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BASIC SCIENCE

Extracorporeal shockwave therapy shows chondroprotective effects in osteoarthritic rat knee

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Abstract
Purpose This study investigated the effects of extracorporeal shockwave therapy (ESWT) on the subchondral bone and articular cartilage in the initiation of osteoarthritis of the knee in rats.
Methods Anterior cruciate ligament transected (ACLT) osteoarthritis (OA) rat knee model was used in this study. Twenty-seven male Sprague-Dawley rats were divided into three groups. The control group underwent sham surgery without ACLT and received no ESWT. The ACLT group underwent ACLT, but received no ESWT. The ACLT plus ESWT group underwent ACLT and received ESWT immediately after surgery. The evaluation parameters included radiograph, bone mineral density, serum levels of cartilage oligometric protein and osteocalcin, and urinary concentration of C-telopeptide of type II collagen (CTX-II), and histomorphological examination.
Results At 12 weeks, OA of the knee was radiographically verified in the ACLT group, but very subtle changes were noticed in the control and the ACLT plus ESWT groups. On articular cartilage, the ACLT group showed significant increases in cartilage degradation and chondrocyte apoptosis compared to the control and ACLT plus ESWT groups. The ACLT plus ESWT group demonstrated significant decrease in the cartilage degradation and an increase in chondrocyte activity comparable to the control. In subchondral bone, the ACLT group showed a significant decrease in bone remodeling as compared to the control and ACLT plus ESWT groups. The ACLT plus ESWT group showed significant improvement in bone remodeling comparable to the control.
Conclusion Extracorporeal shockwave therapy shows chondroprotective effect associated with improvement in subchondral bone remodeling in the initiation of ACLT OA knee model in rats.

Keywords Osteoarthritis · Shockwave · Subchondral bone · Articular cartilage · Knee

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Introduction

Osteoarthritis (OA) of the knee has long being considered primarily a cartilage disease. However, OA is usually accompanied by changes in the subchondral and periarticular bone such as sclerosis, bone cyst, and osteophyte formation [1]. The relationship between the subchondral bone changes and the initiation of OA is still debated [2, 3]. Some studies demonstrated increased subchondral bone turnover accompanied by microarchitecture changes in the subchondral trabecular bone in OA joint [4, 5]. Others reported that subchondral bone remodeling increases in OA [6]. These observations suggested a role of subchondral bone changes in the initiation of OA, and raised the possibility of early intervention to the subchondral bone may reduce or retard the progressive loss of articular cartilage [4–6].

Extracorporeal shockwave therapy (ESWT) has shown effectiveness in the treatment of tendinopathy, non-union

of long bone fracture and avascular necrosis of the femoral head [7–10]. Our previous study demonstrated that application of ESWT resulted in regression of OA changes of the knee in rats [11]. We hypothesized that early application of ESWT may improve subchondral bone remodeling and decrease the articular cartilage degradation, and this in turn may result in chondroprotective effect in the initiation of osteoarthritis of the knee. The purpose of this study was to investigate the effects of ESWT on subchondral bone and articular cartilage in the initiation of anterior cruciate ligament transacted (ACLT) osteoarthritic rat knee.

Materials and methods

The Institutional Review Board on animal experiments approved this study, and all studies were performed in accordance with the guidelines and the care of animals in experiment. We chose ACL transacted osteoarthritic knee model in rat in this study [12]. Twenty-seven 8-week-old male Sprague-Dawley rats with body weight ranging from 251 to 275 g were used in this study. The animals were divided into three groups with nine rats in each group. The control group underwent sham surgery without ACLT and received no ESWT. The ACLT group underwent arthrotomy and ACLT, but received no ESWT. The ACLT plus ESWT group underwent arthrotomy and ACLT and received ESWT immediately after surgery.

