Wogonin and fisetin induce apoptosis in human promyeloleukemic cells, accompanied by a decrease of reactive oxygen species, and activation of caspase 3 and Ca2+-dependent endonuclease

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Abstract

Seven structurally related flavonoids including luteolin, nobiletin, wogonin, baicalein, apigenin, myricetin and fisetin were used to study their biological activities on the human leukemia cell line, HL-60. On MTT assay, wogonin, baicalein, apigenin, myricetin and fisetin showed obvious cytotoxic effects on HL-60 cells, with wogonin and fisetin being the most-potent apoptotic inducers among them. The cytotoxic effects of wogonin and fisetin were accompanied by the dose- and time-dependent appearance of characteristics of apoptosis including DNA fragmentation, apoptotic bodies and the sub-G1 ratio. Treatment with an apoptosis-inducing concentration of wogonin or fisetin causes rapid and transient induction of caspase 3/CPP32 activity, but not caspase 1 activity. Further, cleavage of poly(ADP-ribose) polymerase (PARP) and decrease of pro-caspase 3 protein were detected in wogonin- and fisetin-treated HL-60 cells. An increase in the pro-apoptotic protein, bax, and a decrease in the anti-apoptotic protein, Mcl-1, were detected in fisetin- and wogonin-treated HL-60 cells. However, Bcl-2, Bcl-XL, and Bad all remained unchanged in wogonin- and fisetin-treated HL-60 cells. In vitro chromatin digestion revealed that endonuclease activitywas profoundly enhanced in wogonin- and fisetin-treated HL-60 cells, and the addition of ethylenediaminetetraacetic acid (EDTA) or ethyleneglycoltetraacetic acid (EGTA) into the reaction blocked endonuclease activation and at an optimum pH of 7.5. The caspase 3 inhibitor, Ac-DEVD-CHO, but not the caspase 1 inhibitor, Ac-YVAD-CHO, attenuated wogonin- and fisetin-induced DNA ladders, PARP cleavage, and endonuclease activation. Pretreatment of HL-60 cells with N-acetyl-cysteine or catalase efficiently inhibited H(2)O(2) (200 microM)-induced apoptosis, but showed no inhibitory effect on wogonin- and fisetin-induced DNA ladders, caspase 3 activation, or bax protein induction. Decrease in endogenous ROS production was detected in wogonin- and fisetin-treated HL-60 cells by DCHF-DA assay. In conclusion, our experiments

indicate that a decrease in intracellular peroxide level was involved in wogonin- and fisetin-induced apoptosis; activation of caspase 3 and endonuclease, induction of bax protein and suppression of Mcl-1 protein were detected in the process.