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Original Article

Efficacy of extracorporeal shockwave therapy and low-intensity pulsed ultrasound in a rat knee osteoarthritis model: A randomized controlled trial

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Abstract

Objective: This study aims to assess the efficacy of extracorporeal shockwave therapy (ESWT) and low-intensity pulsed ultrasound (LIPUS) on osteoarthritic rat knees.

Material and Methods: Twenty-four rats were divided into 3 groups: group 1-control (n=8), group 2-LIPUS (n=8) and group 3-ESWT (n=8). Cartilage degeneration was provided using mono-iodo-asetate (MIA). One milligram of MIA was delivered to the right knees in group 1 and both knees in group 2 and 3. A 0.09% saline solution was delivered to the left knees in group 1 for control. Twenty-four hours after the delivery, ESWT was applied once on the right knees in the group 2 rats to the medial tibia plateu with a 1 Hz frequency and 800 impulses. LIPUS was applied to the right knees in the group 2 rats to the medial tibia plateu with a 3 mHz frequency and 40 mW/cm2 intensity for 20 minutes over a period of 15 days. Pain scores were measured with a knee bend test. Bone mineral density measurements and scintigraphic bone scans were performed. Histopathological examination was done using a modified Mankin scale.

Results: There was no difference among the right knee subchondral bone osteoblastic activities ($p>0.05$). The left knee osteoblastic activities in the LIPUS and extracorporeal shockwave therapy (ESWT) groups were higher than those in the control group ($p<0.05$), but there was no difference between the LIPUS and ESWT groups. There was no difference among the groups for both knee subchondral bone BMD values ($p>0.05$). The modified Mankin scores of both the right and left knees of the ESWT and LIPUS groups were lower than those of the control group ($p<0.05$), but there was no difference between the ESWT and LIPUS groups. The pain scores of both knees of the ESWT and LIPUS groups at day 7 were higher than those of the control group ($p<0.05$), but there was no difference between the ESWT and LIPUS groups. There was no difference among the pain scores of the right knees at day 14 ($p<0.05$).

Conclusion: ESWT and LIPUS have systemic proliferative and regenerative effects on cartilage and tissue.

Keywords: Extracorporeal shockwave therapy, low-intensity pulsed ultrasound, osteoarthritis

Introduction

Osteoarthritis (OA) is a heterogenous disease associated with degenerative processes in the joint cartilage and subchondral bone that leads to disintegration of the cartilage along with related clinical signs and symptoms (1). All biochemical and histopathological processes of the joint cartilage may result in cartilage damage with ineffective remodeling during the course of the disease OA may be considered the biochemical and histopathological result of a group of diseases, rather than a single disease (2, 3). Although it is accepted as a noninflammatory disease, moderate inflammation is common, and it results in swelling, effusion, and pain (4). Subchondral bone is a structure that provides mechanical and nutritional support to the overlying joint cartilage. However, the degenerative processes associated with OA originate from the subchondral bone, making it a leading target in the treatment of OA (5).

Traditional treatment modalities of OA are pharmacological (oral and topical non-steroid anti-inflammatory drugs) and non-pharmacological (regulation of daily activities, education and exercises), but none of these can revive the cartilage degeneration. Advanced treatment modalities demonstrating histopathological recovery are required. Low-intensity pulsed ultrasound (LIPUS) and extracorporeal shockwave therapy (ESWT) have been shown to be effective in experimental animal models, but studies have not revealed which one of these modalities is more effective. This study aims to compare the efficacy of these treatment modalities that were shown to be effective in early OA models.

