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Shock wave treatment shows dose-dependent enhancement of bone mass and bone strength after fracture of the femur

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Abstract

Shock wave treatment is believed to improve bone healing after fracture. The purpose of this study was to evaluate the effect of shock wave treatment on bone mass and bone strength after fracture of the femur in a rabbit model. A standardized closed fracture of the right femur was created with a three-point bending method in 24 New Zealand white rabbits. Animals were randomly divided into three groups: (1) control (no shock wave treatment), (2) low-energy (shock wave treatment at 0.18 mJ/mm^2 energy flux density with 2000 impulses), and (3) high-energy (shock wave treatment at 0.47 mJ/mm^2 energy flux density with 4000 impulses). Bone mass (bone mineral density (BMD), callus formation, ash and calcium contents) and bone strength (peak load, peak stress and modulus of elasticity) were assessed at 12 and 24 weeks after shock wave treatment. While the BMD values of the high-energy group were significantly higher than the control group ($P = 0.021$), the BMD values between the low-energy and control groups were not statistically significant ($P = 0.358$). The high-energy group showed significantly more callus formation ($P < 0.001$), higher ash content ($P < 0.001$) and calcium content ($P = 0.003$) than the control and low-energy groups. With regard to bone strength, the high-energy group showed significantly higher peak load ($P = 0.012$), peak stress ($P = 0.015$) and modulus of elasticity ($P = 0.011$) than the low-energy and control groups. Overall, the effect of shock wave treatment on bone mass and bone strength appears to be dose dependent in acute fracture healing in rabbits.

Keywords: Bone mass; Bone strength; Shock wave treatment; Fracture healing; Rabbit

Introduction

Shock wave treatment has been shown to be effective in the treatment of chronic nonunions of human long bone fractures, with success rates ranging from 41% to 85% [1–10]. While many authors have used animal models to investigate the effect of shock waves on bone healing, these studies have focused primarily on radiological and histomorphological findings [11–19]. There is presently only limited data describing the effect of shock wave treatment on the biomechanical properties of bone including bone mass and bone strength. We hypothesized that shock wave treatment would result in improved bone healing, increased bone mass and bone strength. The purpose of this study is to evaluate the effect of shock wave treatment on bone mass and bone strength after fracture of the femur in a rabbit model.

Materials and methods

Animal preparation and fracture model

This study was approved by the Institutional Review Board of our institution and was performed under institutional guidelines for the care and use of animals in research.

Twenty-four New Zealand white rabbits were used in this study. The animals were 12 months of age with a mean body weight of 3.1 kg (range of 2.7–3.6 kg). The rabbits were

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anesthetized with intramuscular ketamine (25 mg/kg) and phenobarbital (30 mg/kg).

The right lower limb was scrubbed and draped in a surgically sterile fashion. A mini-arthrotomy of the knee was made through a medial parapatellar incision. A drill hole was made at the center of the intercondylar notch, and a 1.5-mm Kirschner pin was inserted into the canal of the femur in a retrograde fashion. The proximal end of the pin was exited through a separate incision over the greater trochanter and the tip of the pin was bent to prevent the pin from migration. The distal end of the pin was cut flush with the articular surface of the distal femur condyle. The incisions were closed in a routine fashion.

A standardized closed fracture of the right femur was created with a three-point bend method [20,21]. Using a three-point bending apparatus to support the right hip and the knee, a fracture at the mid-third of the right femur was created with a loading anvil, and the fracture was confirmed with X-ray examination (Figs. 1A and 1B). Special care was undertaken to avoid bending of the intramedullary pin when the fracture occurred, and the overall stability of the fracture was confirmed afterward. Compression dressings were applied to the limb with no additional external immobilization.

