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Kaempferol and rhamnocitrin protect PC12 cells against oxidative stress

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Abstract

Background and Purpose: Oxidative stress has been considered as a major cause of cellular injuries in a variety of clinical abnormalities, especially neural diseases. One of the effective ways to prevent the reactive oxygen species (ROS) mediated cellular injury is dietary or pharmaceutical augmentation of free radical scavengers. Our aim of research is to investigate the protective effects of kaempferol and rhamnocitrin on serum-deprivation and H_2O_2-induced oxidative damage in rat pheochromocytoma PC12 cells. The mechanisms which might underlie the defense effects including free radical scavenging, induction of Heme oxygenase-1 (HO-1) and activation of MAPK/ERK signal transduction pathways, were also studied. **Methods:** xanthine/xanthine oxidase-generated superoxide radicals and Fenton reaction-generated hydroxyl radicals were detected by luminol chemiluminescence (CL) method. PC12 cell viability was measured by MTT. Intracellular ROS was measured by DCFH-DA and flow cytometery. HO-1 gene expression was measured by RT-Q-PCR and Western blotting. Activation of MAPK/ERK signal transduction pathways was measured by Western blotting. **Results**: Kaempferol and rhamnocitrin could strongly scavenged superoxide radicals with IC_{50} 0.04 ± 0.01 and 0.326 ± 0.04 M, respectively. They also compatibly scavenged Fenton-generated hydroxyl radicals with IC_{50} 3-4 M. Only kaempferol (40-80 M) could protect PC12 cells from serum-deprivation induced apoptosis. However, both compounds (40 and 60 M) were able to attenuated H_2O_2-induced cell death by directly scavenging intracellular ROS. Furthermore, both compounds significantly induced HO-1 gene expression and the addition of HO-1 inhibitor, Znpp, markedly reversed the defense effects. Serum - deprivation resulted in decreased phosphorylation of extracellular signal-regulated kinase (ERK) and increased phosphorylations of c-Jun NH_2-terminal kinase (JNK) and p38, of the family of mitogen-activated protein kinases (MAPKs); however, both compounds reversed these phenomena, supporting the antagonistic effects between ERK and JNK-p38 in regulating cell survival. **Conclusions:** Kaempferol dose-dependently protected PC12 from serum-deprivation and H_2O_2-induced cell death. The underlying mechanism may be related to their free radical scavenging activities, activation of phase-2 gene expression and mitogen-activation signaling pathways.