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Original Article

A histomorphometric study of necrotic femoral head in rabbits treated with extracorporeal shock waves

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Abstract. [Purpose] This study aimed to determine the effectiveness and mechanisms of extracorporeal shock wave therapy in the treatment of femoral head osteonecrosis. [Subjects and Methods] Histomorphometric analysis of necrotic femoral head in rabbits treated with shock waves was performed. Bilateral osteonecrosis of femoral heads was induced with methylprednisolone and lipopolysaccharide in eight rabbits. The left limb (study side) received shock waves to the femoral head. The right limb (control side) received no shock waves. Biopsies of the femoral heads were performed at 12 weeks after shock wave therapy. [Results] Necrotic femoral heads treated with shock waves, compared with controls, had higher bone volume per tissue volume, trabecular thickness, trabecular number, osteoblast surface/bone surface, osteoid surface/bone surface, osteoid thickness, mineralizing surface/bone surface, mineralizing apposition rate, and bone formation rate. However, trabecular separation was lower in shock wave-treated femoral heads than in controls. Eroded surface/bone surface and osteoclast surface/bone surface did not differ significantly between groups. [Conclusion] The bone mass of necrotic femoral heads treated with shock waves increases. Extracorporeal shock wave may promote bone repair in necrotic femoral heads through the proliferation and activation of osteoblasts.

Key words: Histomorphometry, Extracorporeal shock wave treatment, Osteonecrosis

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INTRODUCTION

Osteonecrosis of the femoral head is a progressive clinical condition primarily affecting young and middle-aged patients and is the endpoint of a disease process that results from insufficient circulation and bone-tissue necrosis[1, 2)]. Untreated symptomatic osteonecrosis of the femoral head usually leads to joint instability, collapse of the load-bearing segment of the femoral head, severe osteoarthrosis, and permanent deformity of the femoral head[3)]. The severity of the femoral head deformity is the key factor that determines the long-term outcome of a hip joint. Thus, prevention of deformity is fundamental in obtaining a favorable outcome[4)]. Both nonsurgical and surgical treatment options have been used in the treatment of osteonecrosis of the femoral head, such as conservative treatment with analgesics, core decompression, intertrochanteric osteotomy, bone transplants with vascular pedicles, and total hip replacement. Most treatment methods have confirmed limited effects in selected series, but none has demonstrated uniform success[5–7)]. Therefore, a new effective and noninvasive alternative method of treatment would be preferable over the current standard of care.

Extracorporeal shock waves are acoustic waves of extremely high pressure and velocity. They have been shown to be effective in improving bone healing and increasing bone mass and bone strength[8)]. When shock waves are directed at the bone, the reflection and deposition of shock wave energy can stimulate osteogenesis and angiogenesis[9)]. Shock wave treatment can lead to new bone formation in physiological as well as acutely fractured and pseudarthrotic bone[8, 10, 11)]. Shock wave

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treatment has been used for the treatment of nonunion in Europe and Asia since the early 1990s[11]. Recently, the results of extracorporeal shock wave treatment for osteonecrosis of the femoral head have been encouraging. Shock wave treatment appeared to be more effective than core decompression and nonvascularized fibular grafting in patients with early-stage osteonecrosis of the femoral head, although the underlying working mechanism of shock wave inducing bone repair remains to be fully established[12]. The aim of this experimental study was investigate changes in the microarchitecture and bone remodeling in necrotic femoral head of rabbits following shock wave treatment with quantitative bone histomorphometric examination.

SUBJECTS AND METHODS

All procedures performed in the experiment were approved by the local animal care and use committee. Eight New Zealand white rabbits with a body weight ranging from 3.0 to 4.0 kg were used in this study. Using lipopolysaccharide and methylprednisolone, we induced bilateral femoral head necrosis in these animals according to Yamamoto et al[13]. Shock wave treatment was applied to the femoral head of the left limb 6 weeks after injection of methylprednisolone. The right limb received no shock wave treatment and was designated as the control side. On the left side, the femoral head received 2000 shock wave impulses at 0.26 mJ/mm^2 using an Orthospec (Medispec, Gaithersburg, MD, USA). The shock wave dosage was based on the results of previous studies in animals[8, 14]. On days 7 and 2 before necropsy, all rabbits were given intramuscular injections of tetracycline hydrochloride (30 mg/kg; Sigma), which was used as fluorochrome bone markers for histomorphometry. The animals were sacrificed with an overdose of pentobarbital at 12 weeks after shock wave treatment. The bilateral femoral heads were harvested and freed of soft tissue and cartilage. They were bisected along the coronal plane and assigned for undecalcified bone processing. Quantitative bone histomorphometric analysis was performed on all the femoral heads.

Osteonecrosis of the femoral head was induced with methylprednisolone and lipopolysaccharide. The rabbits were given intravenous injection of 10 µg/kg lipopolysaccharide twice at an interval of 24 hours, and after the second injection of lipopolysaccharide, they were injected intramuscularly with 40 mg/kg of methylprednisolone thrice at intervals of 24 hours. Osteonecrosis of the femoral head occurred at 6 weeks after the third injection of methylprednisolone[15].

