Differential Apoptosis-Inducing Effect of Quercetin and its Glycosides in Human Promyeloleukemic HL-60 Cells by Alternative activation of Caspase 3 Cascade

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Abstract

Flavonoids were demonstrated to possess several biological effects including antitumor, antioxidant, and anti-inflammatory activities in our previous studies. However, the effect of glycosylation on their biological functions is still undefined. In the present study, the apoptosis-inducing activities of three structure-related flavonoids including aglycone quercetin (QUE), and glycone rutin (RUT; QUE-3-O-rutinoside), and glycone quercitrin (QUI; QUE-3-O-rhamnoside) were studied. Both RUT and QUI are QUE glycosides, and possess rutinose and rhamnose at the C3 position of QUE, respectively. Results of the MTT assay showed that QUE, but not RUT and QUI, exhibits significant cytotoxic effect on HL-60 cells, accompanied by the dose- and time-dependent appearance of characteristics of apoptosis including an increase in DNA ladder intensity, morphological changes, apoptotic bodies, and an increase in hypodiploid cells by flow cytometry analysis. QUE, but not RUT or QUI, caused rapid and transient induction of caspase 3/CPP32 activity, but not caspase 1 activity, according to cleavage of caspase 3 substrates poly(ADP-ribose) polymerase (PARP) and D4-GDI proteins, and the appearance of cleaved caspase 3 fragments being detected in QUE- but not RUT- or QUI-treated HL-60 cells. A decrease in the anti-apoptotic protein, Mcl-1, was detected in QUE-treated HL-60 cells, whereas other Bcl-2 family proteins including Bax, Bcl-2, Bcl-XL, and Bag remained unchanged. The caspase 3 inhibitor, Ac-DEVD-FMK, but not the caspase 1 inhibitor, Ac-YVAD-FMK, attenuated QUE-induced cell death. Results of DCHF-DA assay indicate that no significant increase in intracellular peroxide level was found in QUE-treated cells, and QUE inhibited the H(2)O(2)-induced intracellular peroxide level. Free radical scavengers N-acetyl-cysteine (NAC) and catalase showed no prevention of QUE-induced apoptosis. In addition, QUE did not induce apoptosis in an mature monocytic cell line THP-1, as characterized by a lack of DNA ladders, caspase 3 activation, PARP cleavage, and an Mcl-1 decrease, compared with those in HL-60 cells. Our experiments provide evidence to indicate that the addition of rutinose or rhamnose

attenuates the apoptosis-inducing activity of QUE, and that the caspase 3 cascade but not free radical production is involved.