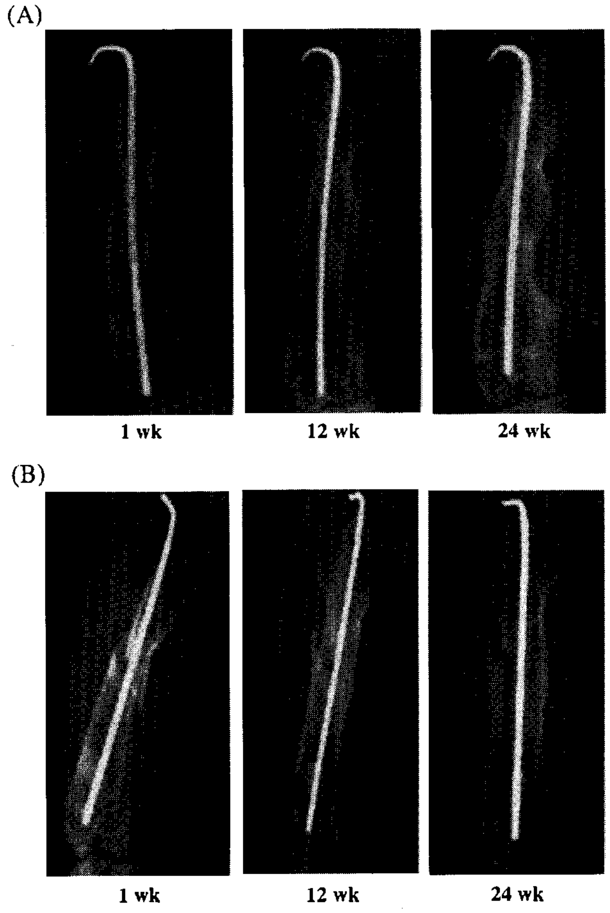

Fig. 1. Radiographs of the right femur taken at 1, 12 and 24 weeks showing progressive fracture healing in shock wave group (A) and control group (B).

Postoperative prophylaxis included ampicillin (50 mg/kg) every 12 h given intramuscularly for 5 days. The surgical wounds, the alignment and stability of the fractured limb and the overall activities of the rabbit were monitored on a daily basis.

Shock wave application

A single shock wave treatment was applied to the right femur 1 week after surgery. The rabbits were sedated with intramuscular phenobarbital (30 mg/kg) while receiving shock wave application. Shock wave treatment was applied using an OssaTron orthotripter (High Medical Technology, Kreuzlingen, Switzerland). The location of the fracture site was focused with a C-arm imaging and the depth was confirmed with the control guide of the machine. Surgical lubricate gel was placed on the skin of contact with the shock wave tube. During shock wave application, the right limb was observed for any unusual movement including muscle twitching. Immediately after the treatment, the limb was inspected for local redness, swelling or hematoma. The rabbits were then returned to housing cages with no additional immobilization. The animals were sacrificed with an overdose of ketamine and phenobarbital at 24 weeks.

Interventions

The animals were randomized to three interventional groups: control, low-energy and high-energy groups. The total acoustic energy of shock wave treatment applied to tissue is presented as the number of impulses multiplied by the energy per pulse. The low-energy group consisted of shock waves at 0.18 mJ/mm^2 energy flux density with 2000 impulses. The high-energy group consisted of shock waves at 0.47 mJ/mm^2 energy flux density with 4000 impulses. Controls received no shock wave treatment.

Radiographic evaluation

AP and lateral X-rays of the right femur were performed at 1, 4, 12 and 24 weeks to confirm adequate callus formation and fracture healing.

Bone mineral density

The measurements of bone mass included bone mineral density (BMD), callus formation, ash and calcium contents. Dual-energy X-ray absorptiometry is considered the gold standard in the measurement of BMD [20,21]. Hologic densitometry (Hologic Inc., Waltham, MA) was used to measure the BMD in this study. The BMD measurement included the proximal and distal margins of the fracture. BMD measurements were performed in 1, 12 and 24 weeks after shock wave application.

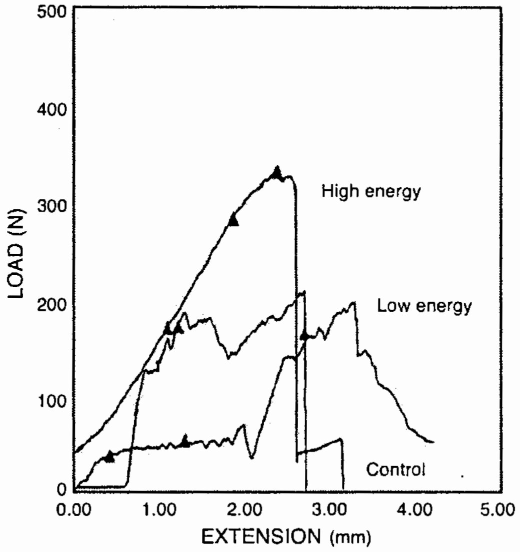

Fig. 2. A load-deflection curve of the femur bone tested on Material Testing System.