The specimens were fixed with 70% ethanol solution at 4 °C for 2 days, run through the alcohol series for dehydration, embedded in methylmethacrylate, and cut into 7- and 15-µm slices parallel to the coronal plane from the center of the specimen. The 7-µm slices were stained with Goldner stain. The 15-µm slices were mounted unstained for fluorescence microscopy. We evaluated the histomorphometric parameters with semiautomatic Simple PCI image analyzer (Compix, USA). All parameters were expressed according to standardized nomenclature[16]. For general bone structure, bone volume per tissue volume (BV/TV, %), trabecular thickness (Tb.Th, µm), trabecular separation (Tb.Sp, µm), and trabecular number (Tb.N, mm^{-1}) were measured and calculated. For bone formation, the following parameters were measured and calculated: osteoid surface/bone surface (OS/BS, %), osteoid thickness (O.Th, µm), mineralizing apposition rate (MAR, µm/day), mineralizing surface/bone surface (MS/BS, %), bone formation rate/bone surface (BFR/BS, µm^3/µm^2/day), and osteoblast surface/bone surface (Ob.S/BS, %). For bone resorption, eroded surface/bone surface (ES/BS, %) and osteoclast surface/bone surface (Oc.S/BS, %) were measured.

Data were expressed as mean ± standard deviation. The indices of histomorphometry were compared by two-tailed t-test analysis of variance between the shock wave-treated femoral heads and the controls. Differences were considered as statistically significant for p values<0.05.

RESULTS

The histomorphometric indices of general trabecular structure are presented in Table 1. The BV/TV, Tb.Th, and Tb.N were significantly higher in shock wave-treated femoral heads than controls. However, Tb.Sp was significantly lower in shock wave-treated femoral heads (Fig. 1).

Parameters reflecting bone formation are given in Table 2. The OS/BS, O.Th (Fig. 1), MS/BS, Ob.S/BS, MAR, and BFR/BS (Fig. 2) significantly increased in shock wave-treated femoral heads compared with controls.

Parameters related to bone resorption are shown in Table 3. There was no significant difference between the two groups in Oc.S/BS and ES/BS.

DISCUSSION

The therapy recommendations for osteonecrosis of the femoral head remain controversial. Current treatment practices vary among conservative treatment, joint-preserving procedures, and total hip replacement. Both conservative and surgical treatment modalities have been used with limited effects[5–7, 9]. Extracorporeal shock wave therapy is a promising technology that has been used in the treatment of various musculoskeletal afflictions, such as tendinopathies, delayed bone healing, pseudarthrosis, and osteonecrosis of the femoral head[11, 12, 17]. Mechanistic studies demonstrated that extracorporeal shock waves have positive influence on osseous biology through enhanced biomechanical properties, angiogenesis, and augmented

Table 1. Bone structural parameters of shock wave-treated and control femoral heads of rabbits

Bone structural parameters	Control group (n=8)	Shock wave-treated group (n=8)	
BV/TV (%)	36.4 ± 4.1	50.5 ± 6.3	**
Tb.Th (μm)	113.3 ± 10.4	145.0 ± 13.2	**
Tb.Sp (μm)	167.1 ± 14.9	130.6 ± 11.5	**
Tb.N (mm^{-1})	3.01 ± 0.28	4.06 ± 0.81	*

Results are shown as mean ± SD. BV/TV: bone volume per tissue volume, Tb.Th: trabecular thickness, Tb.Sp: trabecular separation, Tb.N: trabecular number, *$p<0.05$, **$p<0.01$

Table 2. Bone formation parameters of shock wave-treated and control femoral heads of rabbits

Bone formation parameters	Control group (n=8)	Shock wave-treated group (n=8)	
OS/BS (%)	2.55 ± 0.43	6.13 ± 1.53	**
O.Th (μm)	2.99 ± 0.46	4.14 ± 0.69	*
MAR (μm/day)	1.43 ± 0.24	2.23 ± 0.36	**
MS/BS (%)	6.51 ± 0.52	20.08 ± 2.87	**
BFR/BS ($\mu m^3/\mu m^2$/day)	0.045 ± 0.009	0.126 ± 0.020	**
Ob.S/BS (%)	2.15 ± 0.35	7.52 ± 1.40	**

Results are shown as mean ± SD. OS/BS: osteoid surface/bone surface, O.Th: osteoid thickness, MAR: mineral apposition rate, MS/BS: mineralizing surface/bone surface, BFR/BS: bone formation rate/bone surface, Ob.S/BS: osteoblast surface/bone surface, *$p<0.05$, **$p<0.01$

Table 3. Bone resorption parameters of shock wave-treated and control femoral heads of rabbits

Bone resorption parameters	Control group (n=8)	Shock wave–treated group (n=8)	
ES/BS (%)	15.6 ± 3.3	12.5 ± 2.9	***
Oc.S/BS (%)	3.3 ± 0.7	2.9 ± 0.5	***

Results are shown as mean ± SD. ES/BS: eroded surface/bone surface, Oc.S/BS: osteoclast surface/bone surface, ***$p>0.05$

