Baicalein inhibition of oxidative-stress-induced apoptosis via modulation of ERKs activation and induction of HO-1 gene expression in rat glioma cells

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摘要

Abstract

In the present study, we examined the protective mechanism of baicalein (BE) and its glycoside, baicalin (BI), on hydrogen-peroxide (H(2)O(2))-induced cell death in rat glioma C6 cells. Results of the MTT assay, LDH release assay, and morphological observation showed that H(2)O(2) addition reduced the viability of C6 cells, and this was prevented by the addition of BE but not BI. Incubation of C6 cells with BE significantly decreased the intracellular peroxide level induced by H(2)O(2) according to flow cytometric analysis using DCHF-DA as a fluorescent substrate. Suppression of H(2)O(2)-induced apoptotic events including DNA ladders, hypodiploid cells, and activation of caspases 3, 8, and, 9 by BE but not BI was identified in C6 cells. The cytotoxicity and phosphorylation of ERK proteins induced by H(2)O(2) were blocked by the ERK inhibitor PD98059. Catalase addition prevented H(2)O(2)-induced ROS production, ERKs protein phosphorylation, and cell death, and BE dose-dependently inhibited H(2)O(2)-induced ERK protein phosphorylation in C6 cells. These data suggest that ROS-scavenging activity is involved in BE prevention of H(2)O(2)-induced cell death via blocking ERKs activation. Additionally, BE but not BI induced heat shock protein 32 (HSP32; HO-1) protein expression in both time- and dose-dependent manners, but not heme oxygenase 2 (HO-2), heat shock protein 70 (HSP70), or heat shock protein 90 (HSP90) protein expression. In the absence of H(2)O(2), BE induces ERKs protein phosphorylation, and HO-1 protein expression induced by BE was blocked by the addition of cycloheximide, actinomycin D, and the ERK inhibitor PD98059. The addition of the HO inhibitor ZnPP inhibited the protective effect of BE against H(2)O(2)-induced cytotoxicity in C6 cells according to the MTT assay and apoptotic morphology under microscopic observation, accompanied by blocking the ROS-scavenging activity of BE in C6 cells. However, BE treatment was unable to protect C6 cells from C2-ceramide-induced cell death.

Thesedata indicate that BE possesses abilities to inhibit ROS-mediated cytotoxic effects through modulation of ERKs activation and induction of HO-1 protein expression. The role of HO-1 in ROS-scavenging activity of BE is proposed.