Low-intensity pulsed ultrasound is a representative treatment modality used in the field of orthopedics for the treatment of nonunion (6). Ultrasound has both termogenic and nontermogenic effects. Application of high intensity (1-300 W/cm^2) ultrasound continuously generates termogenic effects in living tissues, while

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low-intensity (<100 mW/cm^2) ultrasound generates non-termogenic effects. Recent data suggests that ultrasound treatment encourages cartilage repair as well as bone healing (7). The repair process mechanism is still unclear. Continuous in vitro exposure to 0.14 mW/cm^2 leads to increased expression of integrin molecules (especially a5 and b1), chondocyte markers, like Sox molecules (especially Sox 5 and Sox 9), collagen II C telopeptides and aggrecan (8). Concerning the molecular restoration process, Cook et al. (9) reported morphologic recovery in osteoarthritic rabbit knees. Huang et al. (10) obtained similar data about rat osteoarthritic knees. It seems that chondrocyte regeneration and matrix proliferation could be provided by exposure to LIPUS *in vivo*. However, the current data about in vitro exposure is insufficient.

Extracorporeal shockwaves are energy waves that have both direct and indirect effects on the tissue (11). ESWT is used in the treatment of various tendinopathies, such as calcific tendonitis of the shoulder, epicondylitis, and plantar fasciitis (12, 13). Treatment indications were extended to cover conditions such as femoral head necrosis, delayed unions, and nonunions of fractures (14, 15). Some studies report reactivation and enhancement of bone healing processes (16, 17). Most of them attribute this enhancement to upgrading the revascularization, which leads to the recruitment of growth factors and possibly stem cells that are necessary for the normal healing process (18). ESWT also has beneficial effects on cartilage tissue (19). Furthermore, some studies demonstrate that ESWT has chondroprotective effects in the early stages of knee OA (20). The effects of ESWT are dose dependent, but the optimal chondroprotective dosage is unknown.

Material and Methods

Study design-animals

The study was conducted after approval was granted by the Ethical Board of Experimental Animal Researches of Gulhane Military Medicine Academy, and all the animals were kept in compliance with the ethics rules. Twenty-four Sprague-Dawney, 8-week-old male rats weighing 200-300g were used and were maintained under climate-controlled conditions on a 12-h light-dark cycle at 22°C with a relative humidity of 50-55%.

Experimental design and mono-iodo-asetate (MIA) injection

Twenty-four rats were divided into 3 groups, randomly: group 1-control group (n=8), group 2-LIPUS group (n=8), and group 3-ESWT group (n=8). The cartilage degeneration model was provided chemically by mono-iodo-asetate (MIA). Four rats (2 from the ESWT and 2 from the LIPUS group) died 24 hours after MIA delivery, so the study was completed with 6 rats in each group.

Before MIA application, all rats were anesthetized with 0.2 mg/kg 2% xylazine and 0.5 mg/kg 10% ketamine delivered intraperitoneally. For each rat, 1 mg of MIA was dissolved in 50 µL 0.09% saline and delivered to the right knees in group 1 rats and both knees in group 2 and 3 rats intraarticularly. Fifty microliters of 0.09% saline was delivered to the left knees in group 1 to check to see if osteoarthritis resulted. Applications were performed with a 27G Hamilton injector.

Application of treatments

Twenty-four hours after MIA delivery, LIPUS was applied to the right knees in the group 2 rats. The application was performed 0.5 cm below the medial tibial plateau in the anteroposterior plane with a 3 mHz frequency and 40 mW/cm^2 intensity (Chattanooga Group Intelect Advance 2005 Encore Medical Corporation; Austin, Texas, U.S.A) at room temperature (22 °C) for 20 minutes a day for 15 days. Ultrasound gel was applied to the skin contacting the ultrasound probe, and all applications were performed under intraperitoneal anesthesia with 0.2 mg/kg 2% xylazine and 0.5 mg/kg 10% ketamine.

Twenty-four hours after MIA delivery, ESWT was applied only once on the right knees in the group 3 rats. The application was performed 0.5 cm below the medial tibial plateau in the anteroposterior plane with 800 impulses of shockwave at 12 kV (equivalent to 0.18 mj/mm^2) (Storz medical duolith SD1; Storz Medical AG, Tagerwillen, Switzerland) at room temperature (22°C). Ultrasound gel was applied to the skin contacting the shockwave tube, and the application was performed under intraperitoneal anesthesia with 0.2 mg/kg 2% xylazine and 0.5 mg/kg 10% ketamine.

