Biomechanical Testing of Spinal Fusion Segments Enhanced by Extracorporeal Shock Wave Treatment in Rabbits

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Background: Extracorporeal shock wave treatment (ESWT) has been proven effective in enhancing spinal fusion in a preliminary animal study. However, biomechanical tests were not performed.

Methods: All 12 rabbits in this study underwent decortication at the bilateral L5 and L6 transverse processes. Bone was chipped off and placed onto the intertransverse space. The rabbits were divided into two groups, a study group (n = 6) and a control group (n = 6). In the study group, the bilateral L5 and L6 transverse processes were treated with 1000 impulses of ESWT at 14 kilovolts (KV) (equivalent to 0.18 mJ/mm^2) at 12 and 18 weeks after surgery. The control group rabbits did not undergo ESWT. A series of radiographic examinations on each rabbit were subsequently performed. All rabbits were killed at 21 weeks, and their spines were harvested for biomechanical tests.

Results: Radiographic examination showed 5 of the 6 rabbits in the study group had callus formation in the fusion masses. Biomechanical tests of the fusion segments showed that the mean flexion stiffness (with internal control) in the study group was 2.11 ± 0.46, while that in the control group was 1.17 ± 0.19. The mean extension stiffness (with internal control) in the study group was 1.70 ± 0.39, while that in the control group was 1.23 ± 0.29. Statistical analysis showed that the fusion segments in the study group had significantly better flexion and extension stiffness than those in the control group ($p < 0.05$).

Conclusion: In this animal study, radiographic examinations showed that ESWT stimulated new bone growth. Biomechanical tests showed that ESWT significantly increased the flexion and extension stiffness of spinal fusion segments.

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Key words: extracorporeal shock wave treatment (ESWT), spinal fusion, biomechanical test

Extracorporeal shock wave treatment (ESWT) has been utilized to treat many orthopedic disorders, including tendinopathies and non-union of long-bone fractures.[1-18] Studies have demonstrated that ESWT causes subperiosteal callus formation by creating small fractures on the cortex (decortication).[19] Additionally, other studies showed that ESWT stimulates expression of growth factors including vascu-

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lar endothelial growth factor (VEGF) and bone morphogenetic protein (BMP).[20-25]

An animal study by our research team proved that ESWT is safe in spinal experiments.[26] Additionally, our preliminary study demonstrated the positive effects of ESWT on rabbit spinal fusion masses.[27] This study evaluates the effects of ESWT on spinal fusion using a biomechanical test. As nonunion is not infrequently encountered in posterolateral fusion in human practice, we hope this study can provide a less drastic method than surgery to repair a fusion gap in failed spinal surgery.

METHODS

The Institutional Committee on Experimental Animals at Chang Gung Memorial Hospital approved this study. All animals were cared for in accordance with the regulations of the National Institute of Health, Taiwan.

Spinal-fusion surgery

This study utilized 1-year-old male New Zealand white rabbits weighing 2.5-3 kg. Bilateral posterolateral intertransverse fusion at the L5–6 level was performed on all rabbits. Rabbits were anesthetized using intramuscular injections of Rompun, an animal anesthetic and muscle relaxant (Bayer, Leverkusen, Germany) (50 mg/kg), and Ketalar (ketamine hydrochloride) (Parke-Davis, Taipei, Taiwan) (50 mg/kg). Following local infiltration with Xylocaine (1% lidocaine) (Fujisawa, Osaka, Japan), a dorsal 7-cm mid-line incision (6 cm above and 1cm below the posterior iliac crest) was made, followed by two paramedian fascial incisions (2 cm lateral to the mid-line). The intermuscular plane was developed to expose the L5 and L6 transverse processes bilaterally. These transverse processes were decorticated using a rongeur. Bone chips excised with the roengeur were placed onto the ipsilateral L5–6 intertransverse space. No additional iliac bone grafts were used in this study as this use may alter the effects of ESWT. The spinal fusion levels were marked with skin stitches. The depth between the spinal fusion and skin was recorded. Wounds were closed with 4-0 absorbable sutures.

Groups

Twelve rabbits were divided into two groups. In the study group (n = 6), the bilateral L5 and L6 transverse processes underwent ESWT, whereas the control group (n = 6) did not.

Shock wave application

The ESWT levels were determined as described before.

