



The effect of extracorporeal shockwaves on cartilage end-plates in rabbits: a preliminary MRI and histopathological study

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Objective: The aim of this study was to examine the changes in the lumbar intervertebral area after extracorporeal shockwave therapy (ESWT) at different energy levels in a rabbit model using magnetic resonance imaging (MRI) and histopathological evaluation.

Methods: The study included 30 male New Zealand white rabbits divided randomly into five groups: Groups A and C received 1,000 shockwave impulses at an intensity of 14 kV, Groups B and D received 1,000 impulses at 21 kV, and Group E was a sham group. Side effects such as subcutaneous and paravertebral soft-tissue injuries were evaluated using MRI one day after ESWT administration. Neovascularization, edema and fibroblast activity in the intervertebral area were evaluated histopathologically.

Results: No change was observed in any group on MRI. Histopathologically, Groups A and C showed no change, whereas Groups B and D showed edema, fibroblast activity and significant neovascularization at the intervertebral end-plate ($p < 0.05$).

Conclusion: Our findings indicate that ESWT caused dose-dependent changes in the intervertebral end-plate. This study constitutes a preliminary evaluation of shockwave therapy to the intervertebral area in an animal model. High-dose ESWT may stimulate angiogenesis at cartilage end-plates in rabbits.

Key words: Biological effect; cartilage end-plate; extracorporeal shockwave; intervertebral disc; rabbit.

The intervertebral area consists of three basic anatomical structures: the nucleus pulposus, annulus fibrosus, and cartilage end-plates. The latter consists of thin layers of hyaline cartilage between the intervertebral discs and vertebral bodies. The intervertebral disc is the largest avascular tissue in the body.^[1,2] The vascular buds and small blood vessels in the end-plates are thought to

play an important role in the nutrition of the nucleus pulposus.^[3-5] The process of disc degeneration accelerates when the permeability of the end-plates is reduced.^[6,7] Cartilage end-plate calcification and vertebral bone sclerosis may decrease the diffusion of nutrients through the end-plates.^[1,8] To prevent disc degeneration, new methods to increase permeability, such as

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growth factor therapy, have been studied.^[9] In addition, some studies have reported attempts to increase the vascularity of degenerated intervertebral discs.^[10,11]

For many years, extracorporeal shockwave therapy (ESWT) was used only to manage kidney and urinary tract stones. In recent years, ESWT has also been used in the treatment of many musculoskeletal diseases and conditions, including tendinopathies and the non-union of long-bone fractures.^[12-18] The mechanism of ESWT is thought to be re-injury of the avascularized bone and fibrous tissue, which triggers neogenesis. The primary advantage of ESWT is its noninvasive nature and seemingly minimal complications when applied to musculoskeletal tissues. Recently, ESWT was shown to create neovascularization by inducing tissue microtrauma.^[19] However, shockwaves may have adverse effects on soft tissues;^[14] a recent review reported that extracorporeal shockwave lithotripsy injured the vital organs and vascular structures.^[20]

Although ESWT is often used to treat musculoskeletal disorders, its use in the vertebral column is very limited.^[21,22] To our knowledge, no study has yet examined the application of shockwaves to the intervertebral area. We hypothesized that biological changes might be seen in cartilage end-plates after ESWT. To test this, we used magnetic resonance imaging (MRI) and histopathological evaluation to examine changes in the lumbar intervertebral area after the application of ESWT at different energy levels.

Materials and methods

Thirty 6-month-old New Zealand white male rabbits weighing 2.5 to 3.0 kg were used in this study. The study was approved by the Harran University Animal Research Local Ethics Committee and performed according to the guidelines of the Association for the Assessment and Accreditation of Laboratory Animal Care.

The animals were divided randomly into five groups of six rabbits (Table 1). Groups A and B received 1,000 shockwave impulses at an intensity of 14kV (Group A) or 21kV (Group B) and were sacrificed after 10 days. Groups C and D received 1,000 shockwave impulses at an intensity of 14kV (Group C) or 21kV (Group D) and were sacrificed after 28 days. Group E (sham group) rabbits received no shockwave application and were sacrificed after 28 days.

Shockwaves were generated using an electrohydraulic shockwave device (Multimed Ortho; Elmed Electronics & Medical Industry & Trade Inc., Ankara, Turkey). Immediately after shockwave therapy, the backs of the rabbits were checked for swelling, edema, or hemorrhage. The activity of the animals was not

restricted and they were returned to their housing cages after therapy and cared for by a veterinarian.

