Histological and genetic changes affected by extracorporeal shockwave therapy after rotator cuff repair in a rat model with chronic rotator cuff tears

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INTRODUCTION: Arthroscopic rotator cuff repair (ARCR) is a widely used surgical treatment for rotator cuff tears. Although the clinical outcomes of ARCR have improved with the development of technology and instrumentation, some studies have reported postoperative retear rates between 20% and 94%. Retears lead to poor clinical outcomes and decreased quality of life. Because insufficient tendon-to-bone healing causes retears, adjunctive therapies such as recombinant human parathyroid hormone, platelet-rich plasma, stem cells, and extracorporeal shock wave therapy (ESWT) are being investigated to facilitate tendon-to-bone healing. ESWT is the least expensive of these treatments and has fewer adverse effects. Recently, its effectiveness has been recognized in the field of orthopaedics and has been used to treat plantar fasciitis, lateral epicondylitis, and other conditions. Previous studies have revealed that ESWT significantly improves load-to-failure test results in rats after rotator cuff tear repair. However, the histological and genetic changes in the tendon-to-bone ratio after ESWT have not yet been investigated. This study aimed to evaluate histological and genetic changes in the rotator cuff caused by ESWT.

METHODS: This study was approved by the Animal Care and Experimentation Committee of our institution. All efforts were made to minimize the number of animals used and their suffering. Twenty-two male retired Wistar rats with left supraspinatus tendon ruptures were created, and three weeks later, rotator cuff repair was performed using a transosseous technique with 5-0 nylon. One week later, the rats were randomly assigned to either the ESWT or control group, with 11 rats in each group. Rats in the ESWT group were irradiated with ESWT (1,000 impulses, 3 Hz, 0.22 mJ/mm^2), while rats in the control group received sham stimulation. The rats were euthanized four weeks after the ESWT or sham stimulation (Fig.1). Six specimens per group were used for histological evaluation, and the remaining five specimens per group were used for the evaluation of gene expression. For histological evaluation, we assessed histological appearance and maturation using a histological scoring system for cellularity, vascularity, and collagen fiber orientation. These parameters were compared to those of the right shoulder. To evaluate gene expression, the expression of tendon-related markers, including Cellular Communication Network Factor 2 (*CCN2*), SRY-box containing gene 9 (*Sox9*), scleraxis (*Scx*), and tenomodulin (*Tnmd*), was quantitatively evaluated by real-time polymerase chain reaction (RT-PCR). Relative expression levels of the target genes were analyzed using the comparative Ct method. The difference between the Ct values of the target genes and glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) gave the delta-Ct values. The results are presented as relative gene expression compared to that of the intact tendon set to 1. Statistical significance between groups was estimated using the Mann–Whitney U test. Statistical significance was set at $P < .05$.

RESULTS: The ratio of cellularity was significantly lower and that of collagen fiber orientation was significantly higher in the ESWT group. However, the number of blood vessels was not significantly different between the two groups (Table I). In the ESWT group, RT-PCR revealed that the expression levels of *CCN2* and *SCX* were significantly upregulated (Fig. 2).

DISCUSSION: This study had two main findings. First, ESWT can accelerate the histological repair of tendons to bones. Second, ESWT upregulated the expression of *CCN2* and *Scx*. After surgery, animal studies have revealed that in weak fibrovascular scars, there is an increase in the number of cells and blood vessels formed at the insertion site, and regeneration of the native fibrocartilaginous layer is not observed in the early stages of the tendon healing process. In this study, there was no significant difference in the number of blood vessels, but the ratio of cellularity was significantly lower and that of collagen fiber orientation was significantly higher in the ESWT group, suggesting that healing of the rotator cuff repair area was accelerated compared to that in the control group. Several recent studies have reported that *CCN2* promotes condylar chondrocyte migration, maturation, and differentiation, and *Scx* is a highly specific marker of the tendon/ligament lineage and appears to be induced at the earliest stage of specification of this lineage. In the present study, we observed an increased expression of *CCN2* and *Scx*, suggesting that ESWT affects the expression of tendon-related markers.

SIGNIFICANCE/CLINICAL RELEVANCE: These findings suggest that ESWT improves rotator cuff tendon healing.

IMAGES AND TABLES:

TABLE Ⅰ Histological Evaluation

	Cellularity (%) [a]	Vascularity [b]	Collagen Fiber Orientation (%) [c]
ESWT	113.1 ± 40.7	0.5 ± 1.2	153.5 ± 33.4
Control	204.2 ± 70.9	0.7 ± 1.6	118.4 ± 21.6
P value	0.041	0.937	0.041

Histological values are expressed as the mean ± standard deviation. (n = 6 per group).
[a] Number of cells per region of interest from each section; percentages represent relative values compared to the values from normal tendon-to-bone sections, which were set to 100%.
[b] Number of blood vessels per low field from each section.
[c] Grayscales per region of interest from each section as measured using Image J; percentages represent the relative values compared to the values from normal tendon-to-bone sections, which were set to 100%.

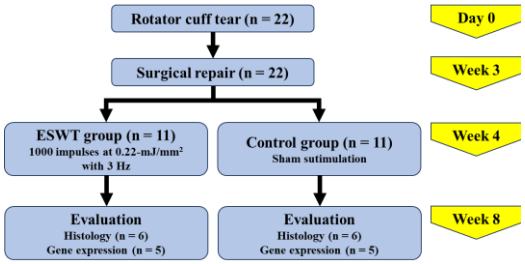

Figure 1. Flowchart of the study design.

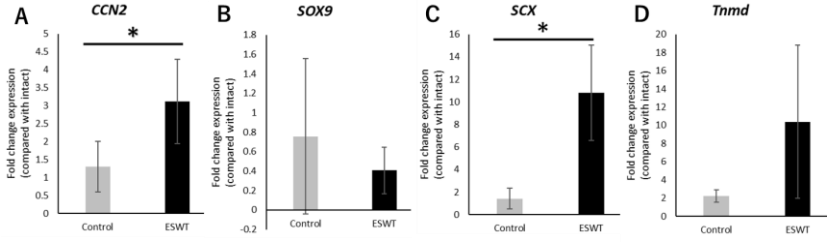

Figure 2. The expression levels of (A) *CCN2*, (B) *SOX9*, (C) *SCX*, and (D) *Tnmd* were analyzed through real-time polymerase chain reaction. The target genes are normalized to *GAPDH* expression and are normalized to that of the intact tendon, which is equal to 1. The bars show the mean for each group, and the error bar represents the standard deviation (SD).
*$P < .05$ (n=5 per group).