

Quercetin, but not rutin and quercitrin, prevention of H₂O₂-induced apoptosis via antioxidant activity and heme oxygenase 1 gene expression in Macrophages.

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Abstract

In the present study, we examine the protective mechanism of quercetin (QE) on oxidative stress-induced cytotoxic effect in RAW264.7 macrophages. Results of Western blotting show that QE but not its glycoside rutin (RUT) and quicitrin-induced HO-1 protein expression in a time- and dose-dependent manner, and HO-1 protein induced by QE was blocked by an addition of cycloheximide or actinomycin D. Induction of HO-1 gene expression by QE was accompanied by inducing ERKs, but not JNKs or p38, proteins phosphorylation. Addition of PD98059, but not SB203580 or SP600125, significantly attenuates QE-induced HO-1 protein and mRNA expression associated with blocking the expression of phosphorylated ERKs proteins. H₂O₂ addition reduces the viability of cells by MTT assay, and appearance of DNA ladders, hypodiploid cells, and an increase in intracellular peroxide level was detected. Addition of QE, but not QI or RUT, significantly reduced the cytotoxic effect induced by H₂O₂ associated with blocking the production of intracellular peroxide, DNA ladders, and hypodiploid cells. QE protection of cells from H₂O₂-induced apoptosis was significantly suppressed by adding HO inhibitor SnPP or ERKs inhibitor PD98059. Additionally, QE protects cells from H₂O₂-induced a decrease in the mitochondrial membrane potential and a release of cytochrome c from mitochondria to cytosol by DiOC₆ and Western blotting assay, respectively. Activation of apoptotic proteins including the caspase 3, caspase 9, PARP, D4-GDI proteins was identified in H₂O₂-treated cells by Western blotting and enzyme activity assay, and that was significantly blocked by an addition of QE, but not RUT and QI. Furthermore, HO-1 catalytic metabolites carbon monoxide (CO), but not Fe²⁺, Fe³⁺, biliverdin or bilirubin, performed protective effect on cells from H₂O₂-induced cell death with an increase in HO-1 protein expression and ERKs protein phosphorylation. These data suggest that induction of HO-1 protein may participate in the protective mechanism of QE on oxidative stress (H₂O₂)-induced apoptosis, and reduction of intracellular ROS production and mitochondria dysfunction with blocking apoptotic events were involved. Differential anti-apoptotic effect between QE and its glycosides RUT and QI via distinct HO-1 protein induction was also delineated.