Hydroxylation at c4’or c6 is essential for apoptosis-inducing activity of flavanone through activation of the caspase 3 cascade and production of reactive oxygen species.

沈杏娟;陳彥州
Ko CH;Shen SC;Chen YC

Abstract

Previous studies demonstrated that hydroxyl groups play important roles in the antioxidative activities of flavonoids; however, the importance of structurally related hydroxylation in their apoptosis-inducing activities is still undefined. In the present study, flavanone with hydroxylation at C4' and C6 had a significant cytotoxic effect in human leukemia HL-60 cells accompanied by the occurrence of DNA ladders, apoptotic bodies, and hypodiploid cells, characteristics of apoptosis. The replacement of a hydroxyl group (OH) by a methoxyl (OCH(3)) group at C4' or C6 attenuated the apoptotic effect in cells, and there was no significant cytotocity of flavanone or flavanone with OH or OCH(3) in C7-treated HL-60 cells. Induction of enzyme activity of caspase-3 and -9, but not caspase-1 and -8, accompanied by release of cytocrome C from mitochondria to cytosol and the appearance of cleaved of PARP (85 kDa), D4-GDI (23 kDa), and caspase-3 (p17/p15) fragments, was identified in 4'-OH- or 6-OH-flavanone-treatedHL-60 cells. Caspase-3 and -9 inhibitors Ac-DEVD-FMK and Ac-LEHD-FMK, but not caspase-1 and -8 inhibitors Ac-YVAD-FMK and Ac-LETD-FMK, attenuated 4'-OH- or 6-OH-flavanone-induced cell death. And, inhibition of capsase-9 activity by Ac-LEHD-FMK suppresses caspase-3 protein procession induced by 4'-OH- and 6-OH-flavanone, indicative of caspase-9 activation locating upstream of caspase-3. A decrease in the antiapoptotic protein Mcl-1 and increases in the pro-apoptotic proteins Baxand Bad were found in 4'-OH- or 6-OH-flavanone-treated HL-60 cells. Induction of endogenous ROS production was detected in 4'-OH- or 6-OH-flavanone-treated HL-60 cells by the DCHF-DA assay. Antioxidants such as N-acetylcysteine (NAC), catalase (CAT),superoxide dismutase (SOD), and allopurinol (ALL), but not pyrrolidine dithiocarbamate (PDTC) or diphenylene iodonium (DPI), significantly inhibited 4'-OH- or 6-OH-flavanone-induced ROS production, with blocking of the apoptosis induced by 4'-OH- or6-OH-flavanone. The apoptosis-inducing activity of 4'-OH- or

6-OH-flavanone was also observed in another leukemia cell line (Jurkat), but was not found in mature monocytic cells (THP-1) and normal human polymorphonuclear neutrophils (PMNs). This suggests that hydroxylation at C4' or C6 is important to the apoptosis-inducing activities of flavanone through ROS production, and that activation of the caspase-3 cascade, downstream of caspase-9 activation, is involved.