β-Cryptoxanthin suppresses the production of matrix metalloproteinases and aggrecanases in human articular chondrocytes and synoviocytes

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[Objective] β-Cryptoxanthin (β-CPX) is a carotenoid pigment mainly contained in citrus fruits. The negative correlation between morbidity of rheumatoid arthritis and daily intake of β-CPX has been shown in an epidemiologic study. However, the effects of β-CPX on the metabolism of articular cartilage matrix have not been clarified. In this study, we investigated the effects of β-CPX on the production of matrix metalloproteinases (MMPs) and aggrecanases (ADAMTS-4 and ADAMTS-5) in cultured human articular chondrocytes and synoviocytes.

[Methods] Confluent human articular chondrocytes and human synoviocytes were treated with IL-1β (10 ng/mL) and/or s-CPX. The amounts of proMMP-1, -3, and -13, and tissue inhibitor of metalloproteinases (TIMP)-1 in culture medium and cyclooxygenase (COX)-1 and -2 in cell lysate were determined by Western blot analysis. Steady-state levels of proMMP-1 mRNA, ADAMTS-4 mRNA and ADAMTS-5 mRNA were determined by quantitative real-time reverse transcription-polymerase chain reaction (RT-PCR).

[Results] β-CPX suppressed IL-1β-mediated production of proMMPs-1 and -3 in chondrocytes and synoviocytes, and that of proMMP-13 in chondrocytes in a dose-dependent manner. Steady-state levels of ADAMTS-4 mRNA and ADAMTS-5 mRNA were also suppressed by β-CPX as well as proMMP-1 mRNA expression in chondrocytes and synoviocytes. On the other hand, β-CPX did not affect the production of TIMP-1. The production of PGE2 in synoviocytes was decreased by the treatment of β-CPX accompanied with the selective suppression of COX-2 production.

[Conclusion] In this study we demonstrated novel evidences that β-CPX directly suppressed the production of MMPs and aggrecanases in human articular chondrocytes and synoviocytes, and also interfered with PGE2 production in synoviocytes. These results suggest that β-CPX possibly prevents cartilage destruction and synovial inflammation in rheumatoid arthritis.

Orientation of synovial fibroblasts in response to cyclic stretch

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Synovial fibroblasts (SFBs) play a crucial role in the maintenance of joint homeostasis, such as transporting small molecules from blood to synovial fluid, lubrication of articular cartilage and producing various extracellular matrixes. SFBs which are in direct contact with the intra-articular cavity are exposed to the continually changing dynamic forces, similar to vascular endothelial cells (ECs). Previous in vitro experiments showed that ECs subjected to cyclic stretch orient perpendicular to the stretch axis. Calcium influx via stretch-activated Ca2+(SA) channels has been shown to be a crucial step in this orientation of ECs when subjected to cyclic stretch. However, the molecular mechanisms by which SFBs respond to mechanical stress have not yet been examined. The aim of this study is to determine whether or not SFBs respond to mechanical stress in vitro. Synovial tissues were obtained from patients with osteoarthritis during knee joint replacement surgery. SFBs (passage two or five) and human umbilical ECs (HUVECs) were transferred onto a silicone chamber coated with type I collagen. They were exposed to mechanical strain by uni-axial cyclic (6 Hz) stretching of a silicone chamber (110%). When SFBs were subjected to cyclic stretch, they oriented perpendicularly to the stretch axis after 3 hours of cyclic stretch. When Gd3+ was added to the medium to blockade SA channels, the orientation of HUVECs in response to cyclic stretch was completely inhibited, as reported previously. On the other hand, SFBs continued to show orientation in response to cyclic stretch after Gd3+ treatment. Next, to examine for the existence of SA channels in SFBs, both SFBs and HUVECs were pre-loaded with Fluo-3, a Ca2+ indicator and mechanically stimulated by using a tungsten needle. Ca2+ influx was induced by mechanical stimuli to the cell membrane in both types of cells. However, while the Ca2+ influx was blocked by application of Gd3+ or GsMTx-4 (a peptide toxin from spider venom), SA channel blocker) in HUVECs, it was not blocked in SFB. Our data show that SFBs respond to mechanical stress in vitro. Although the Ca2+ influx induced by mechanical stimulation of the cell membrane in SFBs is similar to ECs, SA channels previously characterized as being blocked by Gd3+ are not essential for orientation of SFBs responding to mechanical stimuli. Therefore, our data suggest that SFBs may have a distinct mechanism for responding to dynamic forces to which they are exposed.