Anterior cruciate ligament transection

The rats were anesthetized with intraperitoneal injection of phenobarbital (50 mg/kg body weight). The left knee was prepared in surgically sterile fashion. Arthrotomy of the left knee was performed through medial parapatellar approach. The ACL was completely transacted with a scapel in the ACLT and the ACLT plus ESWT groups, but was left intact in the control group. The knee joint was irrigated and closed in routine fashion. The animals were returned to the housing cage under the care of a veterinarian.

Shockwave application

The rats in the ACLT plus ESWT group also received ESWT after ACLT surgery under the same anesthesia. The source of shockwave was from an OssaTron orthotriptor (High Medical Technology, Kruealigen, Switzerland). It is now Sanuwave, Alpharetta, GA). The focus of ESWT tube was 0.5 cm below the medial tibia plateau in anteroposterior projection and 0.5 cm from the medial skin edge (Fig. 1). Surgical lubricate was applied to the skin in contact with the shockwave tube. Each knee was treated with 800 shocks of ESWT at 14 kV (equivalent to 0.18 mJ/mm^2 energy flux density) in a single session. The control group and the ACLT group received no shockwave.

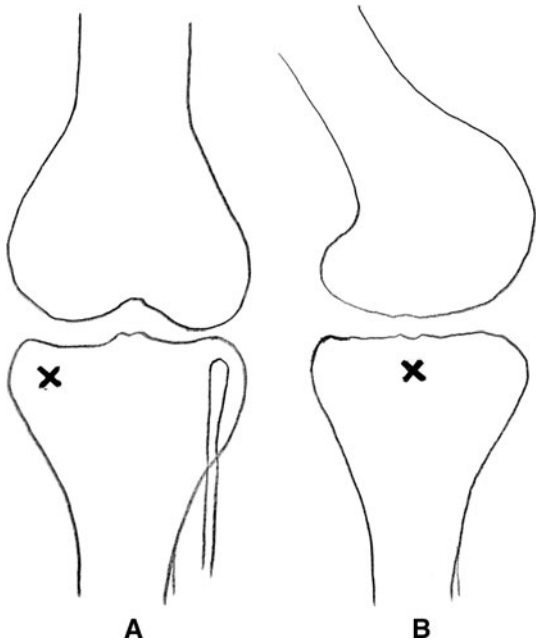

Fig. 1 The knee sketches show the focus of ESWT application to the knee in anteroposterior view (**a**) and lateral view (**b**)

Radiographs and bone mineral density

Serial radiographs of the left knee in anteroposterior and lateral views were performed at 0, 4, 8, and 12 weeks. Radiographs were used to assess the OA changes of the knee. The radiographic osteoarthritis included changes in bony appearance, joint space narrowing, and spur formation. Bone mineral density (BMD) of the left knee was performed using DEXA (dual energy X-ray absorptiometry) device (Fig. 2) at 0 and 12 weeks after treatment. The BMD was used to assess the changes in bone density around the knee especially in the medial half of the proximal tibia.

Blood and urine samples

Two milliliters of blood sample were obtained via cardiac puncture at 12 weeks just before the animals were killed, and the serum levels of cartilage oligometric protein (COMP) and osteocalcin were measured. The urine samples measured the urinary concentration of C-telopeptide of type II collagen (CTX-II).

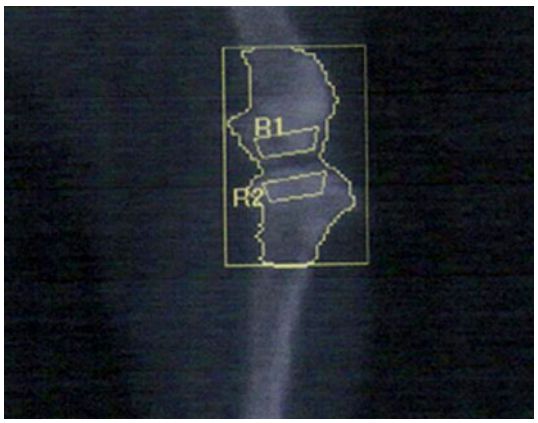

Fig. 2 Bone mineral density (BMD) of the knee with the region of interest (ROI) in medial half of the proximal tibia

