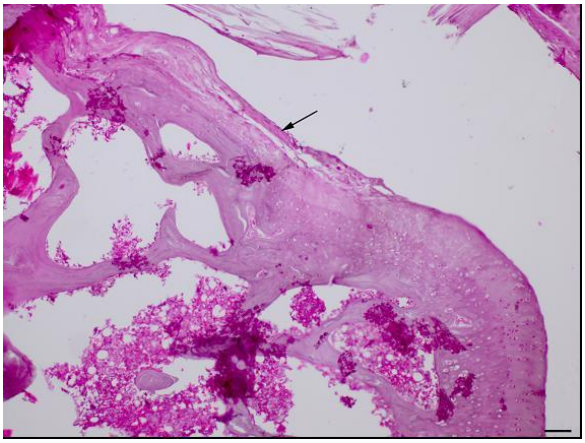

Figure 3. Invasion of hyalene joint cartilage tissue by the fibrous tissue. Fibrous structures (arrows) are observed instead of the hyalene joint cartilage on the 15th and 30th days. Group C-2 30th day; proximal tibia; Hematoxylene&eosine staining; bar=140 µm.

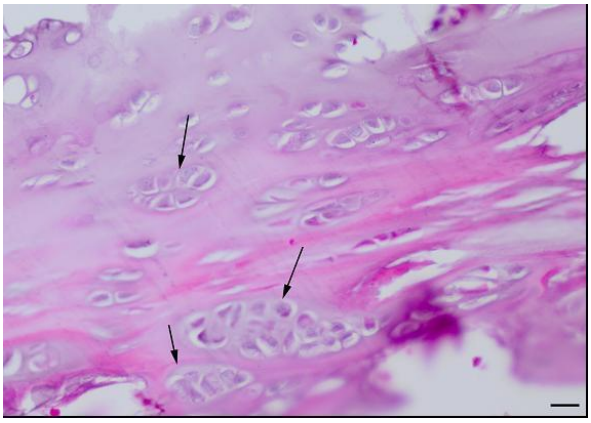

Figure 4. Chondrocytic clones. One of the efforts given by the chondrocytes for compensation is the development of clone (isogen group) formation (shown with arrows). It is seen in both group E and only in group C-2. Group E 30th day; proximal tibia; Hematoxylene&eosine staining; bar=45 µm.

When the general morphology was assessed in the scoring system, the difference in the comparison of three groups was found statistically significant ($p<0.001$). No statistically significant difference is found on the 1st day between C-1 and C-2 groups ($p>0.05$) and as of the 7th and 15th days, morphology scores are found statistically significant in C2 group when compared to C1 ($p<0.001$) . On the 30th day, the difference between the C-1 and C-2 groups are observed as statistically significant ($p=0.001$). When E group and C-2 group are compared, no statistically significant difference is observed ($p>0.05$).

When the sections to which metachromatic staining is applied, the difference between the comparison of three groups is found statistically significant (p<0.001). Between C-1 and C-2 groups, while no statistically significant difference is observed on the 1st day (p>0.05), as of the 7th day, scores are found statistically significantly higher in C2 group when compared to the C1 group (p=0.002). When E group and C-2 group are compared, no statistically significant difference is observed (p>0.05).

In chondrocyte morphology, the difference in the comparison of three groups is found as statistically significant (p<0.001). In C2 group, chondrocyte scores are found statistically significant higher on the 1st-7th-15th-30th days when compared to C1 group (1st day p=0.018, on 7th-15th-30tg days, p=0.001). When E group and C-2 group are compared, no statistically significant difference is observed (p>0.05).

In osteophyte assessment; no statistically significant difference is observed on the 1st day between C-1 and C-2 groups (p>0.05) and on 7th-15th-30th days, scores of group C2 are found statistically significantly higher than group C 1(on the 7th day, p=0.025, on the 15th day, p=0.01, on the 30th day, p=0.027). When E group and C-2 groups are compared, no statistically significant difference is observed (p>0.05).

