INHIBITORY EFFECTS OF IGF-1 AND OP-1 ON FIBRONECTIN FRAGMENT AND IL-1 STIMULATED MMP-13 EXPRESSION BY HUMAN CHONDROCYTES

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Members of the family of matrix metalloproteinasises (MMPs) are key enzymes in matrix degradation. Recently, in vitro, clinical, and transgenic studies have provided evidence that MMP-13 (collagenase-3) is a leading candidate enzyme mediating degradation of type II collagen in OA cartilage (1). A dramatic up-regulation of MMP-13 by inflammatory cytokines such as IL-1β (2) or by fibronectin fragments (Fn-f) (3) has been observed in chondrocytes. Previous studies have shown insulin-like growth factor 1 (IGF-1) and osteogenic protein 1 (OP-1) may exert anabolic/anti-catabolic effects by suppressing transcriptions of MMP-13 gene, stimulating proteoglycan synthesis, or by blocking cartilage damage caused by Fn-f (4-5). The objective of this study was to investigate the inhibitory effects of IGF-1 and OP-1 on the expression of MMP-13 induced by Fn-f or IL-1β in the human chondrocyte cell line C-28I2 and in human primary chondrocytes.

METHODS. Human chondrocytes were isolated by enzymatic digestion of normal ankle articular cartilage obtained from tissue donors through the Regional Organ Bank of Illinois. The immortalized human chondrocyte line C-28I2 was kindly provided by Dr. Mary Goldring. MMP-13 promoter constructs (-1600, -736, -370, and -186) were subcloned into a firefly luciferase vector (pGL2-enhancer, Promega) as was a -562 MMP-1 promoter construct. For transfection, isolated cells were plated 24 h prior to transfection at a density of 1x10^5 cells per plate in 6-well plates and transiently transfected with 2 µg promoter-reporter constructs using FuGene 6 transfection reagent. The reporter construct without promoter (pGL2-basal) was used as a control and a renilla luciferase construct was included as an internal control for transfection efficiency. After 24 h incubation, cells were rinsed in PBS and changed to serum-free conditions for 20-24 h followed by treatment with the 120 kDa Fn-f (1 µM), IL-1β (10 ng/ml), IGF-1 (100 ng/ml) or OP-1 (100 ng/ml). Inhibition of Fn-f-induced activity was measured using 100 ng/ml IL-1 receptor antagonist (IL-1Ra, Anakinra, Amgen). Cells were harvested after 24 h and luciferase activity was assayed using a dual luciferase reporter assay system. All transfection experiments were repeated two times in duplicate.

RESULTS. IGF-1 and OP-1 each significantly reduced the basal level as well as Fn-f- or IL-1β-induced MMP-13 promoter activity in transient transfection experiments in both immortalized chondrocytes (Figs. 1 and 2) and primary human chondrocytes. The combination of IGF-1 and OP-1 further reduced basal promoter activity by 60% and almost completely inhibited Fn-f stimulated activity. The inhibitory effect of OP-1 was further enhanced by the forced expression of a constitutively active OP-1 receptor cDNA construct, BMPR-1B (not shown). IL-1Ra decreased Fn-f- and IL-1β-induced MMP-13 promoter activity by approximately 40 and 70% respectively (Fig 2). When combined with IGF-1 and OP-1, IL-1Ra reduced Fn-f- or IL-1β-induced MMP-13 promoter activity below basal levels. In parallel experiments, similar results were obtained in cells transfected with the MMP-1 promoter construct. Studies were initiated to understand the molecular mechanisms by which IGF-1 and OP-1 might synergistically modulate expressions of the MMP-13 gene. We postulated that OP-1 may regulate IGF-1 expression. Transient transfection of reporter gene constructs containing the human IGF-1 promoter fused to the luciferase gene showed approximately a 2-fold up-regulated promoter activity upon the addition of OP-1.

DISCUSSION. These results are the first to show the additive effects of IGF-1 and OP-1 on Fn-f or IL-1β-induced MMP-13 promoter activity. The combination of IGF-1 and OP-1 was more effective in suppression of Fn-f-induced MMP-13 promoter activity than that induced by IL-1β. On the other hand, IL-1Ra more efficiently inhibited IL-1β-induced MMP-13 promoter activity than that induced by Fn-f. Combining IL-1Ra with the two growth factors maximized the suppressive effect on the Fn-f- or IL-1β-induced MMP-13 expression resulting in a down-regulation of promoter activity below the basal control level. The observation that IL-1Ra more efficiently inhibits the IL-1β-induced MMP-13 promoter activity than Fn-f-induced activity supports our previous finding (3) that signaling from Fn-f is at least in part transduced by an IL-1-independent pathway. Further studies are needed to determine the mechanism of the IGF-1 + OP-1 effect including the potential for autocrine regulation of growth factor expression. The present results indicate that the combination of IGF-1 and OP-1 together with IL-1Ra might represent a more effective treatment for transcriptional suppression of MMP-13 activity and suggest the potential of these compounds for combination anti-arthritis drug therapy.

REFERENCES


ACKNOWLEDGEMENTS

Funded by grants from the Arthritis Foundation and the NIH (AR 16697 and AR 47654). Thanks to ROBI and Arkady Margulis for providing tissue. We thank Stryker Biotech for providing OP-1, Amgen for IL-1Ra, and Dr. Yubo Sun for the MMP-13 promoter constructs used for subcloning.

49th Annual Meeting of the Orthopaedic Research Society
Poster #0549