Wogonin and fisetin induction of apoptosis through activation of caspase 3 cascade and alternative expression of p21 protein in hepatocellular carcinoma cells SK-HEP-1

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Abstract

Wogonin and fisetin are flavonoids, which are widely distributed in plants. Our recent study demonstrated that, among seven structurally related flavonoids, wogonin and fisetin showed the most potent apoptosis-inducing activities in human promyeloleukemic cells HL-60. In the present investigation, we performed molecular studies to assess the apoptotic effects of wogonin and fisetin on hepatocellular carcinoma cells SK-HEP-1. Both wogonin and fisetin showed dose-dependent cytotoxic effects on SK-HEP-1 cells, accompanied by DNA fragmentation. Microscopic observation under Giemsa staining showed that wogonin and fisetin, at the dose of 80 microM, induced cellular swelling and the appearance of apoptotic bodies, characteristics of apoptosis, in SK-HEP-1 cells. Furthermore, flow cytometry analysis showed an increase of hypodiploid cells in wogonin- and fisetin-treated SK-HEP-1 cells. These data demonstrated that wogonin and fisetin were effective inducers of apoptosis in SK-HEP-1 cells. Treatment with an apoptosis-inducing concentration of wogonin or fisetin caused induction of caspase 3/CPP32 activity, but not of caspase 1 activity. In addition, a caspase 3 inhibitor, Ac-DEVD-CHO, but not the caspase 1 inhibitor Ac-YVAD-CHO, reversed the cytotoxic effects of wogonin and fisetin on SK-HEP-1 cells. Further, cleavage of caspase 3 substrates including poly(ADP-ribose) polymerase (PARP) and D4-GDI protein, and decrease of pro-caspase 3 protein were detected in wogonin- and fisetin-treated SK-HEP-1 cells. Increase of p53 protein was associated with wogonin- and fisetin-induced apoptosis; however, a p53-controlled gene, p21(Waf/Cip-1), was only induced in wogonin- (not fisetin-) treated SK-HEP-1 cells. Serum starvation elevated p21(Waf/Cip-1) protein expression, and enhanced the apoptotic induction activity of wogonin (not fiseitn) in SK-HEP-1 cells. Our study has provided molecular evidence to demonstrate that wogonin and fisetin had effective cytotoxic effects through apoptosis induction in hepatocellular carcinoma cells

SK-HEP-1; activation of caspase 3 cascade, induction of p53 protein and alternative expression of p21(Waf/Cip-1) protein were involved.