In synovitis assessment, scores of group C-2 are found statistically significantly higher on 1st-7th-15th and 30th days when compared to group C-1 (p=0.001). When E group and C-2 groups are compared, while no statistically significant difference is found on 7th and 30th days (p>0.05), the scores of E group on 15th day are found statistically significantly lower (p=0.086).

When total scores are asessed, the difference in the comparison of three groups is found statistically significant (p<0.001). Scores of C-2 group are found statistically significantly higher than C1 on 1st, 7th, 15th and 30th days (p=0.002, p=0.001, p=0.001, p=0.001). When E group and ile C-2 groups are compared, no statistically significant difference is observed (p>0.05) (Table 1).

Table 1. Total score.

	Control (Left knee) (n=8)	Control (Right knee) (n=8)	Experiment (Right knee) (n=8)	p*	p⁺	p†
Day 1	2 (2-3)	6 (5-6)			0,002	
Day 7	1.5 (1-2)	12 (9.5-12.75)	10.5 (9.25-11)	<0.001	0,001	>0.05
Day 15	1.5 (0-2)	14 (11.5-20)	12.5 (10.25-14)	<0.001	0,001	0,310
Day 30	1.5 (1-2.75)	19 (14-21.5)	17 (14.5-18)	<0.001	0,001	>0.05

*C-1, C-2, E comparison
⁺ C-1, C-2 comparison
† C-2, E comparison

In control and experimental groups, knee bending test scores between 4th and 28th days are determined as 0. Among the control groups to which knee bending test is applied (C-1,C-2); a significant difference is found in the statistical assessment performed on 1st, 2nd, 3rd and 4th, 28th, 29th, 30th days (p<0.001, p=0.01). While a statistically significant difference is observed on the 2nd and 3rd days between C-2 and E groups (p<0.001, p=0.008), no significant difference is found on other days (p>0.05) (Table 2).

Table 2. Knee bending test scores

	Control (left knee) (n=8)	Control (Right knee) (n=8)	Experiment (Right knee) (n=8)	p⁺	p†
Day 1	3 (2-3)	8 (8-10)	8 (8-10)	<0.001	>0.05
Day 2	0 (0-0)	8 (8-8)	6 (6-8)	<0.001	<0.001
Day 3	0 (0-0)	4 (4-6)	3 (2-4)	<0.001	0,008
Day 4	0 (0-0)	0 (0-1.5)	0 (0-2)	0.01	>0.05
Day 28	0 (0-0)	4.5 (0-5.75)	5 (0-5.75)	0.01	>0.05
Day 29	0 (0-0)	4.5 (0-5.75)	5 (0-5.75)	0.01	>0.05
Day 30	0 (0-0)	4.5 (0-5.75)	5 (0-5.75)	0.01	>0.05

⁺ C-1, C-2 comparison
† C-2, E comparison

DISCUSSION

Animal models play a complementary role in explaining the complex interaction of factors in OA etiopathogenesis and treatment. Animal models are frequently used in observing the effects of treating agents and disclosing the occurrence mechanisms of the disease. In our study, we aim to investigate the effect of laser therapy in knee OA model in the rats.

OA developed by MIA in the rat knee joint is a useful model with significant advantages (8,10). Potential areas of usage of this model are the assessment of the agents designed to inhibit the acute matrix degeneration, the situations in which chondrocytes are not completely eliminated, repair induction and the assessment of the effect of agents regarding the pain and walking changes. Because osteophyte formation is a foreground characteristic of this model, this model can be used in the studies regarding the induction and inhibition of the formation of osteophytes (8,11,).

One of the properties of hyaline cartilage is its best staining with metachromic stains such as Safranin-O, Alcian-blue, Toluidine blue. These metachromic stainings stain GAG and proteoglycans. Thus the loss in metachromic staining shows the GAG and proteoglycan loss (12,13). In our study, we prefer Toluidine blue for metachromic stainings. In the groups to which MIA is applied, especially starting from the 7th day, we have observed metachromasia loss and thus GAG and proteoglycan loss in the joint cartilage. In the group to which laser is applied, despite the metachromasia loss is less than the group to which only MIA injection is applied, this difference is not found statistically significant.

