Radial shockwave effectively introduced NF-kappaB decoy into rat Achilles tendon cells in vitro

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Introduction: Recently, the extracorporeal shock wave (ESW) therapy for pain relief and for stimulating healing of tendinopathies was proved to be effective. We showed that ESW exposure to rat-footpad caused the degeneration and reinnervation of sensory nerve fibers innervating the skin [1]. Pro-inflammatory cytokines such as TNF-alpha IL-1 are known as mediators of inflammation in tendinopathies, Nuclear factor-kappaB plays a crucial role in regulating pro-inflammatory cytokine gene expression. Recent experiments to inhibit pro-inflammatory cytokine gene expression found that NF-kappaB decoy oligodeoxynucleotides (ODNs), which contained synthesized cis elements to block the activation of promoters of proinflammatory cytokine genes, effectively suppressed cytokine expression in chronic inflammatory disease[2]. Thus, NF-kappaB decoy ODNs can be applied for the treatment of tendinopathy. Recently, we reported that newly developed radial shockwave (RSW) increased the efficiency of gene transfer into cultured cells[3], which suggests that RSW may be useful for effective introduction of NF-kappaB decoy ODNs to tendon cells. The purpose of this study was to examine whether RSW can enhance the introduction of NF-kappaB ODNs into rat Achilles tendon cells and suppress NF-kappaB activation in vitro.

Materials and Methods: Cell preparation: The experimental protocol was conducted in accordance with the guidelines of the Ethics Review Committee of Chiba University for Animal Experimentation. The Achilles tendon cells were obtained from the 5-week-old SD rats by an enzymatic preparation and used after first or second passage in all experiments. Radial shock wave exposure: Radial shock waves were generated with a newly developed device, Swiss Dolorclast (Electro Medical System). The value of exposure amplitude and frequency used in this study were 0.3 MPa and 10Hz respectively. The applicator was placed in direct contact with the prepared cell suspension from the upper side of each well during the exposure period. The number of pulse application was varied as follows, (1) control (SW0), (2) one thousand shockwave impulses from the upper side of each well during the exposure period. The number of pulse application was varied as follows, (1) control (SW0), (2) one thousand shockwave impulses from the upper side of each well during the exposure period. The number of pulse application was varied as follows, (1) control (SW0), (2) one thousand shockwave impulses from the upper side of each well during the exposure period.

Effect of RSW on transduction of NF-kappaB ODNs-FITC: RSW were applied to the cells in the presence of NF-kappaB decoy ODNs-FITC in culture media. The cells were incubated for 24 hours at 37 degrees and washed with PBS 3-times, followed by examination under inverted microscopes. The numbers of total cells and FITC-positive cells were counted in microscope field (×100), and average FITC-positive rate was counted from three different fields in each wells.

Treatment of tendon cells with NF-kappaB decoy ODNs and/or RSW: In the absence or presence of NF-kappaB Decoy ODNs (1μg/ml) in culture media, RSW were applied to the cells in variable conditions as described above. After cultivation of the cells for 24 hours, they were stimulated with IL-1beta (10ng/ml) for one hour to activate NF-kappaB. The cells were collected and activation of NF-kappaB was assessed.

Measurement of NF-kappaB activation: Collected cells were separated into cytoplasmic component and nuclear component with Nuclear Extract Kit (Active Motif). NF-kappaB activation was assessed by measuring NF-kappaB p65 subunit in nuclear fraction using ELISA system (Trans AM MTM Kit, Active Motif). Since RSW affects cell viability [3], NF-kappaB activation was normalized with living cell number.

Results: Effect of RSW on transduction of NF-kappaB ODNs-FITC: Simple addition of NF-kappaB Decoy ODNs-FITC to the cell culture media (without RSW exposure) resulted in 4.48% of transduction efficiency. Application of RSW to the cells enhanced transduction of NF-kappaB Decoy ODNs-FITC with increasing its pulse number. The ratio of FITC-positive cells in the SW2000 group was 23.9%.

Discussion: The degeneration of sensory nerve fiber induced by shockwaves is supposed to be an explanation of its analgesic effect in tendinopathy cases. However, reinnervation of nerve fibers occurs within a few weeks after shockwave exposure, which can cause recurrences of the symptom. Considering several pro-inflammatory cytokines take part in the pathogenesis of tendinopathies, the use of NF-kappaB decoy ODNs, which inhibits gene expression of these cytokines, is thought to be feasible to reduce the risk of recurrence. We demonstrated here that RSW significantly enhanced introduction of NF-kappaB decoy ODNs into tendon cells. Although RSW exposure itself slightly activated NF-kappaB, combined application of RSW with NF-kappaB decoy ODNs overcomes this unfavorable effect and markedly inhibited NF-kappaB activation in the tendon cells. In addition to the direct analgesic effect of RSW, local administration of NF-kappaB decoy ODNs before exposure can augment the therapeutic effect of RSW through the decoy transduction to the target tissue.