Assessments

Bone mineral density (BMD) measurements and scintigraphic scanning's performed at day 16 before the animals were sacrified under the same anesthesia procedure (0.2 mg/kg 2% xylazine and 0.5 mg/kg 10% ketamine intraperitoneally). The BMD assessment was performed with a DXA device (QDR 4500 Elite Hologic Inc 1999, U.S.A) after calibration with an Anthropomorphic Spine Phantom (Hologic Inc. 35 Crocbydrie) device. Baseline volumetric subchondral BMD measurements of bilateral proximal (subchondral) tibiae were performed, and the mediolateral BMD ratio was calculated. For scintigraphic scanning, 2 mCi technetium-99 m methylenediphosphate (Tc-99 m-MDP) was administered to each rat through the tail vein. Precisely 190 minutes after injection, planar imaging was performed using a gamma camera (Mediso Nucline Spirit; Budapest, Hungary) for bilateral knees. In the scintigraphy results for evaluation purposes, regions of interest (ROI) of the same size were drawn bilaterally on the knees, and the counts were compared using the statistical analysis of differences.

For pain assessment, the bend test was performed on all subjects twice at day 7 and 14 after MIA delivery. During the knee bend test, all subjects were handled to restrict free motion but to permit passive movements in the knee joints. Vocal responses like squeaking were considered over 20 points.

Histomorphological analysis

Rats were sacrified at day 16 after the BMD measurement and scintigraphic scanning. Proximal tibial segments of the knees were taken, including articular cartilage and subchondral bone, and fixed by a 10% formaldehyde solution. The specimens were decalcified in 8% formic acid/hydrochloric acid solution for 12 hours. After the decalcification procedure, the specimens were immersed in paraffine blocks, and 4 µm thickened samples were cut. Samples were stained with heamtoxylin-eosin stain. The microscopic features of the articular cartilage were presented as chondrocyte activation, proliferation, apoptosis, cartilage fissuring, and pannus formation and assessed by a pathologist using a modified Mankin scale over 14 points. The histological evidence of cartilage degeneration was assessed through the structural changes in the articular cartilage (0, normal; 1, surface irregularities; 2, pannus and surface irregularities; 3, clefts to transitional zones; 4, clefts to radial zones; 5, clefts to calcified zones; and 6, complete disorganization), as well as the cell status (0, normal; 1, diffuse hypercellularity; 2, cloning; and 3, hypocellularity). The total cartilage degeneration score ranged from 0 (normal) to 9 (complete disorganization and hypocellularity of the articular cartilage). All cartilage sections were graded by a blinded pathologist.

Statistical analysis

The Statistical Package for the Social Sciences version 15.0 (SPSS Inc.; Chicago, IL, USA) software package was used in the analysis of the data. The variable distribution was analyzed visually and using analytical methods. The median, minimum, and maximum values by a chi-quare test were used for descriptive analysis. Variables among the groups were analyzed with the Kruskal-Wallis test, while dependent variables were compared with the Wilcoxon test. P values <0.05 were accepted as statistically significant.

Table 1. Osteoblastic activities of the groups

Groups	Osteoblastic Activity median (min-max)	p
Right Knee		
Control	11290.29 (10380.93-14927.26)	
ESWT	13791.29 (8743.12-16757.09)	0.532
LIPUS	12530.29 (10792.66-17139.26)	
Left Knee		
Control	461.515 (358.79-897.29)	
ESWT	13980.85 (9497.53-18653.46)	0.003
LIPUS	14129.31 (10805.97-24999.74)	

min-max: minimum-maximum; ESWT: extracorporeal shockwave therapy; LIPUS: low-intensity pulsed ultrasound