Histomorphological examination

The animals were killed at 12 weeks. The proximal tibiae including bone and cartilage were harvested and the specimens were subjected to histomorphological examination. The specimens were cut into 5-μm thick sections and stained with hematoxylin–eosin and safranin-O stains. The cartilage fissuring, chondrocyte concentration, chondrocyte activity, and chondrocyte apoptosis, Safranin-O stain, and the Mankin score were examined microscopically. The Mankin scores of the articular cartilage were analyzed by the assessment on cartilage structure, cartilage cells, and tidemark integrity [13, 14]. The trabecular morphology and the tissue distributions of trabecular bone and fibrous tissue and the number of osteocyte of the subchondral bone were analyzed microscopically.

Statistical analysis

The data between the control group versus the ACLT group; the control group versus the ACLT plus ESWT group; and the ACLT group versus the ACLT plus ESWT group were compared statistically using ANOVA and LSD post hoc test. The statistical significance was set at $P < 0.05$.

Results

At 12 weeks, radiographs of the left knee showed gross osteoarthritis of the knee in the ACLT group, but very subtle arthritic changes were noticed in the control and the ACLT plus ESWT groups.

The results of BMD and serum COMP and osteocalcin and urinary CTX-II are shown in Table 1. The ACLT group showed significant decreases in BMD and serum osteocalcin; and increases in serum COMP and urinary CTX-II when compared with the control and the ACLT plus ESWT groups ($P < 0.05$). The ACLT plus ESWT group showed significant improvements in BMD and serum osteocalcin; and decreases in serum COMP and urinary CTX-II when compared with the ACLT group

Table 1 The results of BMD, serum COMP and osteocalcin, and urinary CTX-II

$N = 9$	Control	ACLT	ACLT + ESWT	P values
BMD (mg/cm^2)	0.422 ± 0.013	0.315 ± 0.02	0.3381 ± 0.006	$P1 < 0.001$
				$P2 = 0.114$
				$P3 = 0.036$
Serum COMP (μg/ml)	2.15 ± 0.21	3.13 ± 0.22	1.52 ± 0.22	$P1 = 0.001$
				$P2 = 0.78$
				$P3 = 0.002$
Serum osteocalcin (ng/ml)	98.2 ± 5.0	74.4 ± 2.8	177.8 ± 2.7	$P1 = 0.05$
				$P2 = 0.262$
				$P3 < 0.001$
Urinary CTX-II (pg/ml)	70.83 ± 4.44	103.7 ± 9.01	43.8 ± 3.0	$P1 = 0.038$
				$P2 = 0.996$
				$P3 = 0.005$

ACLT anterior cruciate ligament transected, *ESWT* extracorporeal shockwave therapy, *BMD* bone mineral density, *CTX-II* C telopeptide of type II collagen, *COMP* cartilage oligometric protein

*P*1: Comparison between the control group and the ACLT group

*P*2: Comparison between the control group and the ACLT + ESWT group

*P*3: Comparison between the ACLT group and the ACLT + ESWT group

The *P* values were obtained using ANOVA and LSD post hoc test

Table 2 The changes of articular cartilage and subchondral bone of the control, the ACLT, and the ACLT plus ESWT groups