In our study, a joint cartilage destruction has developed as similar to osteoarthritis in the rat knee joint to which MIA is applied. When the knee joins in all three groups are compared by histopathologic scoring system; while the difference between group C-1 and group C-2 to which MIA is applied is found statistically significant, the difference between group C-2 to which MIA is applied and group E to which laser therapy is applied is not found statistically significant.

Although the clinical studies show that laser therapy is effective in reducing pain in OA (14,15,16,17,18), therapeutical effect cannot be exactly approved (16). Chondroprotective effects and inhibition of cartilage degradation after laser-irradiation treatment were observed in some model of OA in rats (7,19). Arves et al. have shown that laser therapy has reduced inflammation in the injury model developed in rats (20). Carlos et al. have shown that it has reduced inflammation in czymosan-induced acute arthritis developed in rats (21). Issa et al showed that, LLLT have analgesic and antiinflamatory effect on arthritis of the knee of mice (22).

Peccin et al. have shown in the study performed on the knee joints of rabbits that laser therapy is efficient in the repair of traumatic cartilage damages (5). In a clinical study, Tascioglu et al reported that LLLT has no effect for in patients with knee osteoarthritis (23).

In the study of Cho HJ et al. performed on rabbits (24)**;** after the application of OA induction via 4% H_2O_2 injection at low-strength laser application; in knee OA treatment, no significant treatment effect is observed 2 weeks later but they have observed that significant recoveries have occured after 4 weeks. Also they have argued that OA should be treated for at least 3 weeks at a low-strength laser application. They have developed this assessment by depending on super oxide dysmutase activity, simple radiography, 3B-CT images, gross observations and histopathology.

In the study of Calatrava et al. performed on rabbits (4), they have compared the effects of He-Ne and IR-Ga laser in the lesion that they have traumatically created on the knee joint. They have observed that when IR-Ga laser is applied at 8 J/cm^2 with 24-hr intervals for a total of 13 sessions, the effect of treatment regarding the inflammatory and analgesic periods is slightly better in deep pathologies. In both treatments, functional recovery is found statistically similar (4).

Huang Z et al. have investigated in the metaanalysis performed in 2015 whether low level laser therapy (LLLT) has a treating effect on knee osteoarthritis or not. They systematically searched randomized controlled trials (RCTs) written in English that compared LLLT (at least eight treatment sessions) with sham laser in OA patients from January 2000 to November 2014. The findings indicate that current evidence does not support the effectiveness of LLLT as a therapy for patients with OA (25). Similarly, we cannot reveal the statistically significant effect of laser therapy.

Wang P et al. demonstrated that in rabbit progressive OA model, at least 6 weeks LLLT therapy may control pain and synovial inflammation (26). In our study, in the OA model that is experimentally developed by applying intraarticular MIA in rats, the effect of IR Ga-Ar laser therapy on cartilage for 15 days was investigated by using pain assessment and histopathological methods. In the experimental group to which laser therapy is applied, similar histopathological changes are observed with group C-2 and despite the strength of the pathological changes is less than group C-2 at all times, it is not found as statistically significant.

Also in this study, we have shown that the intraarticular injection of MIA has induced the histopathogical changes as similar in human OA however we cannot find a correlation between the pain assessment of knee bending test and the level of histopathological changes. On the 2nd and 3rd days of the study; despite a statistically significant difference is found in the knee bending scores between the control group to which MIA is applied and the experimental group; it is a very short-term response and the continuity and increase of the response against increasing histopathological changes cannot be observed. Although the knee bending test seems to be advantageous because it is a simple and rapidly applicable and easily repeatable experimental test (8); we think that the adaptation of the rats to the environment, and their domestication occured in time have clinically affected the pain assessment results.

Most important limitation of our study is the failure of laser application at different doses and periods for different groups. In our study; although no significant positive effect of laser is found on knee OA; the studies to be performed on more number of subjects in which the different dose, application period and effect on pain will be assessed can assess this effect better.

Conflict of interest

No conflict of interest was declared by the authors.

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