Callus size measurement

Callus dimension was determined after euthanization of the rabbit. The fracture site of the femur was identified by direct dissection. The dimension of the callus was measured (mm) in two planes at 90° using a vernier caliper (Prima 505-400, Tokyo, Japan) at the thickest point of the callus. The cross-sectional area of the fracture callus (mm^2) was then calculated from these two measurements in two different planes.

Evaluation of bone strength

Bone strength was evaluated postmortem including peak load, peak stress and modulus of elasticity [22]. The Kirschner pin was removed, and a 5-cm-long bone segment including the fracture callus was obtained from the right femur. Bone strength was measured using a three-point bending method (Material Testing System QT 10, MTS Corp., Minneapolis, MN). A supporting apparatus with two supporting points 30 mm apart was placed on the stage of the machine. The specimens were tested on a supported beam using an actuator displacement rate of 2 mm/min. To avoid rotation, the bending load was applied to the concave surface of the bone. The press head was rounded off to avoid cutting into the bone when loaded. The fracture load (lb) and the deflection of bone (mm) were read from the load-deflection curve (Fig. 2).

Ash and calcium contents

Twenty-millimeter segments of the femur bone including the fracture callus were excised postmortem from each rabbit. The specimens were dried overnight at 110°C, then cooled, weighed and ashed at 700°C in a muffle furnace. The ash was dissolved in 6 M HCl and diluted to fit within the linear portion of the standard curve. Ash content was expressed as the percentage of dry tissue weight. Calcium content (mg/g of dry tissue) was determined using an atomic absorption spectrophotometer (Perkin-Elmer, Norwalk, CT), and was calculated by interpolation from a standard curve made by a series of calcium standard concentrations (Sigma Inc., St. Louis, MO).

Statistical analysis

Data were expressed as mean ± SE and analyzed using a standard ANOVA with Bonferroni post hoc tests to determine differences between individual groups. A P-value of <0.05 was used to determine statistical significance.

Results

Bone mineral density

The results of shock wave energy flux density on BMD of the fracture callus at different time intervals are shown in Fig. 3. At 1 week, BMD values of shock wave groups were comparable as compared with the control, and the difference was statistically not significant. However, at 12 and 24 weeks, a significantly higher BMD value was noted in the high-energy group than the control (P = 0.027, 0.021), whereas that of the low-energy group was comparable as compared with the control group (P = 0.27, 0.358). The BMD values of the high-energy group were significantly higher than those of the low-energy group (P = 0.032, 0.012). The effect of shock waves on BMD changes appeared to be dose dependent.

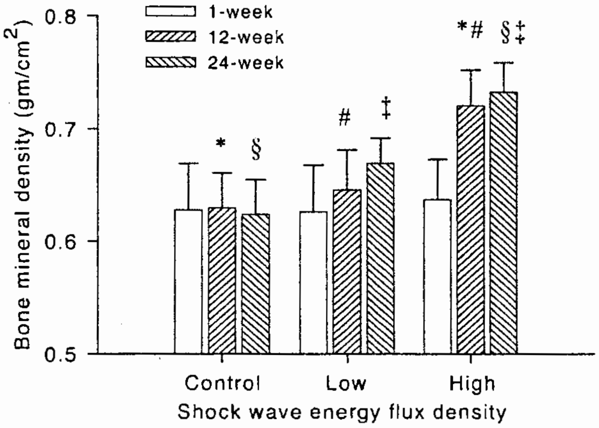

Fig. 3. Effects of shock wave energy flux density on BMD of the fracture callus at different time intervals. High-energy shock waves significantly increased BMD at 12 and 24 weeks after treatment. * (P = 0.027), # (P = 0.012), § (P = 0.037) and ‡(P = 0.031) indicate a significant difference between two groups. Results are presented with mean values ± SE calculated from eight rabbits.

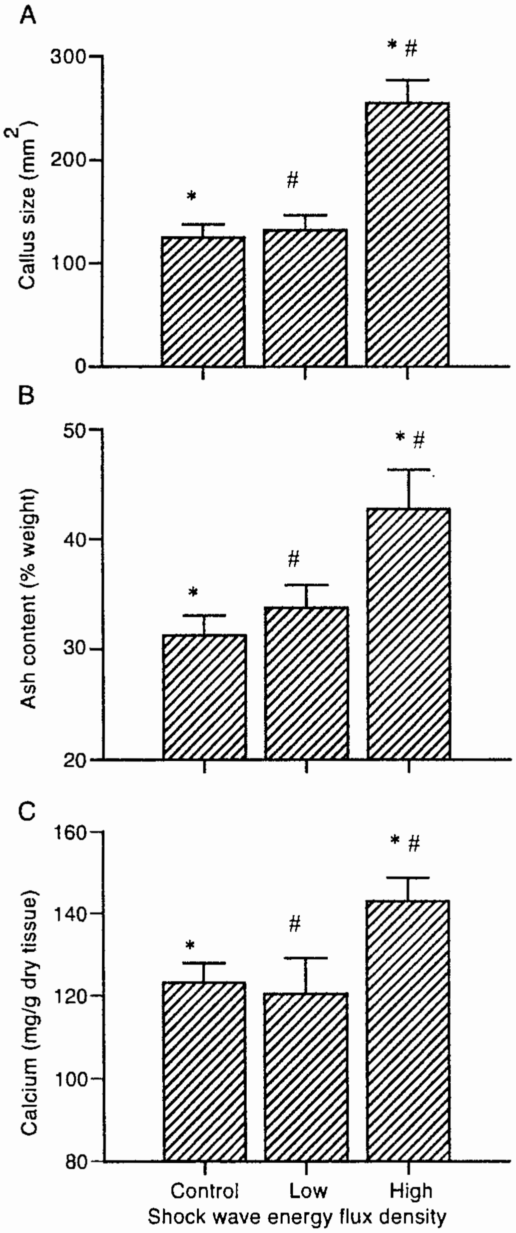

Fig. 4. Effects of shock wave energy flux density on callus size, ash and calcium contents of the fracture callus. (A) High-energy shock waves significantly increased callus size. * ($P < 0.001$) and # ($P < 0.001$) indicate a significant difference between two groups. (B) High-energy shock waves significantly increased ash content. * ($P < 0.001$) and # ($P = 0.012$) indicate a significant difference between two groups. (C) High-energy shock waves significantly increased calcium content. * ($P = 0.003$) and # ($P < 0.001$) indicate a significant difference between two groups. Results are presented with mean values ± SE calculated from eight rabbits.

BMD values significantly increased after high-energy shock waves at 12 weeks ($P = 0.037$) and 24 weeks ($P = 0.034$), whereas no significant changes were noted in the low-energy and control groups at 12 and 24 weeks, respectively ($P = 0.45$, 0.86 for the control, and 0.49, 0.50 for low-energy). The increases in BMD reached the plateau at 12 weeks, and no further increase was noted from 12 to 24 weeks ($P = 0.234$). Therefore, the effects of shock waves on BMD changes appeared to be time interval related.

