

Spine

Application of extracorporeal shock wave treatment to enhance spinal fusion: a rabbit experiment

Tao-Chen Lee, MD^a, Hsiu-Yu Huang, MD^b, Yu-Lin Yang, PhD^c, Kuo-Sheng Hung, MD, PhD^a, Ching-Hsiao Cheng, MD^a, Wan-Ching Lin, BS^a, Ching-Jen Wang, MD^{d,*}

Departments of ^aNeurosurgery and ^bPathology, Kaohsiung Chang Gung Memorial Hospital, Kaohsiung 833, Chang Gung University College of Medicine, Taoyuan 333, Taiwan

^cDepartment of Biological Science and Technology, Chung Hwa College of Medical Technology, Tainan 717, Taiwan

^dDepartment of Orthopedic Surgery, Kaohsiung Chang Gung Memorial Hospital, Niao-Sung, Kaohsiung Hsien 833, Taiwan

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Abstract

Background: Extracorporeal shock wave treatment has been used to treat many orthopedic disorders. However, the effect of extracorporeal shock waves on spinal fusion has not been reported.

Methods: Fifteen rabbits were used in this study. Spinal fusion was performed with decortication of bilateral L5 and L6 transverse processes, and placement of the bone chips onto the ipsilateral L5-L6 intertransverse space. The right L5 and L6 transverse processes in all animals were treated with 1000 impulses of ESWT at 14 kV (equivalent to 0.18 mJ/mm²) at 3 and 6 weeks after surgery. The left transverse processes did not receive ESWT, and were served as controls. Radiographic examinations of the spines were performed at 3, 6, and 12 weeks. Computed tomography was performed at 12 weeks. The rabbits were killed at 12 weeks, and the spinal segments were harvested for histomorphological examination.

Results: Radiographs of the tested rabbits taken at different post-ESWT stages demonstrated repairing effect of ESWT on the fusion gap of the treated (right) sides. Statistical analysis of the image studies indicated that 11 (73%) of 15 rabbits showed superior fusion mass on the ESWT (right) side than that of control (left) side ($P < .001$). The remaining 4 (27%) rabbits showed no discernable fusion difference between the ESWT side and the control side. Histomorphological examination showed good new bone formation in 9 fusion masses. All of these cases were noted on the ESWT (right) sides. Statistical analysis showed that ESWT sides had better new bone formation than the control sides ($P = .001$).

Conclusions: Results of this study demonstrated that ESWT is effective in promoting spinal fusion in rabbits.

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Keywords:

Extracorporeal shock wave treatment (ESWT); Spinal fusion; Rabbits

1. Introduction

Besides its applications in urolithiasis [2,12,21,22], ESWT was introduced for loosening of the bone-cement interface by orthopedic physicians early in 1988 [36].

Abbreviations: CT, computed tomography; ESWT, extracorporeal shock wave treatment.

* Corresponding author. Tel.: +886 7 7317123 8011; fax: +886 7 7335515.

E-mail address: w281211@adm.cgmh.org.tw (C.-J. Wang).

After a thorough investigation of its biologic effects [1,6], ESWT has been further used for the treatment of many orthopedic disorders, including tendinopathies and nonunion of long bone fractures [8,10,11,15,16,18–20,23,24,26].

The exact mechanism of shock waves remains unknown. Some studies demonstrated that ESWT causes subperiosteal callus formation by creating small fractures on the cortex (decortication) [9]. Other studies showed that ESWT stimulates expression of growth factors including vascular endothelial growth factor (VEGF) and bone

morphogenetic protein (BMP) that, in turn, improve blood supply and cell proliferation and eventual tissue regeneration [3,25,31,34,35].

To our knowledge, no study has reported the effect of ESWT on spinal fusion.

2. Methods

The institutional committee on experimental animals of Chang Gung Memorial Hospital approved this study. All animals were cared for in accordance with the regulatory provisions of the National Institute of Health, Taiwan.

