HYALURONIC ACID INHIBITS INTERLEUKIN-1-INDUCED EXPRESSION OF PROINFLAMMATORY CYTOKINES IN SUBACROMIAL SYNOVIAL FIBROBLASTS DERIVED FROM ROTATOR CUFF DISEASE

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ABSTRACT INTRODUCTION:
Rotator cuff diseases occur frequently in middle-aged and elderly patients, and pose a significant problem as they are associated with severe shoulder pain. Voloshin et al demonstrated that proinflammatory cytokines and metalloproteases are expressed in the subacromial bursa in patients with rotator cuff disease.1 Thus, inflammation in the subacromial bursa has been proposed as the mechanism leading to pain in patients with rotator cuff disease.
A steroid is frequently injected locally to alleviate pain because this procedure has been shown to have good analgesic effects. However, many steroid-related complications have been reported. Transient synovitis developed in patients with chronic arthritis after intra-articular injection of steroids. Local injections of steroids have adverse effects not only on the articular cartilage but also on rotator cuff tendons. Despite of these complications, steroid injection has been still used for the conservative treatment of rotator cuff disease because of its excellent analgesic effects.
Hyaluronic acid (HA), a large glycosaminoglycan composed of repeating disaccharides of D-glucuronic acid and N-acetyl-glucosamine, is an important component of the articular cartilage matrix and plays an important role in the lubrication of joints. HA serves not only as a lubricant, but also protects the articular cartilage and suppresses inflammatory process in the joint. In patients with rotator cuff disease, HA injections into subacromial space are often performed. Shibata et al. demonstrated that HA injection is an effective conservative procedure for patients with rotator cuff tears.2 This may suggest the possibility that HA is an alternative to steroid for the conservative management of rotator cuff disease, although the influences of exogenous HA to subacromial synovial fibroblasts (SSF) have not been precisely clarified yet.
Interleukin-1 (IL-1) is considered to be an important reactive factor during infection and inflammation. Gotoh et al reported that IL-1 plays significant role in shoulder pain in rotator cuff disease, inducing subacromial synovitis.3 This study was undertaken to examine the inhibitory effects of HA on the production of proinflammatory cytokines (IL-1beta, IL-6, tumor necrosis factor-alpha (TNF-alpha), and cyclooxygenase2 -COX2) in IL-1-stimulated SSF derived from rotator cuff disease.

METHODS:
Human subacromial synovial fibroblasts (SSF) were obtained during surgery from 7 patients (2 partial-thickness tears and 5 full-thickness tears) with rotator cuff diseases. The average age was 58 years (range 42-72 years). The average duration of pain was 1 year (4 months to 2 years).
The specimens of subacromial synovium were obtained as near the ruptured site as possible. The synovial specimens were cut 3 mm2 in size, and human SSF were cultivated in dulbecco’s modified Eagle’s medium (DME) plus 10% fetal bovine serum (FBS). Second to third passage cells were used in the experiments.
Human SSF were seeded into 6-well plates at a density of 1x105 cells/well in DME plus 10% FBS. At 80% confluency in 6-well plates, SSF were washed with phosphate buffered saline (PBS) 3 times and cultured with IL-1bet (5ng/ml) in serum-free DME in the presence of various concentrations of HA (0.5-4.0mg/ml) (molecular weight: 9x106 Da) for 24 hours.
CD44 and binding of HA to SSF were evaluated by confocal microscopy using fluorescein-conjugated HA and tetramethylrhodamine isomer R (TRITC) – conjugated OS/37 (anti human CD44 antibody).

Real-time PCR was performed to semiquantify the mRNA of IL-1beta, IL-6, TNF-alpha and COX2 in the SSF. The volume of the cytokine-mRNAs were measured and normalized with beta-actin as an internal standard according to the delta-delta CT method. The supernatant media in the dishes were collected, and levels of PGE2 were measured by enzyme-linked immunosorbent assay (ELISA).

The Mann-Whitney U-test was used for comparison of parameters. A p-value less than 0.05 considered significant.

RESULTS:
Prior to IL-1 stimulation, immunofluorescin cytochemistry using TRITC-conjugated OS/37 was performed to confirm the presence of hyaluronic receptor (CD44) on the SSF. The results showed that SSF constitutively expressed CD44. Fluorescent HA also showed similar labeling on SSF. Subclass-matched TRITC-conjugated mouse IgG (negative control) revealed no apparent fluorescence throughout the SSF cells.
Real-time PCR showed that various concentration of HA (0.5-4.0mg/ml) significantly decreased IL-1-induced mRNA expression levels of IL-1beta, IL-6, TNF-alpha and COX2 in a dose-dependent manner.
Measurement of PGE2 in cultured media by ELISA showed that HA significantly decreased IL-1-induced PGE2 production in the SSF.

DISCUSSION:
Upregulated expression of proinflammatory cytokines in SSF in the subacromial bursa is closely associated with the generation of shoulder pain in rotator cuff disease. Subacromial injection of HA showed good clinical results (alleviation of pain) for the treatment of rotator cuff disease, although its precise mechanisms on SSF has not been elucidated.
In this study, we confirmed the inhibitory effect of HA on proinflammatory cytokines produced by IL-1-stimulated SSF derived from rotator cuff disease. In addition, HA significantly decreased the production of PGE2 in the cultured media. Our data therefore support the clinical utility of HA for the conservative treatment in rotator cuff disease.
CD44 plays a major role in multiple physiological and pathological functions. Stimulation of CD44 transmits the signal into the cell, leading to activation of release of cytokine/chemokine from macrocytes and rheumatoid synovial fibroblasts.4 Our immunohistochemical technique identified the CD44 immunoreactivity and binding of HA to CD 44 on the SSF derived from rotator cuff disease. These results may suggest the possibility that HA exerts its effect by downregulation of CD44 level. Studies on these mechanisms are now ongoing in our laboratory.

In the present study, we demonstrated that HA effectively inhibits the production of IL-1beta, IL-6, TNF-alpha, COX2 and PGE2 in IL-1-stimulated SSF derived from rotator cuff disease. These results provide one of the rationale reasons for clinical use of HA in the treatment of rotator cuff disease.

REFERENCES:

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