	Control	ACLT	ACLT + ESWT	*P* values
Cartilage ($N = 9$)				
Cartilage fissuring (Grade: 0–3)*	0.39 ± 0.09	2.89 ± 0.21	1.72 ± 0.4	$P1 = 0.003$
				$P2 = 0.073$
				$P3 = 0.039$
Chondrocyte concentration (Grade: 0–3)*	3.63 ± 0.59	0.75 ± 0.09	2.27 ± 0.23	$P1 = 0.037$
				$P2 = 0.138$
				$P3 = 0.007$
Chondrocyte activity (Grade: 0–3)*	3.77 ± 0.47	0.73 ± 0.04	2.43 ± 0.2	$P1 = 0.022$
				$P2 = 0.089$
				$P3 = 0.006$
Chondrocyte apoptosis (Grade: 0–3)*	0.24 ± 0.05	3.13 ± 0.26	0.4 ± 0.06	$P1 = 0.006$
				$P2 = 0.101$
				$P3 = 0.003$
Mankin Score	2.0 ± 0.58	14.67 ± 2.33	4.0 ± 0.58	$P1 = 0.027$
				$P2 = 0.070$
				$P3 = 0.019$
Safranin-O stain	0 ± 0	15 ± 1.9	0 ± 0	$P1 < 0.001$
				$P2 = 0.659$
				$P3 < 0.001$
Subchondral bone ($N = 9$)				
Trabecular bone (%)	72.34 ± 1.44	45.33 ± 5.81	61.2 ± 3.24	$P1 = 0.037$
				$P2 = 0.058$
				$P3 = 0.047$
Fibrous tissue (%)	0.66 ± 0.07	22.93 ± 1.25	2.57 ± 0.24	$P1 = 0.003$
				$P2 = 0.01$
				$P3 = 0.001$
Osteocytes	54 ± 4.04	23 ± 1.15	41.33 ± 1.45	$P1 = 0.012$
				$P2 = 0.075$
				$P3 < 0.001$

*P*1: Comparison between the control group and the ACLT group

*P*2: Comparison between the control group and the ACLT plus ESWT group

*P*3: Comparison between the ACLT group and the ACLT plus ESWT group

The *P* values were obtained using ANOVA and LSD post hoc test

* The data was graded 0–3 with 0 for the lowest and 3 the highest score

($P < 0.05$), and the results were comparable to that of the control group ($P > 0.05$).

The changes of the articular cartilage and the subchondral bone are summarized in Table 2, and the microscopic features are shown in Fig. 3. In articular cartilage, the ACLT group showed significant increases in cartilage degradation (cartilage fissuring, safranin-O stain, and Mankin score) and chondrocyte apoptosis and decreases in chondrocyte concentration and chondrocyte activity when compared with the control and the ACLT plus ESWT groups ($P < 0.05$). The ACLT plus ESWT group showed significant decreases in cartilage degradation and chondrocyte apoptosis; and an increase in chondrocyte activity when compared with the ACLT group ($P < 0.05$); and the data were comparable to that of the control group ($P > 0.05$). In subchondral bone, the ACLT group showed significant decreases in trabecular bone and osteocyte as compared to the control and the ACLT plus ESWT groups ($P < 0.05$). The ACLT plus ESWT group showed significant improvement in the trabecular bone and the number of osteocyte when compared with the ACLT group ($P < 0.05$) with the results comparable to that of the control ($P > 0.05$).

Discussion

The principal findings of the current study showed that application of shockwave resulted in the improvement in