2.1. Spinal fusion surgery

One-year-old male New Zealand white rabbits weighing 2.5 to 3 kg were used in this study. Bilateral posterolateral intertransverse fusion at the L5-L6 level was performed in all rabbits. Rabbits were anesthetized with intramuscular injections (50 mg/kg) of Rompun (Bayer, Leverkusen, Germany), an animal anesthetic and muscle relaxant, and Ketalar (ketamine hydrochloride; Parke-Davis, Taipei, Taiwan) (50 mg/kg). After local infiltration with Xylocaine (1% lidocaine; Fujisawa, Osaka, Japan), a dorsal 7-cm midline incision (6 cm above and 1 cm below the posterior iliac crest) was made, followed by 2 paramedian fascial incisions (2 cm lateral to the midline). The intermuscular plane was developed to expose the L5 and L6 transverse processes bilaterally. These transverse processes were decorticated with a roengeur. The bone chips bitten off with the roengeur were placed onto the ipsilateral L5-L6 intertransverse space. There was no additional use of iliac bone grafts because this might influence the effect of ESWT in this study. The levels of spinal fusion were marked with stitches on the skin, or a piece of infusion tube embedded in the subcutaneous tissue. The depth of spinal fusion to skin was also recorded. Wounds were then closed with 4-0 absorbable sutures.

2.2. Shock wave application

The levels of ESWT were determined based on stitches marked on the skin or the piece of infusion tube embedded subcutaneously at the time of initial surgery. The depth of treatment from skin to fusion site was based on the measurement during surgery.

An OssaTron machine (HMT High Medical Technologies, GmbH, Kreuzlingen, Switzerland) was used in this study. Shock waves were applied at 3 and 6 weeks after surgery. Rabbits were anesthetized with the same medications as those for fusion surgery. Surgical lubrication gel was applied to the skin in contact with the shock wave tube. ESWT of 1000 impulses at 14 kV (equivalent to 0.18 mJ/mm² of energy flux density) were delivered to the decorticated sites of right L5 and L6 transverse processes of all rabbits. The left L5 and L6 transverse processes did not

receive ESWT and served as controls. Immediately after ESWT, the animals were checked for local skin discoloration and neurologic status.

2.3. Image analysis

All study animals underwent postoperative radiography at 3, 6, and 12 weeks. Posteroanterior radiographs of the spine were obtained by using a standard technique with a tube-to-plate distance of 90 cm. Computed tomography scanning of the lumbar spine was obtained for all animals at 12 weeks before they were killed using a high-speed CT scanner (General Electric, Milwaukee, Wis) with the following variables: 10-cm field of view, 1-mm gap, and 1-mm slice thickness.

Evaluations of the results of radiographs and CT scanning were based on remodeling and trabeculation of the fusion masses, and the continuity of bone bridging between the transverse processes. The comparison of fusion result on the ESWT (right) side and the control (left) side of each animal was performed by 1 radiologist using a single blind method.

2.4. Histomorphological analysis

The animals were killed at 12 weeks, and the spinal segments of L5-L6 were harvested and the fusion mass specimens were sent for examination with light microscopy. They were fixed in 10% buffered formalin and decalcified in a 1:1 mixture of 50% formic acid and 50% sodium citrate. After decalcification, the tissues were embedded in paraffin, and stained with hematoxylin and eosin. Subsequent analysis was performed by 1 pathologist using a single blind method by examining 5 randomized 40× fields of each fusion mass. Specimens showing thick trabecular bone and mature bone marrow, and minimal intervening fibrous tissue were graded as good new bone formation. Specimens showing thin trabecular bone and prominent intervening fibrous tissue were graded as fair new bone formation. Specimens showing no trabecular bone and only fibrous tissue were graded as poor new bone formation.

3. Results

3.1. Clinical analysis

None of the tested rabbits developed neurologic deficit or incontinency throughout the course of this study. Mild ecchymosis of the skin in contact with the shock wave tube was noted on all tested rabbits. However, this skin discoloration generally disappeared in 1 week.

3.2. Image analysis

A series of post-ESWT radiographs of the tested rabbits demonstrated the repairing effect of ESWT by callus formation at the fusion gaps of the ESWT (right) side (Fig. 1). The 3D CT images of the rabbits showed good correlation with the radiographic findings (Fig. 2).

In 11 (73%) of 15 tested rabbits, radiographs and CT scanning showed superior fusion mass on the ESWT (right) side than the control (left) side. In the remaining 4 (27%) tested rabbits, no discernable difference in spinal fusion was

noted between the ESWT side and the control side. Statistical analysis using Pearson χ^2 test showed that ESWT sides had statistically significant better fusion result than the control sides ($P < .001$).

