Influence Of Lidocaine On Torn Rotator Cuff Tendon

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Introduction: Rotator cuff tear (RCT) is a common disease in the middle and elderly person, which often leads to severe shoulder pain. In patients with RCT, pain control is a key element for the conservative treatment of this disease¹.

Local anesthetics and anti-inflammatory agents are frequently injected into the subacromial bursa or glenohumeral joint in patients with RCT. Local anesthetics including lidocaine (LD) can be toxic to tendon fibroblasts in vitro 2, although its effect on RCT remains unclear. Therefore, the purpose of the present study was to determine the effects of LD on tendon fibroblasts in RCT in vitro and in vivo.

Methods: The study protocol was approved by the institutional review board (# H26-210).

Cell culture: Human rotator cuff tissues were obtained from 13 patients (mean age 64.3 ± 8.2 years) during surgery. The tendon fibroblasts from the first and second passages were used for the experiments. Eight specimens were used for cell proliferation assays and 5 specimens for cell viability assays.

Cell proliferation assays: Cultured human tendon fibroblasts were seeded onto 96-well plates at a density of 5,000 cells per well and incubated with DMEM containing 10% FBS for 24 h. The cells were treated with Lidocaine (LD) (AstraZeneca, Osaka, Japan) at various concentration (0, 0.01, 0.1, 0.5, 1.0 mg/ml) for next 24 h. The fluorescence intensity of each wells was measured with a microplate reader (BIO-RAD Model 550, CA, USA) with a wavelength of 450 nm.

Cell viability assays: The cells were seeded onto 6-well plates at a density of 1.5×10⁵ cells per well and incubated with DMEM containing 10% FBS for 24 h. The cells were exposed for the next 24 h to 1.0 mg/ml LD. After incubation for 15 min at 37°C, the numbers of viable and dead cells were obtained using FACS machine (BD Bioscience, USA).

Surgical procedure: Complete tears were made in mid portion of the supraspinatus tendon in bilateral shoulders. After laceration, 0.1 μg/g body weight of LD was applied to the right shoulder (N=20), and PBS to the left shoulder as control (N=20). The animals were sacrificed at 24h, 2w and 4w after the surgery and subjected to the experiments: 2 specimens for electron microscopy, 8 specimens for biomechanical testing and 2 specimens for histological analysis.

Biomechanical testing: The tendon-humerus complex was secured into a uniaxial testing machine (TENSILON RTE-1210, Orientec, USA). The tendon was loaded with a preload 0.1 N, followed by 5 cycles of loading and unloading with 0mm-0.5 N at cross head speed 5mm/min, and then loaded until failure at 1mm/min. The ultimate load to failure was calculated from the resulting load elongation curve.

Histological analysis: The tendon-humerus complex was fixed in 10% buffered formalin with decalcification. The specimens were subjected to hematoxylin-eosin staining.
Ultrastructural analysis: The ultrastructure of the tendon fibroblasts was evaluated 24 h after the surgery, with wide range observation of the flat block face using low voltage scanning electron microscope.

**Results:** Cell proliferation: Compared with control, LD significantly decreased cell proliferation in a dose-dependent manner (p<0.01) (Fig.1).
Cell viability: LD significantly decreased cell-viability (% Live cells: 75.0±7.0), compared with the control (%Live cells: 86.9±8.0).
Biomechanical strength: All specimen tested failed at the tendon-bone interface. At 2 and 4 weeks after surgery, the ultimate load to failure in the LD group was significantly decreased (11.5±1.9N and 18.7±1.6N, respectively) than in the control group (20.9±0.8N and 22.5±0.7N, respectively) (p<0.05). At 8 weeks after the surgery, there was no significant difference between 2 groups (p=0.109). At 2 weeks after the surgery, the stiffness in the LD group (9.4±1.3N/mm) was significantly decreased than in the control group (20.8±3.0N/mm) (p<0.01). At 4 and 8 weeks after the surgery there was no significant difference on the stiffness between 2 groups.

Histological evaluation: At 2 week after the surgery, the injured site was repaired with abundant fibro-vascular tissue at the extra-articular side in both group, although collagen bundles were irregularly arranged and cellularity was relatively less in LD group (Fig.2). Two and 4 weeks after the surgery, the collagen bundles in the control group became more organized than in the LD group. Eight weeks after the surgery, the fibro-vascular tissue was matured in both groups, with a normal arrangement of the collagen bundles.

Ultrastructural evaluation: Twenty-four hours after surgery, collagen necrosis and injured tendon fibroblasts were observed at the edge of the torn tendon where LD was directly injected (Fig.3). These changes were not detected in control group.

**Discussion:** Numerous in vitro studies demonstrate cytotoxic effects of local anesthetics on various cells. Piper et al.2 showed toxic effects of LD on cultured bovine tendon fibroblasts. Lehnoer et al.3 also demonstrated that bupivacaine inhibits viability in cultured rat Achilles tendon-derived cells, leading to a functional damage after peritendinous injection on rat Achilles tendon. However, there have been no studies on the effect of LD on torn rotator cuff tendon; therefore, we evaluated the effects of this agent on the torn tendon, using human-derived tendon fibroblasts and rotator cuff tear model in rat. As a result, LD significantly decreased cell proliferation and viability in the cultured tendon fibroblasts, inducing a delay of the collagen organization and decrease of biomechanical strength in the animal model. Thus, we conclude that physician should recognize these effects of LD on torn rotator cuff tendon when used in the clinical settings.

**Significance:** Physician should recognize the adverse effects of LD on torn rotator cuff tendon when used in the clinical settings.
Fig. 1 In vitro assay

Cell proliferation (%) and Cell viability (%) FACS

Fig. 2 Histology (PO2W)

Lidocain and Control
Fig. 3 Electron microscopy (PO 24h)

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