

**[P-25]****COX-2 Induction by Bovine Type I Collagen in Macrophages  
via C/EBP and CREB Activation**

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Bovine type I collagen (Col-I) is utilized for medical purposes such as cosmetic surgery and wrinkle removal. Cyclooxygenase-2 (COX-2) plays roles in pathophysiological processes including inflammation and tumorigenesis. This study examines the effects of Col-I on the COX-2 expression and the signaling pathways in macrophages. Col-I increased the levels of COX-2 protein and mRNA in serum-stimulated Raw264.7 cells. Treatment of cells with Col-I increased CCAAT/enhancer binding protein (C/EBP) DNA binding. Antibody supershift experiments revealed that C/EBP DNA binding activity induced by Col-I depended largely on C/EBP  $\beta$  and C/EBP  $\delta$ . Immunocytochemistry showed that Col-I induced nuclear translocation of C/EBP  $\beta$  and C/EBP  $\delta$ , whose activation contributes to COX-2 induction. Overexpression of the dominant-negative mutant form of C/EBP abolished COX-2 induction by Col-I. Col-I also increased cAMP response element binding protein (CREB) binding to DNA. Inhibition of FAK or downstream PI3-kinase and p70S6 kinase prevented COX-2 induction by Col-I, and C/EBP and CREB from binding to their consensus DNA oligonucleotides. Experiments using chemical inhibitors or dominant negative mutant vectors showed that the MAP kinase pathways including p38 kinase and ERK1/2, but not JNK, simultaneously regulated COX-2 induction by Col-I. This was in agreement with inhibition of Col-I-inducible C/EBP and CREB DNA binding by concomitant treatment with SB203580 and PD98059. These results provide evidence that Col-I induces COX-2 in serum-stimulated macrophages and that the multiple cell signaling pathways involving focal adhesion kinase, phosphoinositide 3-kinase and MAP kinases regulate COX-2 induction by Col-I via C/EBP and CREB activation.

**Keyword** : Bovine type I collagen (Col-I), Macrophages, Cyclooxygenase-2 (COX-2), CCAAT/enhancer binding protein (C/EBP), cAMP response element binding protein (CREB)