Nuclear Factor-κB Activation by Type II Collagen Peptide in Rheumatoid Arthritis Chondrocytes: Its Inhibition by Hyaluronan via CD44 and ICAM-1

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Introduction: Degradation products of cartilage matrix are important amplifiers or catabolic players in diseased joints like rheumatoid arthritis (RA). Excessive degradation of cartilage matrix in RA involves enhanced cleavage of type II collagen by collagenases, especially matrix metalloproteinase (MMP)-13, resulting in denaturation of the triple helix of this collagen. Denatured and degraded type II collagen leads to an increase in proteolytic products of type II collagen. A 24-mer synthetic peptide of type II collagen (CB12-II) can stimulate type II collagen cleavage with MMP-13 induction in cartilage explant culture [1, 2]. The intracellular signaling that leads to cartilage destruction is mediated by catabolic pathways including nuclear factor-κB (NF-κB). The prototypical NF-κB complex consists of a p65-p50 heterodimer. Activity of NF-κB is regulated by the phosphorylation and degradation of IκB, an endogenous inhibitor that binds to NF-κB in the cytoplasm. Phosphorylation of p65/RelA is also required to activate NF-κB-dependent transcription. The released NF-κB translocates to the nucleus where it binds to specific NF-κB DNA binding sites and initiates gene expression. NF-κB activates gene expression from NF-κB sites in association with the transactivation domains located in the carboxyl-terminus of the p65 protein. At present, little is known of how CB12-II affect NF-κB in chondrocytes. Hyaluronan (HA) of high molecular weight is now used in the treatment of RA by intra-articular injection in Japan. HA can bind several cell surface receptors. The principle HA receptor is CD44, which is up-regulated in in vivo articular cartilage from patients with RA. Intercellular adhesion molecule-1 (ICAM-1), another HA receptor, is also expressed on chondrocytes. There is evidence that such inhibitory effects by HA are mediated through its cell surface receptors. Accumulating data indicate that HA down-regulates intracellular signals activated by catabolic stimuli. This study was aimed to examine NF-κB activation by CB12-II and its inhibition by HA via its receptors, CD44 and ICAM-1 in RA chondrocytes.

Methods: Cartilage explants harvested from RA knee joints or isolated chondrocytes in monolayer were incubated with CB12-II or its scramble peptide with or without pretreatment with 2700 kDa HA. In another set of experiments, following preincubation with the anti-ICAM-1, anti-CD44, a combination of both antibodies, or non-specific IgG, chondrocytes or cartilage explants were incubated with or without 2700 kDa HA, followed by coincubation with CB12-II or the scramble peptide. Enzyme-linked immunosorbent assays for phosphorylated p65 NF-κB and MMP13 were performed using total cell lysates and culture supernatants, respectively.

Results: Treatment of RA cartilage explants with CB12-II resulted in enhanced MMP-13 production in a dose-dependent manner. In contrast to CB12-II, the scramble peptide failed to enhance the collagenase production. When RA cartilage explants were pretreated with the NF-κB inhibitor, CB12-II-stimulated MMP-13 production was significantly suppressed in a dose-dependent manner. HA also significantly suppressed CB12-II-stimulated MMP-13 production. Similar to the findings in cartilage explant cultures, CB12-II stimulated MMP-13 production in RA chondrocyte monolayer cultures. NF-κB inhibitor and HA individually suppressed the collagenase production by the peptide. CB12-II caused significant phosphorylation of p65 NF-κB, leading to enhanced NF-κB translocation into the nucleus in chondrocytes. HA down-regulated NF-κB phosphorylation activated by CB12-II. In order to elucidate the involvement of CD44 in HA action on CB12-II-activated NF-κB, chondrocytes in monolayer were preincubated with anti-CD44 antibody, and subsequently incubated with HA before CB12-II stimulation. Anti-CD44 antibody significantly but partially reversed the inhibitory effect of HA on CB12-II-stimulated phosphorylation of p65 NF-κB. Similarly, anti-ICAM-1 antibody partially reversed HA action. When chondrocytes were pretreated with both antibodies to CD44 and ICAM-1 in combination, the HA inhibitory effect on CB12-II-stimulated phosphorylation of p65 NF-κB was almost completely attenuated. The antibodies to HA receptors caused similar blocking effects on inhibition of MMP-13 production by HA in CB12-II-stimulated chondrocytes in monolayer. The inhibitory effect of HA on CB12-II-induced MMP-13 was partially cancelled with treatment with the antibody to CD44 or ICAM-1 alone, and almost completely reversed with both antibodies in combination. Thus, NF-κB down-regulation by HA via CD44 and ICAM-1 could result in a decrease in enhanced MMP-13 production in CB12-II-stimulated chondrocytes. Because HA penetrates into cartilage explants and directly binds to CD44 and ICAM-1 on chondrocytes [3, 4], blocking experiments using the antibodies to HA receptors were conducted to elucidate the contribution of CD44 and ICAM-1 to the inhibitory effects of HA on MMP-13 production by CB12-II in RA cartilage explant culture. The antibody to CD44 or ICAM-1 partially cancelled HA suppression of CB12-II-increased MMP-13 production. A combination of both antibodies almost completely reversed the inhibitory effect of HA on CB12-II action. The antibodies in the absence of HA had no clear effect on CB12-II-stimulated MMP-13 production. From the findings in monolayer cultures, HA could inhibit NF-κB activation by CB12-II through interaction with CD44 and ICAM-1, leading to the suppression of enhanced MMP-13 production in RA cartilage explant
Discussion: The present study has demonstrated for the first time that CB12-II stimulates NF-κB in RA chondrocytes. The concentrations of the peptide used in the present study (1–50 μM) are within the estimated levels of denatured type II collagen in arthritic cartilage (~60 μM) [1]. This specific peptide sequence of type II collagen is excessively exposed in situ in osteoarthritic cartilage [1]. Thus, once type II collagen fragments containing the CB12-II domain reaches sufficient levels, NF-κB activation could be induced in chondrocytes. Because NF-κB is utilized as a common pathway to induce MMP-13 in response to proinflammatory cytokines and matrix degradation products, NF-κB may be one of the primary target molecules for treatment of arthritis. In the present study, antibodies to the HA receptors effectively reversed the inhibitory action of HA on MMP-13 and the phosphorylation of p65 NF-κB. Whereas cancellation of HA effect with the individual antibody was partial, treatment with both antibodies to CD44 and ICAM-1 in combination resulted in almost complete block of HA action. These data suggest that both CD44 and ICAM-1 could be required for full exertion of HA effect in chondrocytes. Overall, this study clearly demonstrated that CB12-II activates NF-κB for MMP-13 induction and that HA inhibits the CB12-II action through interaction with CD44 and ICAM-1 in RA chondrocytes. Administration of HA into RA joints could suppress the catabolic action of matrix degradation products like CB12-II as a potent NF-κB inhibitor.

Significance: While type II collagen peptide that could be generated from elevated proteolysis in arthritic joints induces collagenase through nuclear factor-κB activation in chondrocytes, hyaluronan works as an inhibitor of nuclear factor-κB for prevention of the catabolic action by type II collagen peptide.

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