Each rabbit was anesthetized with an intramuscular injection of a mixture of xylazine hydrochloride (5 mg/kg; Rompun® 23.32 mg/ml; Bayer Turk, Istanbul, Turkey) and ketamine hydrochloride (50 mg/kg; Ketalar 50 mg/ml; Parke-Davis, Eczacıbaşı, Istanbul, Turkey) and its back was shaved. The rabbit was placed in the prone position and ESWT applied at the L4-L5 intervertebral level. The shockwave was focused using C-arm imaging and depth was confirmed with the control guide of the machine. Surgical lubrication gel was placed on the skin in contact with the shockwave tube. In the study groups, the L4-L5 intervertebral area was bilaterally treated 500 pulses each to the left and right sides in a paravertebral direction. ESWT was applied in a single session of 1,000 pulses at an intensity of 14 or 21 kV. The sham group received the anesthetic agents (50 mg/kg ketamine HCl and 5 mg/kg xylazine) and exposed to fluoroscopy for the same time as the study groups, but no shockwave was applied. ESWT dosage was based on the safe, effective doses used in previous studies.^[23,24] Immediately after ESWT, the back of each rabbit was examined for redness, swelling, or hematoma and returned to its cage.

Intervertebral area tissue and concomitant soft-tissue reaction in the lumbar area was evaluated using a 1.5T 16-channel system (MAGNETOM Symphony. A Tim System; Siemens, Erlangen, Germany). MRI was performed 24 hours after ESWT, as in a previous study.^[14] Animals in Groups A, B, and E were anaesthetized and placed in the prone position. T1-weighted spin-echo (one acquisition in the sagittal plane; repeat time (TR), 671 ms; echo time (TE), 11 ms; rectangular field of view (FOV), 250 mm with a 320x320 matrix; slice thickness, 3 mm) and short tau inversion recovery (STIR; two acquisitions in the axial and sagittal planes; TR, 3000 ms; TE, 107 ms; inversion time, 150 ms; rectangular FOV, 250 mm with a 320x320

Table 1. Study groups (Six rabbits per group).

Group	kV	MRI (days after ESWT)	Histopathological examination (days after ESWT)
A	14	1	10
B	21	1	10
C	14	-	28
D	21	-	28
E*	-	1	28

*Sham group. MRI: magnetic resonance imaging, ESWT: extracorporeal shockwave therapy.

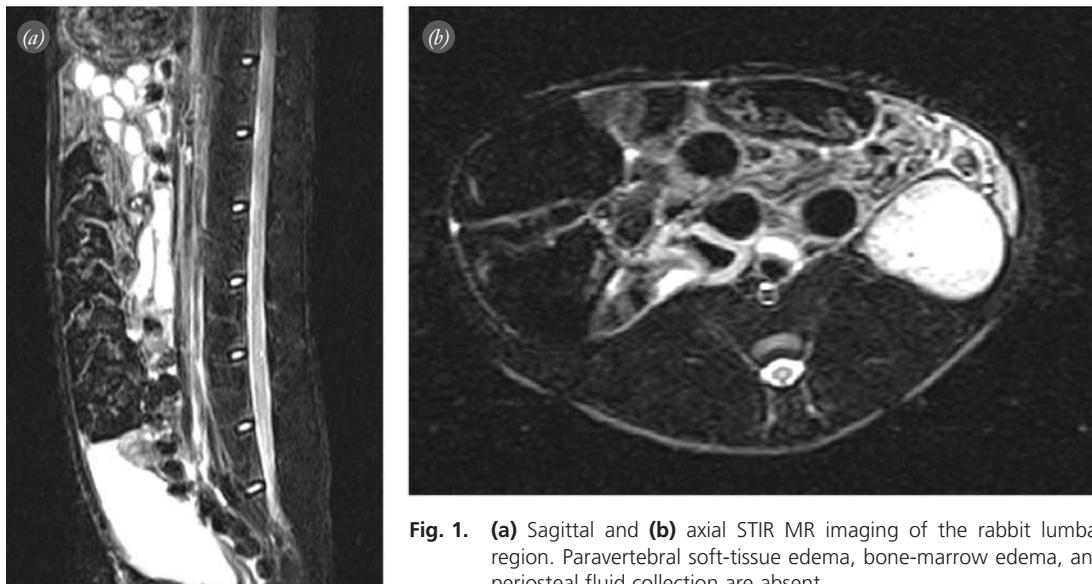


Fig. 1. (a) Sagittal and (b) axial STIR MR imaging of the rabbit lumbar region. Paravertebral soft-tissue edema, bone-marrow edema, and periosteal fluid collection are absent.

matrix; slice thickness 3 mm) were obtained. MR images were evaluated blind by two radiologists with musculoskeletal imaging experience. The presence or absence of lumbar soft-tissue edema, bone-marrow edema, disc and end-plate hemorrhage or edema and periosteal fluid collection was recorded.

