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2'-Amino-3'-Methoxyflavone(PD98059) inhibits 3-Methylcholanthrene Induction of CYP1A1 through CCAAT/Enhancer Binding Protein-β activation

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Previously, we reported that 2'-amino-3'-methoxyflavone(PD98059), an MKK1 inhibitor, induces glutathione S-transferase A2, which is mediated with nuclear translocation of C/EBP β and activation of the C/EBP binding site in the gene promoter. In view of the facts that the putative C/EBP binding site is located in the CYP1A1 promoter region and that C/EBP β activation negatively regulates AhR-mediated CYP1A1 expression, this study investigated whether PD98059 inhibits CYP1A1 induction by 3-methylcholanthrene (3-MC) via C/EBP β activation. 3-MC induced CYP1A1 in H4IIE cells in a concentration-dependent manner, which was inhibited by concomitant treatment of the cells with PD98059. PD98059 also repressed induction of CYP1A1 promoter-luciferase gene by 3-MC. Gel shift analysis showed that PD98059 prevented the increase in the band intensity of protein binding to the XRE by 3-MC although the agent alone slightly increased formation of protein-XRE complex. PD98059 increased the band intensity of C/EBP DNA binding, which was supershifted with anti-C/EBP β antibody. PD98059 almost completely inhibited 3-MC induction of CYP1A1 in cells stably transfected with dominant negative mutant of MKK1(-), which provided evidence that PD98059 blocks CYP1A1 induction by 3-MC irrespective of the inhibition of MKK1/ERK1/2. We then determined the role of C/EBP β activation by PD98059 leads to repression of CYP1A1 induction. Overexpression of dominant-negative mutant C/EBP significantly abolished the ability of PD98059 to suppress 3-MC-inducible CYP1A1 in H4IIE cells. These results showed that PD98059 suppresses 3-MC induction of the CYP1A1 gene expression and that activation of C/EBP β by PD98059 contributes to suppression of 3-MC-inducible AhR-mediated CYP1A1 expression irrespective of the inhibition of MKK1/ERK activity.

Keyword : PD98059, C/EBPβ, CYP1A1