3.3. Histomorphological analysis

Thirty fusion masses from 15 rabbits were harvested from both sides of the L5-L6 intertransverse spaces. Histomorphological examination graded 9 fusion masses as good new bone formation (Fig. 3A). All these cases were noted on the ESWT (right) side. The other 21 fusion masses were graded as fair new bone formation (Fig. 3B). Among them, 15 were noted on the nontreated (left) side, and 6 on the ESWT (right) side. None of the fusion masses were graded as poor new bone formation.

Statistical analysis using Fisher exact test showed that ESWT (right) sides had better new bone formation than the control (left) sides ($P = .001$).

4. Discussion

Extracorporeal shock wave treatment has been used for treating urolithiasis for more than 20 years [2,22]. In recent years, it has been used in tendinopathy of the heel, elbow, and shoulder, as well as nonunion of long bone fracture [26-29,33].

Shock waves are high-amplitude sound waves with a sudden pressure increase at the wave front [5,7,9,17,25,36]. The width and depth of the focus areas are approximately 8 and 10 mm [9]. Our research team has demonstrated the good effect of ESWT in treating nonunion fractures, shoulder tendonitis, elbow epicondylitis, and plantar fasciitis [25,27-30,33]. In animal experiments, we have proven that ESWT is effective in enhancing expression of BMP [4,35], transforming growth factor beta 1 [3], and VEGF [31,34]. In addition, Ikeda et al [9] observed ESWT induced periosteal detachment, small fracture of the cortex, and callus formation at the sites of nonunion fractures in a human study. According to the literatures cited earlier, we hypothesize that the fundamental mechanism of ESWT could be a stimulation of growth factor expression or microdecalcification. Because our research team has had good experience in rabbit spinal fusion experiments [13,14], and no study has