Table 2. The subchondral BMD values of the groups

Groups	Subchondral BMD scores median (min-max)	p
Right Knee		
Control	1.460 (1.170-1.830)	
ESWT	1.285 (1.190-2.050)	0.557
LIPUS	1.710 (1.350-2.020)	
Left Knee		
Control	1.625 (1.190-1.960)	
ESWT	1.515 (1.140-1.700)	0.232
LIPUS	1.455 (1.170-1.860)	

min-max: minimum-maximum; ESWT: extracorporeal shockwave therapy; LIPUS: low-intensity pulsed ultrasound

Table 3. The pain scores of the groups at day 7

Groups	Pain Scores median (min-max)	p
Right Knee		
Control	12.0 (8.0-16.0)	
ESWT	15.5 (14.0-18.0)	0.044
LIPUS	13.0 (10.0-18.0)	
Left Knee		
Control	5.5 (4.0-8.0)	
ESWT	16.5 (12.0-20.0)	0.001
LIPUS	16 (13.0-19.0)	

min-max: minimum-maximum; ESWT: extracorporeal shockwave therapy; LIPUS: low-intensity pulsed ultrasound

Table 4. Pain scores of the groups at day 14

Groups	Pain Scores median (min-max)	p
Right Knee		
Control	14.5 (12.0-17.0)	
ESWT	13.5 (10.0-17.0)	0.774
LIPUS	14.0 (10.0-17.0)	
Left Knee		
Control	3.0 (1.0-4.0)	
ESWT	16.5 (14.0-18.0)	0.004
LIPUS	15.0 (13.0-18.0)	

min-max: minimum-maximum; ESWT: extracorporeal shockwave therapy; LIPUS: low-intensity pulsed ultrasound

Table 5. Modified Mankin Scale scores of the rats according to groups

Groups	Modified Mankin Scores median (min-max)	p
Right Knee		
Control	9.0 (7.0-10.0)	
ESWT	2.0 (2.0-5.0)	0.002
LIPUS	4.0 (2.0-5.0)	
Left Knee		
Control	0.5 (0-2.0)	
ESWT	4.5 (3.0-8.0)	0.004
LIPUS	6.5 (5.0-7.0)	

min-max: minimum-maximum; ESWT: extracorporeal shockwave therapy; LIPUS: low-intensity pulsed ultrasound

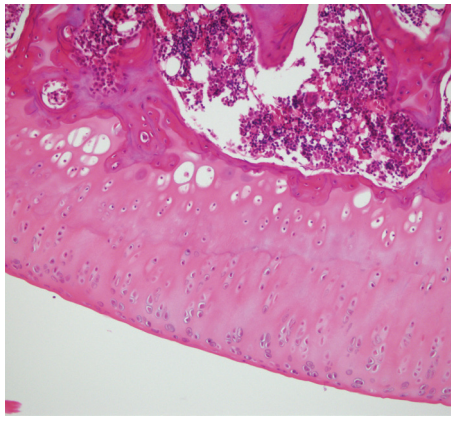

Figure 1. Normal cartilage tissue. Normal carilage surface is smooth. Deep layers of the cartilage tissue consist of proliferating chondroctes, but the density of the cells decreases through the cartilage surface.

Results

Radiographs of the knee in the anteroposterior and lateral projections were performed at day 0 and day 16. At day 16, osteoarthritic features like narrowing of the joint space and spur formation were seen.

The osteoblastic activities measured by scintigraphic scanning are given in Table 1. There was no significant difference among the right knee osteoblastic activities (p=0.532). The left knee osteoblastic activity of both the LIPUS and ESWT groups was significantly higher than those in the control group (p=0.003 for each), but there was no difference between the LIPUS and ESWT groups (p=0.631).

The subchondral BMD values are given in Table 2. There was no difference among the groups for the right and left knee BMD values (p=0.557 and p=0.232, respectively).

The pain scores obtained by the knee bend test at day 7 are given in Table 3. The pain scores of both the right and left knees of the ESWT and LIPUS groups are significantly higher than those in the control group (p=0.044 and 0.001, respectively), but there was no significant difference between the ESWT and LIPUS groups (p=0.936 and 0.620, respectively).

