INTERCELLULAR ADHESION MOLECULE-1 MEDIATES THE INHIBITORY EFFECTS OF HYALURONAN ON INTERLEUKIN-1β-INDUCED MATRIX METALLOPROTEINASE PRODUCTION IN RHEUMATOID SYNOVIAL FIBROBLASTS VIA DOWN-REGULATION OF NF-κB AND p38

Objective. In rheumatoid arthritis (RA), it is well known that rheumatoid synovial fibroblasts (RSFs) produce matrix metalloproteinases (MMPs) stimulated by cytokines such as interleukin-1β (IL-1β), which causes joint destruction, and up-regulate adhesion molecules expression.

It has been reported that hyaluronan (HA) injection into RA joints has clinically beneficial effects, but how it works remains to be clarified. We have previously shown that HA inhibits IL-1β actions in RSFs via CD44, one of HA receptors, but that CD44 only mediates the HA effects partially, which led us to speculate that other receptors of HA do as well. Intercellular adhesion molecule-1 (ICAM-1), one of adhesion molecules, is one of HA cell surface receptors, but is not revealed how it works.

Thus, we investigated whether HA affected matrix metalloproteinase (MMP) -1 and -3 productions induced by IL-1β in RSFs and whether ICAM-1, one of HA cell surface receptors, was involved in the HA effects via which intracellular pathways.

Methods. Human rheumatoid synovial tissues were obtained from patients with RA at total knee replacement surgery. RSFs were isolated from the rheumatoid synovial tissues by enzymatic digestion and were cultured in monolayer. The confluent cells were incubated for 48 hours with IL-1β at 2 ng/ml, IL-1β at 2 ng/ml plus HA at the various concentrations (0.1, 1, 2, 3, or 5 mg/ml), IL-1β at 2 ng/ml plus HA at 3 mg/ml after the pretreatment of anti-ICAM-1 antibody at the various concentrations (5 or 50 µg/ml), or without any treatment.

Immunoblotting analyzed MMP-1 and MMP-3 secretions of the cell lysates of RSFs treated with monensin. Immunofluorescent cytochemistry was performed to evaluate HA binding to ICAM-1. The phosphorylation of nuclear factor (NF) -κB and mitogen-activated protein kinases (MAPKs) was analyzed by immunoblotting. All data are expressed as mean ± SD.

The data of densitometric analysis were evaluated by Mann-Whitney’s U test. Significant differences were set at p<0.05.

Results. MMP-1 and MMP-3 were markedly produced by IL-1β at 2 ng/ml stimulation, and HA at 2 mg/ml and more significantly inhibited MMP-1 and MMP-3 productions induced by IL-1β in a dose dependent manner. Moreover the pretreatment with anti ICAM-1 antibody at 50 µg/ml significantly attenuated HA effect for IL-1β action on RSFs (Fig. 1). In monensin-treated RSF, HA decreased MMP levels enhanced by IL-1β. Pretreatment with 50 µg/ml anti-ICAM-1 antibody reversed the MMP levels in the cell lysates with treatment with both IL-1β and HA.

Immunofluorescent cytochemistry demonstrated that, indeed, HA bound to RSFs (Fig. 2A), and that the pretreatment with anti ICAM-1 antibody partially prevented HA from binding to RSFs (Fig. 2D). It also showed that anti ICAM-1 antibody did not bind to RSFs after the pretreatment with HA (Fig. 2C). Therefore HA bound to RSFs, at least partially, via ICAM-1.

Inhibition study revealed the requirement of NF-κB, p38, and c-jun NH2-terminal kinase (JNK) for IL-1β-induced MMP production. IL-1β activated all the three transcription factors, while HA down-regulated their phosphorylation. Pretreatment with anti-ICAM-1 antibody reversed the inhibitory effects of HA on activation of NF-κB and p38 without affecting JNK.

Conclusion. This study has clearly demonstrated that HA suppresses IL-1β-enhanced MMP-1 and MMP-3 synthesis in RSF via ICAM-1 through down-regulation of NF-κB and p38. Exogenous HA of high molecular weight by intraarticular injection may work through such a mechanism in RA joints.

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