Effects of Shock Wave on Tenocytes Proliferation and Extra-cellular Matrix Metabolism

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Introduction: Shock waves are focused, high-energy acoustic waves and represent one of the most effective approaches to the treatment of renal calculi. Since 1990s, extracorporeal shock wave therapy (ESWT) has been found to be an effective non-invasive treatment for resolving various tendon pathologies. Despite the efficacy in clinical application, scientific evidence of shock wave therapeutic effects and biochemical mechanisms on tenocytes remain limited. The effect of shock wave on enhancing tendon healing is well recognized in animal studies. Shock wave induces neovascularization with an early release of angiogenesis-related markers such as nitric oxide synthase, proliferating cell nuclear antigen (PCNA) and vascular endothelial growth factor (VEGF) at the Achilles tendon-bone junction in rabbits [Wang CJ, et al. 2003]. The hydrosyrupine concentrations and tensile strength of injured patellar tendon has been shown to increase after shock wave exposure. Nitric oxide (NO) and transforming growth factor-β (TGF-β) are two well-known mediators of wound healing. The study aims to elucidate the effects of shock wave on cultured tenocytes, and to further investigate the molecular and biochemical mechanisms of shock wave promoting tenocytes proliferation and extra-cellular matrix synthesis in vitro.

Materials and Methods: Primary Culture of Rat Tenocytes were harvested from Achilles tendons of Sprague–Dawley rats. Tenocytes under passages 3 with normal fibroblast-shape were used in the following experiments. Dornier EMSE 220F shock wave equipment was employed for studies. The test tubes containing cell suspension were mounted into a degassed, water-filled container that can be attached directly to the shock wave generator. The focus of the shock wave is pointed correctly to the test tube. 1x10^6 Cells were suspended in a 2 ml polystyrene round-bottom tube containing 1 ml culture medium. The experiment was done using two different energy levels (0.36, 0.68 mJ/mm²) and four total impulses (50, 100, 250, 500 impulses) for each level. The shock wave triggering frequency was 120 impulses/minute. Tenocytes in the test tubes without shock wave stimulation were used as control group. At the end of treatment, cells from all tubes were counted with a hemocytometer and cell viability was determined by a 0.4% trypan blue exclusion assay. The mitochondria activity of the tenocytes after shock wave exposure was determined by colorimetric (MTT) assay. Total collagen in culture medium was analyzed by Sircol collagen assay and the NO synthesis was measured correctly to the test tube. 1

Results: Effects of Shock Wave on Cell Viability

Cells in test tubes were exposed to two levels of energy flux density (0.36 and 0.68 mJ/mm²) and four numbers of impulses (50, 100, 250 and 500 impulses). Control group was treated under the same condition without shock wave treatment. After shock wave exposure, cell viability assay was done immediately. The results showed 0.36 mJ/mm² (50, 100 and 250 impulses) revealed normal viability, whereas 0.36 mJ/mm² (500 impulses) and 0.68 mJ/mm² (50, 100, 250 and 500 impulses) significantly suppressed cell viability in a dose-dependent manner.

Shock Wave Promoted Tenocyte Proliferation

The viable cells in each group were further cultured for 24, 48 and 96 hours for cell proliferation. Low energy level with low impulses (0.36 mJ/mm² with 50, 100 impulses) in this study showed positive stimulatory effects and high energy level with high impulses (0.68 mJ/mm² with 250, 500 impulses) resulted in a significant inhibitory effects in cell proliferation.

Shock Wave Stimulated Collagen Synthesis by Tenocytes

According to the viability and proliferation results, shock wave with low energy flux density (0.36 mJ/mm²) showed normal viability and significant cytostimulatory effects. We further studied with 0.36 mJ/mm² for subsequent experiments. An optimal dose of shock wave treatment at 0.36 mJ/mm² for 100 impulses showed greater collagen synthesis (p<0.01) than other experimental groups.

Shock Wave Stimulated Nitric Oxide Release

The NO released into cell culture medium in each group was measured by Greiss reaction assay. At 24 hours, greater NO production was shown in 50 (p<0.05) and 100 (p<0.01) impulses, whereas 250 and 500 impulses revealed no effect in NO production.

Shock Wave Stimulated TGF-β1 Production

Using different shock wave impulses, we found that TGF-β1 production in 250 and 500 impulses group was less than control group significantly at 24 hours. The TGF-β1 concentration of conditioned medium of tenocytes treated with shock wave showed significant increase at 0.36 mJ/mm² for 100 impulses at 48 and 96 hours.

Shock Wave Induced Early Expression of PCNA

PCNA was essential for DNA replication and repair. Detection of PCNA was chosen to reflect tenocyte proliferation in our study. Upregulation of PCNA expression was shown after shock wave treatment in all experimental groups at 6 hours, 100 impulses group showed stronger expression than other groups. At 24 hours, 250 and 500 impulses group showed stronger expression than control, 50 and 100 impulses group (p<0.05). Experimental groups showed no more stimulatory effects than control group at 48 hours. The sequence of PCNA expression at 6 and 24 hours consisted with 24 and 48 MTT results.

Shock Wave Stimulated the Expression of Type I Collagen, Type III Collagen, and TGF-β1 at Transcriptional Level

Shock wave stimulated the gene expression of collagen type I of tenocytes in 100 impulses group at 24 (p<0.01) and 48 (p<0.05) hours. The strong expression of collagen type I shifted to 250 (p<0.05) and 500 (p<0.01) impulses group at 96 hours. Collagen type III mRNA expression after shock wave treatment showed significant increase in 100 impulses group (237.47±68.92 % of control, p<0.05) at 24 hours. TGF-β1 mRNA expression after shock wave treatment showed significant increase in 100 impulses group at 24 hours. Gel electrophoresis of PCR products showed similar results. The mRNA expression of TGF-β1 consisted with protein level TGF-β1 concentration in conditioned medium. Furthermore, the increase in TGF-β1 expression at the mRNA and protein level after shock wave treatment correlated well with the stimulatory effect on collagen synthesis of tenocytes.

Discussion: In conclusion, our study demonstrates that shock wave stimulates tenocyte proliferation and collagen synthesis. The shock wave stimulates tendon cell proliferation is mediated by early upregulation of PCNA. Shock wave stimulates collagen synthesis both in protein and mRNA level in a process that is likely mediated by NO production and upregulation of TGF-β1.