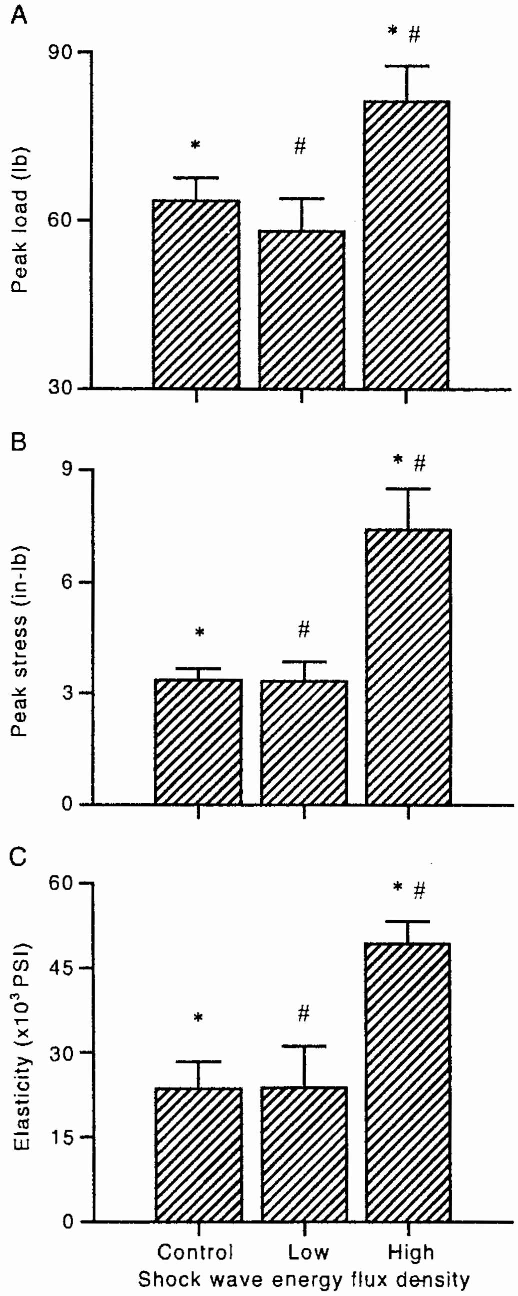

Fig. 5. Effects of shock wave energy flux density on peak load, peak stress and modulus of elasticity of the healing femur. (A) High-energy shock waves significantly increased peak load. * ($P = 0.018$) and # ($P = 0.012$) indicate a significant difference between two groups. (B) High-energy shock waves significantly increased peak stress. * ($P = 0.013$) and # ($P = 0.015$) indicate a significant difference between two groups. (C) High-energy shock waves significantly increased modulus of elasticity. * ($P = 0.002$) and # ($P = 0.011$) indicate a significant difference between two groups. Results are presented with mean values ± SE calculated from eight rabbits.

Ash and calcium contents

The results of shock wave energy flux density on callus size, ash and calcium contents are shown in Fig. 4. The high-energy group showed significantly larger callus size and higher ash and calcium contents than the control group ($P < 0.01$, <0.01 and 0.003), whereas the low-energy group showed comparable results as compared with the control ($P = 0.87$, 0.81 and 0.31). The callus size, ash and calcium contents of the high-energy group were significantly higher than those of the low-energy group ($P < 0.01$, 0.012 and <0.001). It appeared that the effects of shock waves on bone mass were dose dependent.

Bone strength

All specimens showed a typical load-displacement curve with an initial nonlinear response followed by an upward linear slop and a failure response at fracture. The results of shock wave energy flux density on bone strength including peak load, peak stress and modulus of elasticity are shown in Fig. 5. The high-energy group showed significantly higher peak load, peak stress and elastic modulus than the control ($P = 0.018$, 0.013 and 0.0021), whereas the low-energy group showed comparable results as compared with the control ($P = 0.82$, 0.77 and 0.83). The bone strength of the high-energy group was significantly higher than the low-energy group ($P = 0.012$, 0.015 and 0.011). Therefore, the effects of shock waves on bone strength appeared to be dose dependent.

Discussion

Many factors had been investigated on the influence of bone healing including electric stimulation, electromagnetic fields, piezoelectricity, ultrasound and mechanical factors such as intermittent tension, immobilization, continuous passive motion and hormonal factor, and most factors had demonstrated limited effects in selected series, but none had shown universal success [23–27]. Shock wave treatment is a new therapeutic modality that has shown promise as a non-surgical treatment for nonunions of long bones [1–10]. However, the effects of shock waves on acute fractures have not been established. In animal experiments, many studies have shown a positive effect of shock waves on fracture healing including intense formation of new cortical bone, ingrowth of neovascularization and promotion of bone morphogenetic protein [11–19,28–30], whereas others have reported negative results including reduction of mechanical stability, damage of osteocytes, bone marrow necrosis and delay of fracture healing [31–34]. The results of this study showed that shock wave treatment promotes bone healing with increased bone mass and bone strength after acute fractures in a rabbit model.

While many efforts have been made to define the mechanism of shock wave treatment, most investigations in both human and animal models have been based on radiological and histomorphological findings. These studies have not quantitatively assessed biomechanical parameters such as bone mass and bone strength on the effect of shock waves in fracture healing were based on radiologic and histomorphological findings, and they did not provide quantitative assessments of bone mass and bone strength [11–19]. Bone mass and bone strength are important outcome measures because they best describe the mechanical property and quality of bone [35]. The results of this study verified that high-energy shock wave treatment improves bone mass and bone strength, whereas the results of low-energy shock wave were less prevailing. Therefore, the effect of shock wave treatment appeared to be dose dependent. The results of this study are in agreement with that of Rompe et al. [36] who showed dose-dependent changes in the tendon and paratenon after extracorporeal shock wave treatment in a rabbit model.

In conclusion, shock wave treatment results in significant changes in bone mass and bone strength after fracture of the femur in rabbit. The effect of shock waves on fracture healing appeared to be dose dependent. This study demonstrates that the radiological and histomorphological changes observed in shock wave treatment are in fact reflected by changes in the biomechanical properties of the healing bone.

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