Animals were sacrificed with a pentobarbital overdose on Day 10 (Groups A and B) or 28 (Groups C, D and E) after ESWT. L4-L5 spinal segments were harvested for histopathological examination. All specimens were subjected to a gross examination, including surface appearance, color, consistency, and structural integrity. Specimens were then fixed in 10% formalin for 24 hours and decalcified in 3% nitric acid. Samples were embedded in paraffin and cut with a knife blade along the coronal plane. Sections (4- μ m thickness) were obtained using a standard rotatory microtome and stained with hematoxylin & eosin. Histological analysis to demonstrate alteration of the tissue in the end-plates was performed as described in previous work.^[23,25] Histopathological changes, such as changes in cellularity, myxoid alteration of the matrix (edema), fibroblast proliferation, and the presence of neovascularization in the end-plates, were evaluated semi-quantitatively under a light microscope. Each parameter was scored as Grade 0 (no change), 1 (slight alteration), 2 (moderate change) or 3 (marked change).

Statistical analyses were conducted using SPSS software for Windows v.16 (SPSS Inc., Chicago, IL, USA). Histopathological differences among the groups were tested for significance using the chi-squared test. P values of less than 0.05 were considered significant.

Results

No device-related problem occurred and no rabbit tested developed a neurological deficit throughout the course of this study.

The short tau inversion recovery MR images showed no sign of subcutaneous or paravertebral soft-tissue edema, effusion, or hemorrhage in Groups A, B and E. The vertebral structure in the two study groups was similar to that in the sham group (Fig. 1).

No histological difference was observed between the end-plates in the two low-dose groups (Groups A and C) and those in the sham group (Group E).



Fig. 2. Photomicrograph of a coronal section of the L4-L5 intervertebral area of a rabbit from the sham group (no extracorporeal shockwave application). The intervertebral disc and cartilage end-plates appear normal (H-E x40). [Color figure can be viewed in the online issue, which is available at www.aott.org.tr]

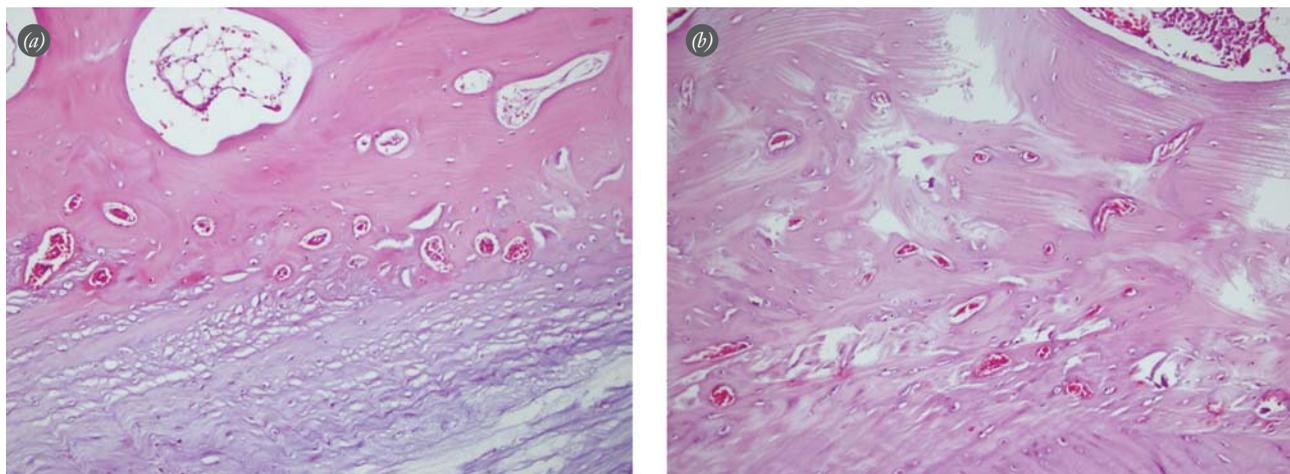


Fig. 3. (a, b) Photomicrographs of coronal sections of the L4-L5 intervertebral area of the rabbits that received extracorporeal shockwave application. Neovascularization is apparent in the cartilage end-plate (H-E x200). [Color figure can be viewed in the online issue, which is available at www.aott.org.tr]

Histopathologically, the findings were similar to that of the normal end-plate found in the sham group (Fig. 2). In contrast, the differences between the sham and the two high-dose groups (Groups B and D) were significant in terms of the presence of neovascularization at the intervertebral end-plate ($p < 0.05$) (Fig. 3). Histopathological changes were present in two of the six study specimens in Group B and four of the six study specimens in Group D. Edema and fibroblast activity were also observed in the high-dose groups (Table 2), although no histological change was apparent in the nucleus pulposus or annulus fibrosus in any group.