An OssaTron machine (High Medical Technologies HMT, GmbH, Kreuzlingen, Switzerland) was employed. Shock waves were applied at 12 and 18 weeks following surgery. Rabbits were anesthetized using the same medications as in fusion surgery. Surgical lubrication gel was applied to the skin contacting the shock wave tube. In the study group, ESWT of 1000 impulses at 14 kilovolts (KV) (equivalent to 0.18 mJ/ mm^2 of energy flux density) were applied to the decorticated sites of the bilateral L5 and L6 transverse processes. The timing of ESWT was determined based on our previous experiment that showed fusion defects remained obvious at 6 weeks, and a second ESWT significantly increased callus formation.[27] The dosage of ESWT was determined according to our previous studies that showed 14 KV is a safe and effective dose to promote spinal fusion.[26,27] Immediately following ESWT, the rabbits were checked for local skin discoloration and neurological status.

Image analysis

All study animals underwent postoperative radiography at 9, 15, and 21 weeks. Spinal posteroanterior radiographs were obtained using a standard technique and a tube-to-plate distance of 90 cm. Callus formation in the fusion gaps on either side of the intertransverse space was considered evidence of an ESWT effect.

Biomechanical analysis of fusion segments

After rabbits were killed at 21 weeks, spinal segments L4–6 and L1–3 (for internal control) were harvested from each rabbit. The soft tissue was grossly cleaned off the osseous tissue. The L4 and L6 bodies were placed into a mold, and cerro metal was poured around the bodies and allowed to cool. The cerro metal, an alloy of bismuth, tin, cadmium, and lead, melts at 70°C and, therefore, is ideal for specimen mounting. Each entire spinal segment with the molds was fixed to a custom-made apparatus for

biomechanical testing, including flexion and extension, with a material testing machine (Qtest10, MTS Systems Co., Minneapolis, MN, U.S.A.). The torque was 0.3 N m and the load rate was 25 mm/min during flexion and extension testing. Each test was repeated with the load applied 0.1 m anterior to the rotation center to generate a maximum bending moment of 0.3 N m under flexion. The load was then applied posterior to the rotation center during the extension test (Fig. 1). All stiffness values were estimated to assess the biomechanical effects of the specimens.

Stiffness of the L1–3 segments, which was studied using the same method as for the L4–6 segments, served as an internal control.

Data were analyzed by an independent-sample t-test to compare relative flexion and extension stiffness of the fusion segments in the control and study groups.

All statistical results were significant at $p < 0.05$.

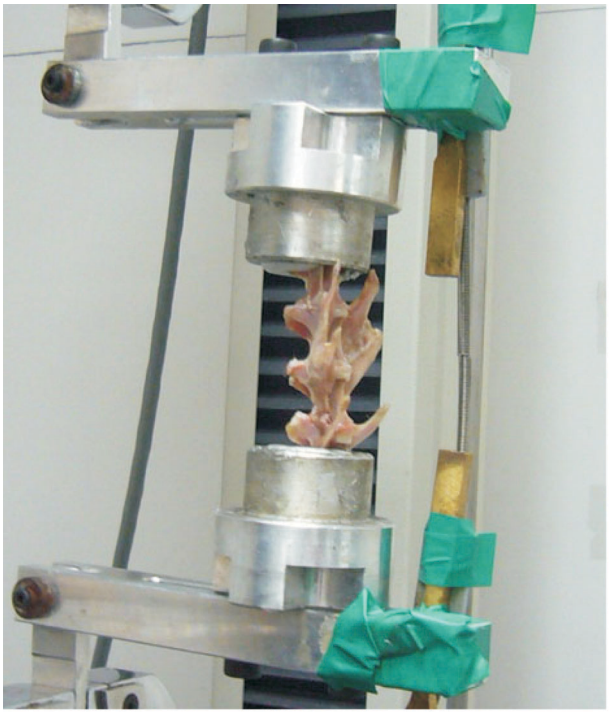

Fig. 1 After the rabbits were killed, each entire spinal segment underwent biomechanical testing with a material testing machine.

RESULTS

Clinical analysis

No rabbit tested developed neurological deficits or incontinency throughout the course of this study. Mild ecchymosis of the skin where it contacted the shock wave tube was noted in all rabbits tested. However, this skin discoloration generally disappeared in 1 week.

Image analysis

Scheduled radiographs revealed that no rabbit in the control group showed callus formation on either side of the L5–6 intertransverse space (Fig. 2). However, 5 of the 6 (83%) rabbits in the study group had callus formation in the fusion gaps on at least one side of the L5–6 intertransverse space (Fig. 3).

Biomechanical testing

The relative flexion stiffness (with internal control) of the fusion segments was 0.97–1.39 (mean ± SD, 1.17 ± 0.19) in the 6 control group rabbits and 1.36–2.67 (mean ± SD, 2.11 ± 0.46) in the study group rabbits. These experimental data indicate significantly increased flexion stiffness in the fusion segments in the study group compared with that in the control group ($p < 0.001$).

The relative extension stiffness (with internal control) of the fusion segments was 0.98–1.67 (mean ± SD, 1.23 ± 0.29) in the control group and 1.30–2.33 (mean ± SD, 1.70 ± 0.39) in the study group. These experimental data also indicate significantly increased extension stiffness of the fusion segments in the study group compared with that in the control group ($p = 0.0419$).

DISCUSSION

Shock waves are high-amplitude sound waves with a width and depth at focus areas of approximately 8-10 mm.[18,19,22,28-30] Notably, ESWT, which is effective in clinical orthopedic practice[22,31-35] and basic research, enhances new bone formation and expression of growth factors.[20,23-25,36]

As high-energy ESWT can crack animal femurs and injure animal arteries,[7,37] potential injury to neural tissues has to be clarified before applying it for spinal experiments. One of our previous studies

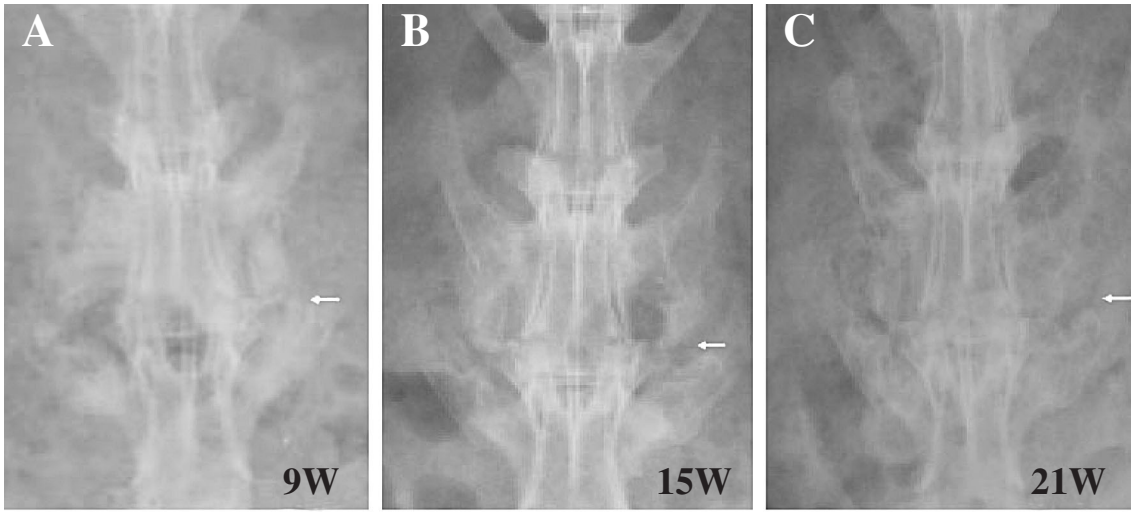

Fig. 2 A series of radiographs for 1 control group rabbit reveals a fusion gap (arrow) and no callus formation on either side of the L5–6 intertransverse space at 9, 15 and 21 weeks.

