Effects Of High Molecular Weight Hyaluronan For Joint Capsule In An Immobilized Rat Knee Model

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Introduction: Joint immobilization causes joint contracture. The cause of joint contracture is divided in arthrogenic and myogenic components. Among these, the joint capsule plays an important role in the progression of joint contracture. Joint immobilization decreases synovial fluid and hyaluronan (HA) expression levels [1], and it also causes prolonged inflammation, adhesion, and fibrosis of the joint capsule [2][3]. A number of studies have suggested that the use of HA can prevent postoperative adhesions. However, the influence of HA on immobilized synovial tissue is still unknown. The purpose of this study was to elucidate preventive effects of high molecular weight HA on the joint capsule of immobilized knees in rats.

Methods: Animals: The unilateral knee joints of adult male rats were immobilized with an internal fixator for 1, 2, 4, 6, 8, and 16 weeks (6 rats/each group). Fifty μl of high molecular weight HA or saline (control) was administered intra-articularly on the day of surgery and once a week until euthanasia [4]. Tissue Preparation: Sagittal sections of 5 μm were prepared from the medial midcondylar region of the knee joints and assessed by histological, histomorphometric and immunohistochemical methods. Histology & Histomorphometry: The sections were stained with Hematoxylin Eosin, and morphological changes were observed. The length of the superficial layer of the synovial membrane in the antero-superior, antero-inferior, postero-superior, and postero-inferior subdivisions were separately measured [3].

Scanning acoustic microscope (SAM): The tissue elasticity of the capsule of both groups were examined by SAM. We set the region of the anterior and posterior capsule (1, 2, 4, 8, 12, and 16 weeks: n=6/each period) and their average sound speed was calculated with gray scale SAM images with image analysis software (PhotoShop CS4, Adobe Systems Inc., San Jose, CA) [5].

Number of the macrophage: To elucidate the localization of the macrophage-like type A synoviocyte, we used Cluster of differentiation 68 (CD68) as a marker [6]. Primary antibodies was mouse anti-rat CD68 monoclonal antibody (Serotec, MCA341R, dilution 1:100 ). The number of CD68 positive cells in the posterior capsule was counted.

Immunohistochemistry (IHC): Primary antibodies were rabbit anti-rat IL-6 (Abcam, ab6672, dilution 1:400 ) and mouse anti-rat CD68 antibody (Serotec, MCA341R, dilution 1:100 ) for double staining.

qRT-PCR: PCR efficiencies and relative expression levels of interleukin-6 (IL-6), IL-1β, connective tissue growth factor (CTGF), transforming growth factor-β (TGF-β), secreted protein acidic and rich in cysteine (SPARC), collagen types Ia (col1a) and IIIa ( col3a) as a function of elongation factor 1α1 (EF1α1) were calculated as previously described [4].
Results: Histology: Adhesion was observed primarily between the postero-superior synovial fold and the SM after 2 weeks both in the control and Im-HA group (Fig. 1A and D). After that, the adhesion area extended to the posterior side, diminishing the residual joint space. These changes were more progressive in the control group than those in the Im-HA group (Fig. 1B, C, D, and F). Histomorphometry: The length of the postero-superior SM was significantly longer between 4 to 8 weeks in the Im-HA group than that of the control group. SAM: The average sound speed of the anterior and posterior capsule gradually increased in both groups (Fig. 2). However, the sound speed of the control group was already at a high level at 2 weeks (Fig. 2A and D). The average sound speed of the anterior and posterior capsule in the control group were significantly higher than that in the Im-HA group in the early experimental periods. Inflammatory conditions: CD68 positive cells were mainly located at the surface layer of the SM and the fibrous layer of the capsule at 1 week in both groups. The positive cells were observed in the adhesion area after 2 weeks and gradually disappeared in both groups (Fig. 3A). Though the Im-HA group showed similar changes, the numbers of positive cells were fewer than those in the control group (Fig. 3A). CD68 positive cells at 4 weeks to 8 weeks in the control groups were significantly higher than that in the Im-HA group (Fig. 3A). In double staining of CD68 (red) and IL-6 (green), IL-6 were especially observed at 1 and 2 weeks in the control group, there is little visible in the Im-HA group, existing around CD68 positive cells, and extended adhesion area of posterior SM (Fig. 3B and C). qRT-PCR of posterior capsule: The gene expression of IL-6 and IL-1β in the control group was significantly higher at 1 week when compared with that in the Im-HA group. The gene expressions of SPARC and TGF-β were significantly higher in the control group when compared with that in the Im-HA group. The gene expressions of Col1a, Col3a, and CTGF were significantly higher in the control group when compared with that in the Im-HA group.

Discussion: Our study examined the effects of high molecular weight HA on the joint capsule of immobilized knees in rats. Adhesion and shortening of the SM is a key cause of joint contracture and induces limitation in the range of motion [4]. Several prior studies have identified concerning inflammation and fibrosis after joint immobilization [2][7]. In the present study, the intra-articular high molecular weight HA injections could prevent adhesion and shortening of the postero-superior SM. Additionally, it suppressed inflammatory and fibrotic conditions of the joint capsule in immunohistochemistry and gene expression, and influenced the elasticity of the joint capsule as a result. High molecular weight HA injections may be clinically useful to prevent changes of joint contractures in patients who need joint immobilization.

Significance: The most important finding of the present study was that intra-articular high molecular weight HA injections could suppress inflammatory and fibrotic conditions in the joint capsule, which might lead to joint contracture. Our results indicate an availability of clinical benefits of HA injections for contracture caused by joint immobilization.
Control

2-week 4-week immobilized 8-week immobilized

Im-HA

Fig. 1. Histological appearance of the posterior SM and capsule of the immobilized knee joint at 2 weeks, 4 weeks, and 8 weeks in the control groups (A–C) and in the Im-HA groups (D–F). Adhesion (asterisk) was observed primarily between the postero-superior synovial fold and the SM around the posterior horn after 2 weeks in the control groups and Im-HA groups. These changes were more progressive in the control group than those in the Im-HA group. The adhesion area (asterisk) was replaced by fibrofatty loose connective tissues. P: Femur, T: Tibia, M: Meniscus, JS: Joint space. * Asterisks: Adhesion area. Hematoxylin Eosin staining. Scale bars = 500 μm.

Control

2-week immobilized 4-week immobilized 8-week immobilized

Im-HA

Fig. 2. Scanning acoustic microscopy (SAM). The sound speed of the anterior and posterior capsule gradually increased in both groups. However, the sound speed of the control group was already at a high level in the early experimental periods (Fig. 3A to F).
Fig. 3. The number of CD68 positive cells (A). Double staining of CD68 (red) and IL-6 (green) at 2 weeks in the control group (B) (Scale bars = 200μm), and the same section as in figure B (Scale bars = 50μm) (C). CD68 positive cells at 4 weeks to 8 weeks in the control group were significantly higher than that in the Im-HA group. In double staining of CD68 (red) and IL-6 (green), IL-6 were especially observed at 1 and 2 weeks in the control group (Fig. 6B, C), existing around CD68 positive cells, and extended adhesion area of posterior SM (Fig. 6D). M: Meniscus, JS: joint space, Arrow head: Positive cells of CD68