Discussion

In this *in vivo* study, the biological effects of ESWT to the intervertebral area in rabbits were investigated. Histologically, an increase in the number of fibroblasts, edema in the matrix and new capillaries on the cartilage end-plate were observed. The increases were significant in groups receiving 21kV shockwaves while there were no changes in the sham group or in the groups receiving 14kV treatment, possibly due to the low energy of the shockwaves.

The determination of a standard ESWT dose posed a challenge at the beginning of this study. While ESWT doses have been established in the literature for tendons and bones,^[13,26-29] there are no published standards for the intervertebral area, which has significantly different anatomical and mechanical properties. For this reason, we deferred to the reports by Wang et al. and set ESWT levels at 14 and 24kV, based on their data.^[23,24]

While no histological change in the nucleus pulposus or annulus fibrosus due to the avascular nature of these

tissues was observed, new blood vessel formation in the cartilage end-plate was found. In the human embryo, the intervertebral disc has vascular channels running between the lamellae of the annulus fibrosus. Soon after birth, these vascular channels disappear.^[1] The activity of disc cells depends on diffusion from blood vessels at the cartilage end-plate.^[6] Consequently, calcification or subchondral sclerosis of the cartilage end-plate may induce degeneration of the nucleus pulposus.^[1,8]

The functions of tendons and spinal end-plates differ. Tendons are mostly exposed to tensile forces and end-plates to compressive forces. However, both have an avascularized nature and consist of a connective tissue structure. Therefore, degeneration in the cartilage end-plate may resemble the pathology in tendinosis. The etiology of tendonitis is multifactorial and includes degenerative changes, inflammatory process, metabolic disturbances with hypovascularity, neuronal origin, and neo-

Table 2. Grading of histopathological changes. Animals were sacrificed 10 (Group B) or 28 (Group D) days after 21kV extracorporeal shockwave application.

Case number	Edema (grade)	Fibroblast activity (grade)	Presence of new vessels (grade)
Group B			
21	1	1	2
24	1	1	1
Group D			
8	0	2	2
9	1	1	1
10	0	2	2
11	0	2	2

vascularization.^[19] Neovascularization may improve the blood supply and increased blood circulation in the tissues may play a role in the healing process, including tissue regeneration, osteogenesis and the secretion of local growth factors.^[19,25,30] Wang et al. reported that the biological mechanism of ESWT in bone healing involves the ingrowth of neovascularization and the upregulation of angiogenic and osteogenic growth factors.^[24] In addition, some studies have shown that ESWT stimulates the cascade of angiogenic and osteogenic transcription factors in osteoblast cells and promotes bone healing via significant increases in the serum nitric oxide level and osteogenic growth factors.^[31-33] Shockwave therapy is thought to work by inducing tissue microtrauma, which may initiate a healing response that causes blood vessel formation and increased delivery of nutrients to the affected area. Therefore, ESWT may stimulate the repair process and induce new blood vessel formation in hypovascular tissues. This increased vascularity on the end-plate may be used to treat disc degeneration. Modic changes, which can be rendered reversible by ESWT, can be determined in future disc degeneration animal model studies. Maier et al. observed the distal femurs of rabbits 1 day after ESWT and found soft-tissue edema, epiperiosteal fluid, and bone-marrow edema using MRI.^[14] In contrast, we observed no pathology in any of our groups which may be due to the anatomical differences between the knee and the lumbar region. Additionally, no neurological deficit was observed in this study. The absence of this side effect may be due to the paravertebral direction of the ESWT application. The application of low-energy ESWT directly to the interspinous area from the posterior through the neural canal has not been found to produce a neurological side effect on the rat spinal cord.^[34]

One limitation of this study was our inability to conduct immunohistochemical studies for technical reasons. Therefore, our study could not be confirmed by other stains for neovascularity.

In conclusion, ESWT may induce new blood vessel formation in the cartilage end-plate and was effective in the intervertebral area. Future studies using microangiography and immunohistochemistry to assess the role of ESWT in the vascularity of the end-plate in different animal species to confirm our results are necessary.

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Conflicts of Interest: No conflicts declared.

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