Hylan Treatment Inhibits PGE2 Production by Regulating COX-2 Expression in Chondrocytes and Synoviocytes

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Introduction: Intra-articular injections with hyaluronan (HA) and hylan (cross-linked HA) have been used clinically to treat osteoarthritis (OA) pain. The cellular and molecular mechanisms of HA in OA pain relief are largely unknown. Accumulating evidence has suggested that prostaglandin E2 (PGE2) directly contributes to pain hypersensitivity by sensitizing peripheral nociceptors, reducing threshold and/or increasing responsiveness. We hypothesize that HA relieves OA pain in part by inhibiting PGE2 production in chondrocytes and/or synoviocytes. In this study, we examined the effects of a high molecular weight (Mw) chemically cross-linked HA, hylan G-F 20, on PGE2 production and COX-2 gene expression using cartilage explant and synoviocyte cultures in response to IL-1β and using a rabbit model of OA.

Materials and Methods: Experimental OA model: Male New Zealand White rabbits (8-12 months old, 3.2-4.5 kg body weight) underwent either sham surgery or anterior cruciate ligament transection (ACLT) to induce OA. All animal studies were approved by the Institutional Animal Care and Use Committee.

RNA isolation and real-time PCR analysis: Total RNA was isolated from cultured cells using RNeasy kit (Qiagen) and from tissues using Trizol (Invitrogen). Human COX-2 gene specific primers and probes were purchased from Applied Biosystems. Rabbit COX-2 gene specific primers and probes were designed using Primer Express software (Applied Biosystems). The PCR reactions were carried out in triplicate using ABI Prism 7900 Sequence Detector. The Ct value of the target gene was subtracted by that of the internal control to generate ΔΔCt. The relative expression levels of the target gene were expressed as fold change over normal tissues or untreated controls as baseline (2^-ΔΔCt).

Statistical analysis: Student’s t-test was used to analyze the significance of difference. A p value less than 0.05 was considered significant.

Results: To examine the effects of hylan on PGE2 production in vitro, bovine cartilage explant cultures were treated with IL-1β (5 ng/ml) for 2d, and hylan G-F 20 (1 mg/ml) was added in the presence of IL-1β for another 3d. Since hylan G-F 20 is composed of 80% hylan A fluid and 20% hylan B gel, these components were also tested separately. PGE2 levels in the medium were determined by ELISA and normalized to cartilage wet weight (Figure 1). Bovine cartilage explants produced low basal levels of PGE2, which were increased by IL-1β. Co-incubation of IL-1β with a COX-2 selective inhibitor, NS-398, completely abrogated PGE2 up-regulation, suggesting that IL-1β induced PGE2 production is mediated by COX-2. In IL-1β treated cultures, the addition of hylan G-F 20 reduced PGE2 levels. Furthermore, hylan A inhibited IL-1β induced PGE2 significantly (p<0.05), whereas hylan B had no significant effect. To confirm these results, we performed a similar experiment using cartilage explants from a 79-yr old donor without OA symptoms. Similar to our findings in the bovine cartilage, hylan G-F 20 treated human explant cultures had decreased levels of PGE2 in the conditioned medium compared with non-treated IL-1β controls (0.90 vs. 2.62 ng/ml).

We next examined if hylan treatment had a similar effect on synoviocytes. Human synoviocytes were pre-incubated with hylan G-F 20 at different concentrations, 0.04, 0.2 and 1 mg/ml, for 16h. IL-1β together with hylan G-F 20 was added for another 24h. Medium was collected for the PGE2 assay and RNA was isolated for COX-2 gene expression analysis by real-time PCR. Hylan G-F 20 at 0.2 and 1 mg/ml, but not 0.04 mg/ml, inhibited IL-1β induced PGE2 production. The reduction in PGE2 levels as a result of hylan G-F 20 treatment correlated with decreased levels of COX-2 mRNA. Taken together, we concluded that hylan G-F 20 inhibited IL-1β mediated PGE2 production by regulating COX-2 expression.

To examine if hylan G-F 20 inhibits COX-2/PGE2 pathway in vivo, we used a rabbit ACLT model of OA. Four weeks after the surgical induction of OA, a series of 3 weekly intra-articular injections was performed in the operated knee of 0.5 ml of hylan G-F 20 (8 mg/ml) or Lactated Ringer’s Solution (LRS) as vehicle control. Animals were sacrificed at one week after the final injection. Synovial fluid (SF) was collected by joint lavage and PGE2 levels were determined by ELISA. Total RNA was isolated from synovium and COX-2 gene expression was analyzed using real-time PCR. We found that hylan treated OA joints had a trend of decrease in PGE2 levels in the SF and COX-2 mRNA expression in the synovium. Interestingly, SF from an OA patient had reduced PGE2 levels one week after the first hylan G-F 20 injection, which remained low one week after the second injection.

Discussion: Using culture systems, we have demonstrated that hylan G-F 20 inhibits IL-1β induced PGE2 production in chondrocytes and synoviocytes. HA may exert its role by negatively regulating COX-2 gene expression in these cells. In the rabbit ACLT model of OA, PGE2 levels in SF and COX-2 expression in synovium have a trend of increase in OA joints and a trend of decrease after intra-articular injections with hylan. PGE2 levels in SF from an OA patient show a similar trend of decrease after hylan treatment in comparison with pre-treatment. Taken together, these findings suggest that one of the mechanisms by which HA relieves OA pain clinically may be by modifying COX-2/PGE2 pathway in chondrocytes and synoviocytes. Due to the small sample size and inter-animal variations, no statistical significance was found in SF PGE2 levels from the OA animals with or without hylan treatment. Future studies will be planned to compare PGE2 levels from the same animal prior and post treatment to minimize the variation. More data will be collected from human OA patients to continue the current work.

Figure 1. Hylan treatments inhibited IL-1β-induced PGE2 production in bovine cartilage explants. Kineret, IL-1β antagonist. NS-398, selective COX-2 inhibitor. The data are expressed as mean ± SD. * p<0.05 and # p=0.07 in comparison with IL-1β treatment controls (n=3).