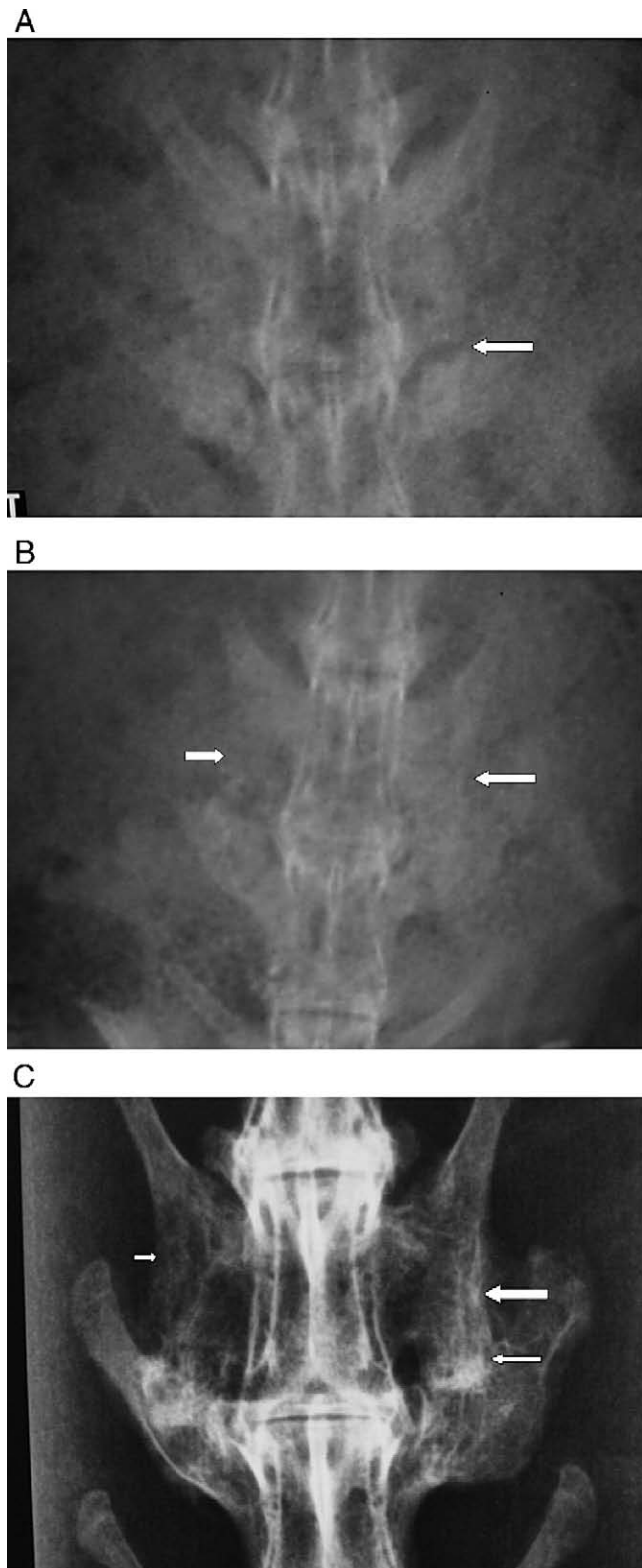


Fig. 1. A: Radiograph of the spine of rabbit no. 6 taken at 3 weeks (before shock wave treatment) exhibited comparable bony formation on both sides of the L5-L6 fusion masses. A small gap (incomplete healing) was noted at the lower portion (arrow) of the fusion mass of the ESWT (right) side. B: Radiograph of the same rabbit taken at 6 weeks (ie, 3 weeks after ESWT) demonstrated better bony formation on the ESWT (right) side (big arrow) than on the control (left) side (small arrow). The area of incomplete healing on the right fusion mass disappeared. C: Radiograph of the harvested lumbar segment of the same rabbit taken at 12 weeks revealed good fusion mass on ESWT (right) side (big arrow). The continuity, remodeling, and trabeculation of this fusion mass were superior to those of the left fusion mass (small arrow). There was callus formation (median arrow) on the previous incomplete healing area.



Fig. 2. A: Right oblique view of the 12 weeks 3D CT of the same rabbit (no. 6) shown in Fig. 1, revealing good new bone formation at L5-L6 intertransverse space (big arrow) of ESWT (right) side. There were paravertebral calcified strips (median arrow) that were speculated to be intramuscular calcified hematoma induced by ESWT. The small arrow indicates a segment of infusion tube that had been implanted during surgery as a landmark for planning ESWT. B: Left oblique view of 12 weeks 3D CT of the same rabbit reveals a depression defect (big arrow) and a fusion gap (median arrow) at the fusion mass of the L5-L6 intertransverse space of control (left) side. The small arrow indicates the implanted infusion tube.

investigated the effect of ESWT on spinal fusion, we therefore designed the current study.

The potential injury to bony structures, neural tissues, or large vessels by ESWT poses a risk when it is used in spinal fusion. Sukul et al [12] reported that ESWT, 1000 impulses at 0.6 mJ/mm^2 energy flux density, will crack rabbit femurs. Results of our studies demonstrated that high-energy ESWT, 5000 impulse at 20 kV (equivalent to 0.47 mJ/mm^2 of energy flux density), may injure the femoral artery, but causes very little damage to vein and nerve [32]. A recent study by our research team found that low-energy ESWT, 1000 impulse at 14 kV (equivalent to 0.18 mJ/mm^2

of energy flux density), causes no neurologic symptoms, with minimal microscopic myelin damage and neuronal loss, when applied directly to the rabbit spinal cord (unpublished data). It has also been noted that ESWT dosage requirement is generally higher for treating nonunions than for soft tissues or enhancing new bone formation [4,16,27-31,33,34]. Because ESWT of 500 impulses at 0.12 to 0.16 mJ/mm^2 energy flux density induces bone formation associated with a persistent increase in transforming growth factor beta 1, VEGF, and BMP expression [3,4,34,35], we decided to use a safe and effective dose of 1000 impulses at 14 kV (equivalent to 0.18 mJ/mm^2 energy flux density) to induce new bone growth in this spinal fusion study.

We did not harvest iliac bone as a graft for intertransverse fusion. Rather, decortication of the transverse

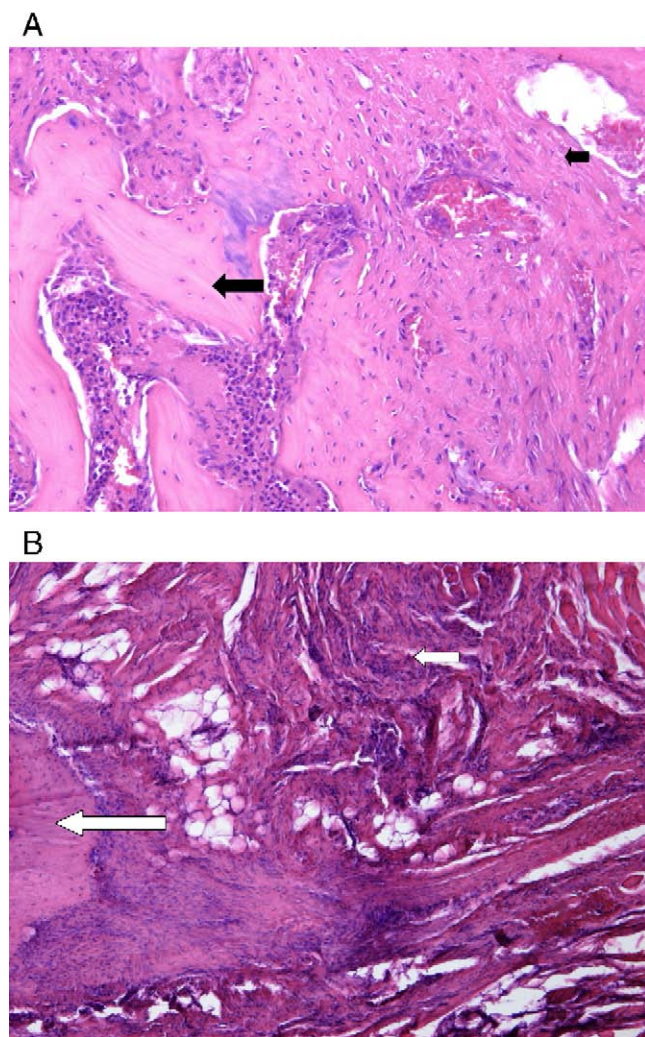


Fig. 3. A: Microphotograph ($\times 40$; hematoxylin and eosin stain) of the fusion mass at the ESWT (right) side of a rabbit shows good fusion with thick trabecular bone (large arrow) and minimal intervening fibrous tissue (small arrow). B: Microphotograph ($\times 40$, hematoxylin and eosin stain) of the fusion mass at the control (left) side of a rabbit shows fair fusion with thin trabecular bone (large arrow) and prominent intervening fibrous tissue (small arrow).

processes was performed by chipping the dorsal cortical portion of the transverse processes with a roengeur. These bone chips were then placed at the intertransverse spaces. Based on our previous observations, intertransverse fusion with a large amount of graft bone ($>2.0 \text{ cm}^2$) usually leads to good fusion in rabbits without additional treatment. By doing so, it is difficult to ascertain the pure effect of ESWT.

Biomechanical testing was not performed in the current study because the study design included ESWT on 1 (right) side of L5-L6 intertransverse space. The other (left) side of L5-L6 intertransverse space served as a control. Consequently, the biomechanical test of L5-L6 motion segments could not be performed with an materials testing system machine that we used to introduce for assessing a fusion result [14].

Based on a series of radiographs of the tested rabbits taken at different post-ESWT stages, ESWT demonstrated its effect of enhancing spinal fusion by repairing fusion gaps at the treated (right) sides. Statistical analysis of the results from image and histomorphological studies also demonstrated that ESWT (right) sides had significantly better fusion result than the control (left) sides. We therefore conclude that ESWT is effective in promoting spinal fusion in a rabbit model. We believe there is a potential role for shock wave in human spinal fusion as well.

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Commentary

Lee et al have provided a very interesting approach to enhancing bone fusion. Their results are impressive. The strategy is truly unique and very different from those

currently used. This type of innovative research is truly commendable. I am most certainly looking forward to future articles in this arena. I hope that the authors and others can provide such reports in the near future, and this manuscript should stimulate such efforts. For their efforts and quality work, the authors are to be commended.

Edward C. Benzel, MD
Department of Neurosurgery
The Cleveland Clinic Foundation
Cleveland, OH 44106, USA