The pain scores at day 14 are given in Table 4. There was no difference among the pain scores of the right knees (p=0.774). However, the pain scores of the left knees in both the ESWT and LIPUS groups were significantly higher than those in the control group (p=0.004 for each), but there was no significant difference between the ESWT and LIPUS groups (p=0.216).

The modified Mankin scores of the rat knees are given in Table 5. The modified Mankin scores of the right knees in the ESWT and LIPUS groups were significantly lower than those in the control group (p=0.002 for each), but there was no difference between the ESWT and LIPUS groups (p=0.131). The modified Mankin scores of the left knees in the ESWT and LIPUS groups were significantly higher than those in the control group (p=0.002 for each), but there was no difference between the ESWT and LIPUS groups (p=0.072).

Figures 1-4 show the microscopic examples of the knee cartilage tissues of the subjects.

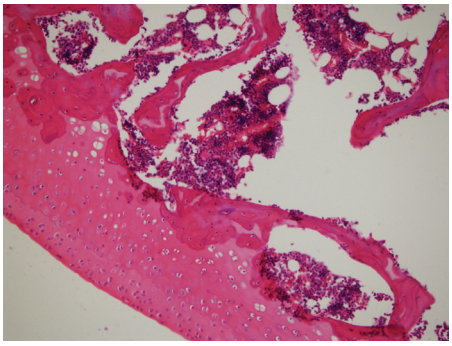

Figure 2. Cellular irregularity and picnosis. The early morphological features of osteoarthritis are cellular irregularity and picnosis. Both picnosis and cellular irregularity are unique findings for chondrocyte apopitosis. Chondrocyte loss results in decreased cartilage regeneration.

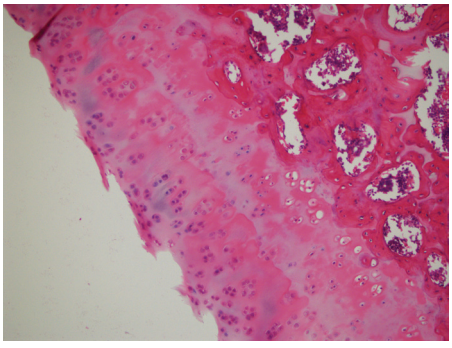

Figure 3. Surface irregularity. Decreased regeneration makes cartilage tissue sensitive to mechanical stress. Mechanical stress results in microfractures in deep layers. Excessive load and mechanical trauma without regeneration of the cartilage tissue results in surface irregularity.

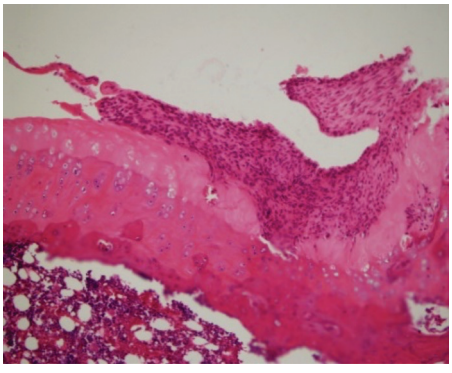

Figure 4. Cleft and pannus formation. Union of the surface irregularity areas results in deep clefts on the surface of the cartilage tissue. Insufficient chondrocytes with poor proliferative response to mechanical trauma evoke pannus formation.

Discussion

Our study results suggest that both ESWT and LIPUS have proliferative, regenerative effects on both bone and cartilage tissue in rats. Both modalities have analgesic effects besides the mitogenic activities. These findings confirmed the results of prior studies. However, we also found that both modalities have systemic effects and, to the best of our knowledge, this is the first study that demonstrates the systemic effect of LIPUS and ESWT in OA *in vitro*.

Hence, the basic pathological finding of OA is cartilage degeneration, and it has been recently accepted as a joint cartilage disease. There are increasing data indicating that morphological changes in OA arise from subchondral and underlying trabecular bone before cartilage degeneration, which makes subchondral bone an important target for new treatment modalities.

