

Mitochondrial-dependent, reactive oxygen species-independent apoptosis by myricetin: role of protein kinase C, cytochrome C and caspase cascade

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摘要

Abstract

Abrogation of mitochondrial permeability and induction of reactive oxygen species (ROS) production have been observed in chemical-induced apoptosis; however, the relationship between the mitochondria and intracellular ROS levels in apoptosis is still unclear. In the present study, myricetin (ME) but not its respective glycoside, myricitrin (MI; myricetin-3-O-rhamnose) reduced the viability of human leukemia HL-60 cells via apoptosis, characterized by the occurrence of DNA ladders and hypodiploid cells. Results of Western blotting and caspase activity assays showed that activation of caspases 3 and 9 but not caspases 1, 6 or 8 with cleavage of PARP and D4-GDI proteins is involved in ME-induced apoptosis. A reduction in mitochondrial functions characterized by a decrease in the Bcl-2/Bax protein ratio and translocation of cytochrome c (cyt c) from the mitochondria to the cytosol in accordance with a decrease in mitochondrial membrane potential were observed in ME-treated HL-60 cells. No significant induction of intracellular ROS levels by ME was observed by the DCHF-DA assay, DPPH assay or plasmid digestion assay, and antioxidants including N-acetyl-cysteine (NAC), catalase (CAT), superoxide dismutase (SOD), and tiron (TIR) showed no protective effects on ME-induced apoptosis. A PKC activator, 12-O-tetradecaoylphorbol-13-acetate (TPA) significantly attenuated ME-induced apoptosis via preventing cytochrome c release to the cytosol and maintaining the mitochondrial membrane potential by inhibiting the decrease in the Bcl-2/Bax protein ratio; these effects were blocked by protein kinase C (PKC) inhibitors including GF-109203X, H7, and staurosporin. Removing mitochondria by ethidium bromide (EtBr) treatment reduced the apoptotic effect of ME. Results of SAR studies showed that the presence of OH at C3', C4', and C5' is important for the apoptosis-inducing activities of ME, and that ME induces apoptosis in another leukemia cell line, Jurkat cells, but not in primary human polymorphonuclear (PMN) cells or in

murine peritoneal macrophages (PMs). The results of the present study suggest that apoptosis induced by ME occurs through a novel mitochondrion-dependent, ROS-independent pathway; TPA protects cells from ME-induced apoptosis via PKC activation which prevents the occurrence of mitochondrial destruction during apoptosis.