Hyperoside Protects Cells against Gamma Ray Radiation-Induced Apoptosis in Hamster Lung Fibroblast†

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Abstract – Ionizing radiation, including that evoked by gamma (γ)-rays, induces oxidative stress through the generation of reactive oxygen species, resulting in apoptosis, or programmed cell death. This study aimed to elucidate the radioprotective effects of hyperoside (quercetin-3-O-galactoside) against γ-ray radiation-induced apoptosis in Chinese hamster lung fibroblasts, V79-4 and demonstrated that the compound reduced levels of intracellular reactive oxygen species in γ-ray-irradiated cells. Hyperoside also protected irradiated cells against DNA damage (evidenced by pronounced DNA tails and elevated phospho-histone H2AX and 8-oxoguanine content) and membrane lipid peroxidation. Furthermore, hyperoside prevented the γ-ray-provoked reduction in cell viability via the inhibition of apoptosis through the increased levels of Bel-2, the decreased levels of Bax and cytosolic cytochrome c, and the decrease of the active caspase 9 and caspase 3 expression. Taken together, these results suggest that hyperoside defend cells against γ-ray radiation-induced apoptosis by inhibiting oxidative stress.

Keywords – Hyperoside (quercetin-3-O-galactoside), γ-Ray radiation, Apoptosis, Reactive oxygen species, Oxidative stress

Introduction

Ionizing radiation increases the production of reactive oxygen species (ROS), including superoxide anions, hydroxyl radicals, and hydrogen peroxide (H2O2), leading to oxidative stress and cell damage (Sies, 1983). ROS can oxidatively modify lipids, proteins, DNA, and small intracellular molecules. For example, the reaction of ROS with lipids generates lipid peroxides, leading to increased membrane permeability (Yang et al., 2007). Furthermore, the reaction of ROS with proteins gives rise to decreased protein synthesis due to ribosomal translocation and/or destruction of proteins, resulting in impaired cellular metabolism and accumulation of cellular waste products (Tuder et al., 2003). ROS can also cause damage to nucleic acids by altering purine and pyrimidine bases and by enhancing DNA strand breaks (Dizdaroglu et al., 2002).

Hyperoside (quercetin-3-O-galactoside), a flavonoid compound, possesses many defensive properties against ROS-induced damage, such as nitric oxide synthase inhibitory activity (Luo et al., 2004) and antioxidant activity (Zou et al., 2004). The compound also demonstrates antifungal activity (Li et al., 2005), antiviral activity (Chen et al., 2006; Wu et al., 2007), antidepressant activity (Premer et al., 2007), and cardioprotective (Trumbeckaite et al., 2006) and neuroprotective effects (Liu et al., 2005). Recently, we reported that hyperoside decreases H2O2-induced cell damage through activation of an antioxidant system (Piao et al., 2008). However, little information is available regarding the protective capacity of hyperoside against cell damage stemming from gamma (γ)-ray radiation. Therefore, the present study investigated this question, as well as the mechanism of action of hyperoside against γ-ray-generated oxidative stress.

Experimental

Reagents – Hyperoside (quercetin-3-O-galactoside, Fig. 1) compound purified from aerial parts of Metaplexis japonica (Lee et al., 2012), 2',7'-Dichlorodihydrofluorescein diacetate (DCF-DA), propidium iodide, and Hoechst 33342 were purchased from Sigma Chemical Company
were exposed to 100 µM and were exposed to γ-ray irradiation at 1.5 Gy/min from a γ-ray source (MDS Nordion C-188 standard source, St. Louis, MO, USA), and diphenyl-1-pyrenylphosphine (DPPP) from Invitrogen (Poole, Dorset, UK). The primary anti-Bcl-2, -Bax, -cytochrome c, -caspase 9, -caspase 3 and -poly ADP-ribosyl polymerase (PARP) antibodies were purchased from Cell Signaling Technology (Beverly, MA, USA), and the primary anti-phospho H2A.X antibody applied (300 mA, 25 V) for 20 min at 4°C to draw the negatively charged DNA toward an anode. After the electrophoresis, the slides were washed three times for 5 min at 4°C in a neutralizing buffer (0.4 M Tris, pH 7.5), followed by staining with 75 µl of propidium iodide (20 µg/ml). The slides were observed with a fluorescence microscope and image analyzer (Kinetic Imaging, Komet 5.5, UK). The percentage of the total fluorescence in the tail and the tail length of the 50 cells per slide were recorded.

Western blot analysis – Cells were treated with hyperoside at 5 µM and with γ-ray radiation at 10 Gy, an hour later. The cells were incubated for an additional 48 h at 37°C, and harvested, followed by washing twice with PBS. The harvested cells were then lysed on ice for 30 min in 100 µl of a lysis buffer [120 mM NaCl, 40 mM Tris (pH 8), 0.1% NP 40] and centrifuged at 13,000 × g for 15 min. The supernatants were collected from the lysates and the protein concentrations were determined. Aliquots of the lysates (40 µg of protein) were boiled for 5 min and electrophoresed in 10% SDS-polyacrylamide gel. The blots in the gels were transferred onto nitrocellulose membranes (Bio-Rad, Hercules, CA, USA), and subsequently incubated with anti-primary antibodies. The membranes were further incubated with secondary anti-immunoglobulin-G-horseradish peroxidase conjugates (Pierce, Rockford, IL, USA), followed by exposure to X-ray film. The protein bands were detected using an