The effects of ESWT on connective tissue are controversial. Although proliferative effects were indicated, some studies have reported no significant effects but some of the recent animal experiments have indicated that ESWT application has chondroprotective effects and relieves symptoms such as pain by improving motor functions (21-24). Zhao et al. (25) suggested that these effects were due to the inhibition of nitric oxide (NO) release and partial apopytosis. Mayer-Wagner et al. (26) reported that ESWT down-regulates Tenascin-C whose expression increases at the superficial layer of the joint cartilage in OA. In the same study, researchers found that the Chi3L1 gene, which mediates the release of proinflammatory cytokines like TNF α and IL-1 regarding the subchondral bone reorganization, upregulates with ESWT application in 10 weeks. In their studies with a dog tendinopathy model, Wang et al. (17, 18) indicated that the chondroprotective effects of ESWT could be attributed to angiogenesis, neovasculation, and osteogenesis mediated by growth factors such as eNOS, VEGF, PCNA, and BMP-2.

The therapeutic use of ultrasound was first introduced in the orthopedic field in nonunion and delayed union cases. The following studies indicated that LIPUS might have beneficial effects on cell metabolism and tissue regeneration (27). Xueping et al. (28) reported that LIPUS inhibited signaling pathways like ERK1 ERK2 and p38, which mediate the matrix metalloproteinase-13 (MMP-13) release.

Our study results suggest that both ESWT and LIPUS have proliferative and regenerative effects on both bone and cartilage tissue in rats. Both modalities have analgesic effects besides the mitogenic activities. These findings confirm prior studies. Despite obvious histopathological recovery in cartilage tissue, there was no significant difference among the control, ESWT and LIPUS groups in subchondral BMD and knee joint osteoblastic activity suggesting that these treatment modalities may not have the same beneficial effects in subchondral bone. This may be due to the fact that LIPUS application was limited to 15 times and ESWT to one time. Longer applications may have more beneficial effects.

We found no difference among the pain scores of the right knees in the control, ESWT and LIPUS groups at day 7 and 14, suggesting that neither of the treatment modalities had an effect on pain in the early OA model. We found that the pain scores at day 14 were significantly lower than those at day 7 in both the ESWT and LIPUS groups. Several mechanisms, such as anti-inflammatory effects and increased angiogenesis, may be involved through the analgesic effects of both modalities (24, 26). In a recent study, Maier et al. (29) reported an increased release of substance P and prosotglandin E2, which might implicate analgesic effects.

The modified Mankin scale scores of the untreated knees in either the ESWT or LIPUS group were significantly lower than those in the right knees of the control group. However, there was no significant difference among the osteoblastic activities. These findings suggest that both treatment modalities have systemic effects.

The LIPUS and ESWT modalities are shown to have proliferative effects and prevent apoptyosis in chondrocyte cell culture models (30). However, these beneficial effects are limited to experimental animal studies *in vitro*. Moreover, no other studies have indicated their systemic effects before. We found histopathological improvement in both treated and untreated knees. To the best of our knowledge, this is the first study to compare the effects of these two modalities, and we have found that both of them have regenerative, proliferative, and protective effects on osteoarthritic rat knees.

Osteoarthritis is a common cause of morbidity in the world. The disease may be asymptomatic in early stages, and its irreversible features are common when it is diagnosed. Current treatment modalities are palliative and do not improve morphology. Research is still underway for a "disease modifying treatment" supporting morphological recovery. ESWT and LIPUS are effective, safe and cheap treatment modalities for OA. They seem to be effective before irreversible changes take place in the cartilage. Therefore, the early stages of OA must be discovered to determine when the morphologic features become irreversible. Further studies must be designed to assess the efficacy *in vitro*.

Although a power analysis was performed before the study, the main limitation of this study was the limited number of subjects. Similar studies with a larger number of subjects may have more significant results. Another limitation was the method of ESWT application. Some studies have used several ESWT applications, and some have used only one. We used only one application to present the effect of minimal use. Nevertheless, our results are remarkable, and these modalities seem to be promising for the treatment of OA.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Gülhane Training and Research Hospital.

Informed Consent: N/A.

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