Rutinoside at C7 attenuates the apoptosis-inducing activity of flavonoids.

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Abstract

Rutinoside (rhamnoglucoside; rhamnose+glucose) addition has been examined extensively in the metabolism of flavonoids, however the effect of rutinoside on apoptosis-inducing activity of flavonoids is still unknown. In the present study, the two pairs of flavonoids of hesperetin (HT) and hesperidin (HD; HT-7-rutinose), and naringenin (NE) and naringin (NE-7-rutinose), were used to study their apoptosis-inducing activities in HL-60 cells. Both HD and NI are flavonoids which contain a rutinoside at the C7 of HT and NE, respectively. Results of the MTT assay showed that HT and NE, but not HD and NI, exhibited significant cytotoxic effect in HL-60 cells, accompanied by the dose- and time-dependent appearance of characteristics of apoptosis including an increase in DNA ladder intensity, morphological changes, appearance of apoptotic bodies, and an increase in hypodiploid cells by flow cytometry analysis. HT and NE, but not HD and NI, caused rapid and transient induction of caspase-3/CPP32 activity, but not caspase-1 activity, according to the cleavage of caspase-3 substrates poly(ADP-ribose) polymerase and D4-GDI proteins, the appearance of cleaved caspase-3 fragments detected in HT- or NE-, but not in HD- or NI-treated HL-60 cells. A decrease in the anti-apoptotic protein, Mcl-1, was detected in HT- and NE-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 HT- and NE-induced cell death. Interestingly, neither HT nor NE induced apoptosis in the mature monocytic cell line THP-1 and primary human polymorphonuclear cells, as characterized by a lack of DNA ladders, caspase-3 activation, poly(ADP-ribose) polymerase cleavage, and Mcl-1 decrease, compared with those in HL-60 cells. In addition, the rutinoside group in HD and NI was removed by hesperidinase and naringinase, accompanied by the production of HT and NE, respectively, according to HPLC analysis. Accordingly, hesperidinase and naringinase digestion recovered the apoptosis-inducing activity of HD and NI in HL-60 cells. Our experiments provide the first evidence to suggest that rutinoside in flavonoids prevents the induction of apoptosis, and that activation of the traditional caspase-3 cascade participates in HT- and NE-induced apoptosis.