

12-o-Tetradecanoylphorbol 13-acetate prevents baicalein-induced apoptosis via activation of protein kinase C and JNKs in human leukemia cells

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摘要

Abstract

In the present study, we found that baicalein (BE), but not its glycoside baicalin (BI), induced apoptosis in human leukemia HL-60 and Jurkat cells, but not in primary murine peritoneal macrophages (PMs) or human polymorphonuclear (PMN) cells, by the MTT assay, LDH release assay, and flow cytometric analysis. Activation of the caspase 3, but not caspase 1, enzyme via inducing protein processing was detected in BE-induced apoptosis. The ROS-scavenging activity of BE was identified by the anti-DPPH radical, DCHF-DA, and in vitro plasmid digestion assay, and none of chemical antioxidants including allpurinol (ALL), N-acetyl-cystein (NAC), and diphenylene iodonium (DPI) affected BE-induced apoptosis in HL-60 cells. This suggests that apoptosis induced by BE is independent of the production of ROS in HL-60 cells. Interestingly, the apoptotic events such as DNA ladders formation and activation of the caspase 3 cascade were significantly blocked by TPA addition in the presence of membrane translocation of PKC α , and TPA-induced protection was reduced by adding the PKC inhibitors, GF-109203X and staurosporin. TPA addition induces the phosphorylation of JNKs and ERKs, but not p38, protein in HL-60 cells, and incubation of HL-60 cells with JNKs inhibitor SP600125, but not ERKs inhibitor, PD98059 or the p38 inhibitor SB203580, suppressed the protective effect of TPA against BE-induced apoptotic events including DNA ladders, apoptotic bodies, caspase 3 and D4-GDI protein cleavage in according with blocking JNKs protein phosphorylation. In addition, PKC inhibitor GF-109203X treatment blocks TPA-induced ERKs and JNKs protein phosphorylation, which indicates that activation of PKC locates at upstream of MAPKs activation in TPA-treated HL-60 cells. Additionally, a loss in mitochondrial membrane potential with a reduction in Bcl-2 protein expression, the induction of Bad protein phosphorylation, and translocation of cytochrome c from mitochondria to the cytosol were observed in BE-treated HL-60 cells, and these events were prevented by the addition of TPA. GF-109203X and SP600125 suppression of TPA against cytochrome c release induced by BE was

identified. This suggests that activation of PKC and JNKs participate in TPA's prevention of BE-induced apoptosis via suppressing mitochondrial dysfunction in HL-60 cells..