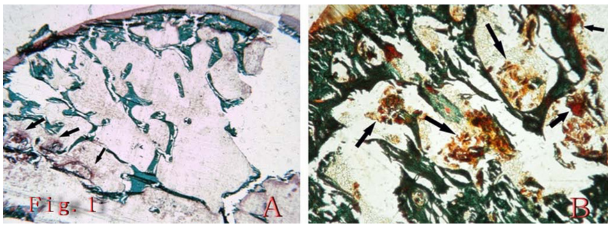

Fig. 1. Representative photomicrographs of Goldner-stained bone for untreated control (A) and shock wave–treated (B) necrotic femoral heads.
(A) Bone of the necrotic femoral heads untreated with shock waves was sparse and osteoid (arrows) was scarce. (B) Bone of the necrotic femoral heads treated with shock waves had greater bone volume. Abundant osteoid (arrows) was also observed in these femoral heads. (Goldner stain, original magnification ×40).

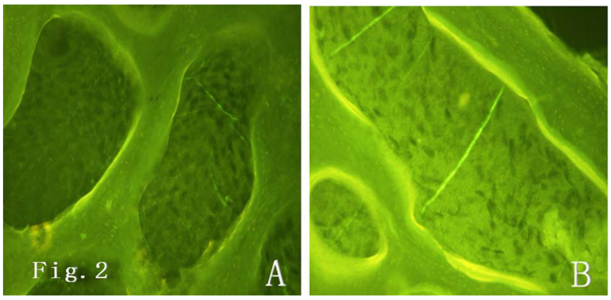

Fig. 2. Representative micrographs demonstrating vital stain of bone in untreated control (A) and shock wave–treated (B) necrotic femoral heads.
(A) Narrow distances between double fluorescent tetracycline labels in untreated femoral heads were observed. (B) Remarkable distances between double fluorescent tetracycline labels in shock wave–treated femoral heads were marked. (Original magnification ×100).

osteogenetic differentiation of mesenchymal stem cells[8, 18, 19]. However, the effects of extracorporeal shock waves on the microarchitecture and bone remodeling of the necrotic femoral heads have not been established. The results of this study showed that shock wave treatment promotes bone formation with increased bone mass in necrotic femoral heads of rabbits.

Many authors had studied the mechanism of shock wave treatment, and they documented that shock wave therapy was effective in clinical orthopedic practice and basic research because it enhances new bone formation and expression of growth factors[8, 10, 11, 20]. Our previous studies showed that extracorporeal shock wave treatment significantly upregulated the expression of bone morphogenetic protein 2 and vascular endothelial growth factor in necrotic femoral heads and accelerated the repair process of femoral head necrosis[15, 21]. Microarchitecture evaluation was considered as an interesting approach to histological evaluation of bone[22]. We applied histomorphometry in the current study to investigate the detailed changes in subchondral bone of necrotic femoral heads treated with extracorporeal shock waves. The results of this study demonstrate significant structural changes in trabecular bone volume, Tb.Th, Tb.Sp, and Tb.N of subchondral bone of necrotic femoral heads treated with extracorporeal shock waves. Increases in Tb.Th and Tb.N and a decrease in Tb.Sp result in a significant increase in trabecular bone volume. The data provide strong evidence that extracorporeal shock wave therapy promotes marked new bone formation in the necrotic femoral heads.

The effectiveness of extracorporeal shock wave therapy in promoting new bone formation observed in our study is similar to that reported for long bone fracture treated with shock waves[8]. Changes in bone remodeling may be proposed to explain the increased bone mass of the necrotic femoral heads treated with shock waves. In our study, we found that necrotic femoral heads treated with shock waves, when compared to controls, were characterized by higher Ob.S/BS, OS/BS, and O.Th, MS/BS, MAR, and BFR. These increases in MAR and BFR are indicative of an increase in osteoblastic activity at the cellular level[23]. Therefore, shock wave treatment appears to have stimulated the proliferation of osteoblasts as well as their activation to produce more and denser bone. Moreover, more osteoid observed in shock wave-treated femoral heads also indicates that shock wave treatment promotes new bone formation. However, there was no significant difference between shock wave-treated femoral heads and the controls in ES/BS and Oc.S/BS. The unchanged resorption of trabecular bone and increased formation of new trabecular bone resulted in increased bone mass and enhanced bone repair in necrotic femoral heads treated with shock waves. The results of this study are identical with those of Tamma et al.[24], who showed that shock wave treatment induced bone repair through the proliferation and differentiation of osteoblasts and the reduction of their secretion of pro-osteoclastogenic factors. It is therefore highly likely that the positive effect of shock wave treatment on osteoblasts proliferation and their activation in necrotic femoral heads contribute to the mechanism by which shock waves increase new bone formation and promote repair of necrotic femoral heads.

In summary, this study documents the general trabecular structure, trabecular formation, and trabecular resorption changes in necrotic femoral heads treated with extracorporeal shock waves. The results of denser trabecular structure and increased bone remodeling demonstrate increased bone repair in shock wave-treated femoral heads, due to proliferation and activation of osteoblasts.

Conflict of interest

No competing interest declared

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