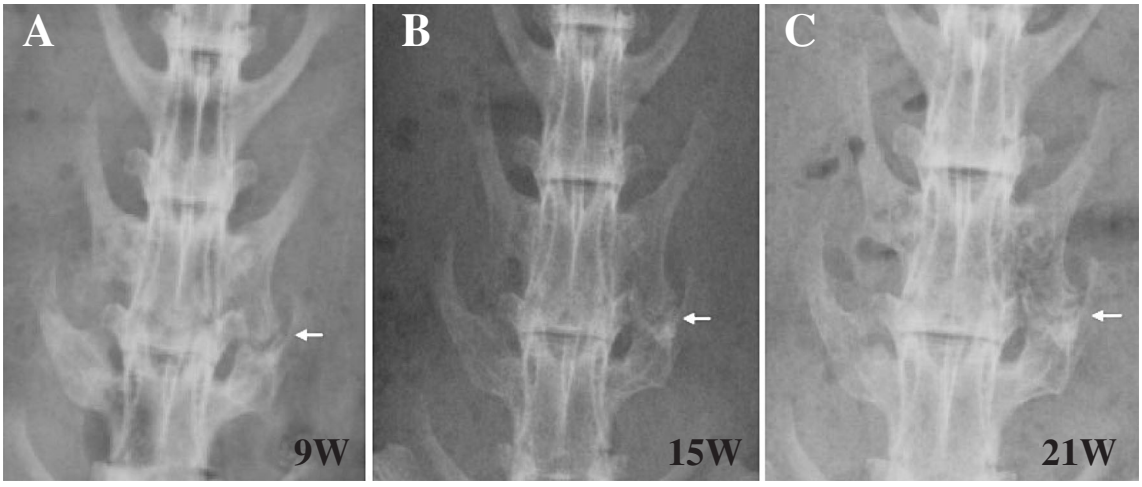

Fig. 3 A series of radiographs of 1 study group rabbit reveals a fusion gap (arrow) in the right L5-6 intertransverse space on the 9 week (before ESWT) radiograph (A). Post-ESWT callus formation (arrow) repairing the fusion gap is noted on the 15- and 21-week radiograms.

showed low-energy ESWT at 14 KV (equivalent to 0.18 mJ/mm^2 of energy flux density) is safe when applied directly to the spinal cord.[26] Therefore, this study applied this ESWT dose for the spinal fusion study.

Another animal study by our research team found that low-energy ESWT at 14 KV is effective in enhancing spinal fusion.[27] However, in that study, the fusion results were assessed only by radiographic and histological examinations. As the researchers in this study have experience in biomechanical testing,[38] this digitized modality was introduced to assess fusion results from ESWT to further confirm the effects of ESWT in spinal fusion.

Biomechanical test results demonstrate animals subjected to ESWT had significantly better stiffness of fusion segments compared with that of the control group. This experimental result is encouraging for ESWT in humans in cases of poor postoperative fusion at the intertransverse spaces.

This study did not harvest iliac bone as a graft for intertransverse fusion. Rather, decortication of the transverse processes was performed by chipping the dorsal cortical portion of the transverse processes using a rongeur. These bone chips were then placed at the intertransverse spaces. Based on observations in a previous animal study, intertransverse fusion with a large amount of graft bone (> 2.0 cm^2) typically leads to good fusion in rabbits without additional treatment.[27] Thus, ascertaining the pure effect of ESWT is difficult.

The L4–6 segments were used for biomechanical testing in this study instead of the L5–6 segments (ESWT levels) because the transverse processes are located at the rostral site of the vertebra and, therefore, the portion from the L5 body rostral to the transverse processes is too short to be securely mounted in a mold for biomechanical testing. The L4 vertebral body was therefore utilized after removal of the transverse processes, as the rostral end of the fusion segment could be mounted securely in the mold. For the same reason, this study used the L1–3 segment for internal control.

In this study, a series of radiographic examinations of tested rabbits taken at different post-ESWT stages demonstrated the effects of ESWT by callus formation in fusion gaps. The ESWT repair effect in the fusion gaps was considered to be the cause of better fusion stiffness in the study group compared with that in the control group.

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以生物力學檢測體外震波對脊椎融合的功效：兔子的動物實驗

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背 景： 體外震波已經被證明可以有效的促進動物的脊椎融合，但這項實驗尚未經生物力學的檢測。

方 法： 本實驗將 12 隻兔子執行第 5 第 6 腰椎兩側橫突去皮質手術。取下的骨頭置放於橫突之間。兔子分爲兩組。在實驗組的 6 隻兔子，兩側的第 5 第 6 橫突於 12 和 18 週接受 1000 次的低劑量 (0.18 mJ/mm^2) 體外震波治療。對照組的 6 隻兔子則不接受體外震波治療。所有兔子都接受一系列的放射線檢查。兔子於第 21 週犧牲後，手術部位的脊椎被取出作生物力學檢查。

結 果： 放射線檢查發現實驗組的 6 隻兔子中，有 5 隻在融合體上產生骨痂。生物力學檢測發現實驗組兔子的平均彎曲韌度 (經內部比照) 爲 2.11 ± 0.46，而對照組爲 1.17 ± 0.19。實驗組兔子的平均伸展韌度 (經內部比照) 爲 1.70 ± 0.39，而對照組爲 1.23 ± 0.29。統計學分析顯示實驗組在脊椎融合體的彎曲和伸展韌度皆優於對照組 ($p < 0.05$)。

結 論： 本實驗的放射學檢測發現體外震波能刺激新骨生成。生物力學檢測也證明體外震波明顯的增加脊椎融合部位的彎曲和伸展韌度。

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關鍵詞： 體外震波治療，脊椎融合，生物力學檢測

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