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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Abstract

Abstract In the present study, we found that baicalein (BE), but not its glycoside baicalin (BI), induced apoptosis in hu-man leukemia HL-60 and Jurkat cells, but not in primary murine peritoneal macrophages (PMs) or human polymor-phonuclear (PMN) cells, by the MTT assay, LDH release assay, and flow cytometric analysis. Activation of the cas- pase 3, but not caspase 1, enzyme via inducing protein pro- cessing 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 pro- duction 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 ad-dition in the presence of membrane translocation of PKCα, and TPA-induced protection was reduced by adding the PKC nhibitors, GF-109203X and staurosporin. TPA addition in- duces 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 PKClocates 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.