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Potentiation of Lipopolysaccharide-Inducible COX-2 Expression by C2-ceramide: The Role of JNK- and AP-1-Mediated C/EBPβ Activation

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Ceramide, formed by sphingomyelinase, is involved in the expression of cyclooxygenase-2 (COX-2). This study examines the effect of C2-ceramide (C2), a cell-permeable ceramide analog, on the LPS-inducible COX-2 expression and signaling pathways. C2 did not induce COX-2, but potentiated LPS-inducible COX-2 expression in Raw264.7 cells, whereas dihydro-C2 was inactive. Treatment of cells with C2 notably increased LPS-inducible CCAAT/enhancer binding protein (C/EBP) DNA binding. Antibody supershift experiments revealed that LPS-induced C/EBP DNA binding activity depended on C/EBPβ and C/EBPδ, but not C/EBPα, C/EBPε or CBP/p300. C/EBPβ contributed to C2-enhanced DNA binding activity. SB203580, a p38 kinase inhibitor, completely inhibited LPS-inducible and C2-potentiated LPS- inducible COX-2 expression. Enhancement of LPS-inducible COX-2 expression and C/EBP DNA binding by C2 was abrogated in dominant negative mutant of JNK1 [JNK1(-)] cells. PD98059 or stable transfection with dominant negative mutant of MKK1 [MKK1(-)] decreased COX-2 induction by LPS, but failed to inhibit C2-enhanced LPS induction of COX-2. C2 increased the level of nuclear C/EBPβ with a decrease in C/EBPδ in LPS-treated cells. JNK1(-) transfection abolished the increase in the nuclear C/EBPβ by LPS+C2. Transfection with dominant-negative mutant of C/EBP inhibited the ability of C2 to enhance LPS induction of COX-2. These results demonstrate that C2 increases C/EBPβ activation and COX-2 induction by LPS and that the pathway of JNK1, but not ERK1/2, is responsible for the increase in nuclear C/EBPβ and C/EBPβ-mediated potentiation of COX-2 induction.

Keyword : COX-2, ceramide, JNK1, AP-1, C/EBPβ