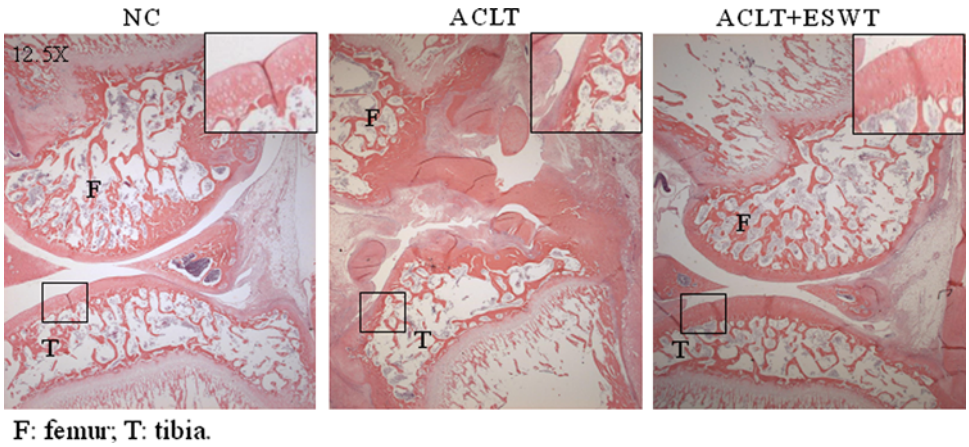

Fig. 3 Histomorphological examination with H–E stain. In articular cartilage, the ACLT group showed significant increases in cartilage fissuring and degradation, chondrocyte apoptosis and Mankin score, and decreases in chondrocyte concentration and activity when compared with the control and the ACLT + ESWT groups. The ACLT plus ESWT group showed significant increases in chondrocyte concentration and activity; and decreases in chondrocyte apoptosis, cartilage degradation and Mankin score as compared to the ACLT group and the data were comparable to the control group. In subchondral bone, the ACLT group showed significant decreases in trabecular bone and the number of osteocyte as compared to the control and the ACLT plus ESWT groups. The ACLT plus ESWT group showed significant improvement in subchondral bone remodeling as compared to the ACLT group and the results are comparable to the control

subchondral bone remodeling; and a decrease in cartilage degradation after ACLT of the knee in rats. Previous study demonstrated that shockwave showed regression of established osteoarthritis of the rat knee when ESWT was applied later in the disease [11]. The results of the current study showed that early application of ESWT results in chondroprotective effect in the initiation of ACLT osteoarthritis of the rat knees.

Emerging evidence indicates that bone turnover increases in patients with OA [5]. The subchondral bone changes have been reported in early OA [2, 5, 15]. Radin et al. [2] proposed the potential role of subchondral bone in the initiation and progression of osteoarthritis of the knee. Remodeling of the subchondral bone plate that is exposed to excessive non-physiological mechanical load results in stiffer bone of inhomogeneous density with poor shock absorption. The denser and less compliant bone can generate shear stress that alters the physiological deformation and cartilage damage. It was suggested that increased subchondral bone stiffness can reduce the ability of knee joint to dissipate the load and distribute the forces within the joint, and subsequently increases the force loads on the overlying articular cartilage, which in turn accelerates the cartilage damage over time [3, 4]. Articular damage to full thickness cartilage loss can occur upon repetitive loading over stiffened subchondral bone. Therefore, the functional integrity of the articular cartilage depends on the mechanical properties of the subchondral bone. The results of the current study support other reports that subchondral bone may play an important role in the initiation of OA of the knee with secondary articular cartilage changes [4, 5, 15].

The exact mechanism of shockwave remains unknown. Recent studies demonstrated that application of shockwave induces neovascularization and promotes angiogenesis and osteogenesis growth factors (eNOS, VEGF, PCNA, and BMP-2) that may lead to bone and joint remodeling [16, 17]. Other studies showed that substance P release from the periosteum of the femur was increased 6 and 24 h after ESWT, but was decreased in 6 weeks, however, ESWT did not alter the prostaglandin E(2) release, and suggested that substance P might be involved in the biologic action of ESWT in musculoskeletal system [18]. The results of this study revealed that ESWT improved the subchondral bone remodeling and decreased the articular cartilage degradation that may either prevent or retard OA changes of the knee. It appears that ESWT acts as a mechanotransduction that produces therapeutic benefits through complex biological pathways leading to tissue regeneration.

There are limitations in this study. The results of this study were based on small animals. The anatomy and physiology of the knee in rat may not necessarily resemble that of the human subject. Therefore, the effects of ESWT in human subjects may be different. In addition, the dosage of ESWT in this study was based on the pilot study. The optimal dosage of ESWT in OA of the knee is yet to be determined. Only one treatment was performed in each knee in this study. The results after multiple treatments may differ. Additional studies are needed to establish the therapeutic regimen of ESWT in OA of the knee.

Conclusion

Early application of ESWT shows improvement in subchondral bone remodeling and chondroprotective effect of the articular cartilage in the initiation of ACLT OA knee model in rats.

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Conflict of interest The authors declared that they did not receive any honoraria or consultancy fees in writing this manuscript. One author (CJW) had served as a member of the scientific advisory committee of Sanuwave until November 2010. The remaining authors declared no conflict of interest.

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