HYALURONIC ACID INHIBITS mRNA EXPRESSION OF PRO-INFLAMMATORY CYTOKINES AND CYCLOOXYGENASE-2/PROSTAGLANDIN E2 PRODUCTION VIA CD44 IN INTERLEUKIN-1-STIMULATED SUBACROMIAL SYNOVIAL FIBROBLASTS FROM PATIENTS WITH ROTATOR CUFF DISEASE

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Introduction: Rotator cuff disease occurs frequently in middle-aged and elderly individuals, and poses a significant problem as it is associated with severe shoulder pain. Concomitant subacromial synovitis contributes to the generation of shoulder pain in patients with rotator cuff disease.⁴ Proinflammatory cytokines and enzymes (interleukin-1 beta: IL-1β, tumor necrosis factor alpha: TNF-α, interleukin-6: IL-6, and Cyclooxygenase-2: COX-2) produced by subacromial synovial fibroblasts (SSF) play a major role in the generation of shoulder pain.⁵ Our previous studies have demonstrated that the level of IL-1β mRNA expression in SSF correlates well with the degree of the shoulder pain in rotator cuff disease.⁶

Hyaluronic acid (HA) suppresses the production of IL-1β, IL-6, TNF-α and COX-2/ProstaglandinE2(PGE2) in the osteoarthritic joint.⁴ In rotator cuff disease, Shibata et al. have uniquely demonstrated that HA injection is an effective conservative procedure for patients with rotator cuff disease⁵, although the mechanism of its anti-inflammatory effect is not fully understood.

On the basis of these background factors, it has been hypothesized that HA may have an anti-inflammatory effect in patients with subacromial synovitis associated with rotator cuff disease. In the present study, we investigated the effect of HA on the expression of mRNAs for proinflammatory cytokines and COX-2/PGE2 production in IL-1-stimulated SSF from patients with rotator cuff disease.

Materials and Methods: Specimens of the subacromial synovium were obtained during surgery from 7 patients with rotator cuff disease. The average age of the patients was 58 years (range 42-72 years), and the average duration of symptoms was 1 year (4 months to 2 years).

The synovial specimens were cut into pieces 3 mm2 in size, and human SSF were cultivated in Dulbecco's modified Eagle medium (DMEM). Second to third passage cells were used in the experiments.

The cells were fixed with 4% paraformaldehyde in PBS for 30 min. After blocking, the samples were then incubated with anti-human CD44 antibody OS/37 or subclass-matched nonspecific mouse IgG1 for 12 h at 4°C. After washing, the cells were stained with polyclonal rabbit anti-mouse immunoglobulins conjugated with tetramethylrhodamine isomer R (TRITC). To investigate the binding of HA (molecular weight 90x104, Seikagaku Co., Tokyo, Japan) to the cells, SSF were incubated with fluorescein-conjugated HA in DMEM for 30 min at 4°C.

All samples were subjected to confocal microscopy analysis.

Total RNA was isolated from the cells using a Qiagen mini kit in accordance with the manufacturer's instructions.

After reverse transcription, real-time PCR was performed. The amounts of mRNA for IL-1β, IL-6, TNF-α, and COX-2 were measured and normalized against β-actin as an internal standard according to the delta-delta-CT method.

After incubation, the supernatant medium in the dishes was collected, and its level of PGE2 was measured using an ELISA kit. Assays were performed in accordance with the manufacturer's recommendation.

Paired t-test or repeated measurement ANOVA was used for comparison of parameters. Spearman correlation rank test was used to evaluate relationships among different parameters. Differences at p<0.05 were considered significant.

Results: Immunofluorescence cytochemistry confirmed the binding of HA and the presence of CD44 on SSF. Exogenous HA significantly and dose-dependently decreased the expression of proinflammatory cytokine mRNAs and COX-2/PGE2 production in IL-1-stimulated SSF. Pretreatment with OS/37 reversed the inhibitory effects of HA on expression of proinflammatory cytokine mRNAs and COX-2/PGE2 production in IL-1-stimulated SSF. Thus, HA inhibited the expression of proinflammatory cytokine mRNAs and COX-2/PGE2 production via CD44 in IL-1-stimulated SSF derived from patients with rotator cuff disease.

Discussion: A clinical benefit of HA in rotator cuff disease has been demonstrated by Shibata et al.[⁵], although little is known about its anti-inflammatory effect in the disease. The present study clearly demonstrated that HA inhibits not only the expression of mRNA for proinflammatory cytokines (IL-1β, IL-6, and TNF-α) but also COX-2/PGE2 production via CD44 in IL-1-stimulated SSF. These results suggest that HA has an anti-inflammatory effect on the expression of proinflammatory cytokine mRNAs and COX-2/PGE2 production produced by IL-1-stimulated SSF in this disease. To our knowledge, such findings have not been reported previously.

In order to investigate whether the mechanism of HA action is biologically mediated by CD44 on IL-1-stimulated SSF, we performed a HA-binding inhibition experiment using the anti-CD44 blocking antibody OS/37. We found that pretreatment of SSF with OS/37 reversed the inhibitory effects of HA on the expression of mRNAs for pro-inflammatory cytokines and COX-2/PGE2 production in IL-1-stimulated SSF from patients with rotator cuff disease. These findings suggest that the inhibitory effects of HA on the expression of proinflammatory cytokine mRNAs and COX-2/PGE2 production are mediated through interaction with CD44. How HA interacts with CD44 in SSF remains to be elucidated in a future study.

The present study demonstrated an anti-inflammatory effect of HA in IL-1-stimulated SSF from patients with rotator cuff disease, which involved down-regulation of proinflammatory cytokine mRNAs (IL-1β, IL-6, and TNF-α) and COX-2/PGE2 production. Thus, our results provide a basis for explaining why HA is effective for the treatment of rotator cuff disease, and support its clinical utility.

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References: