Role of 7,8-dihydroxyflavone on γ- ray radiation-induced reactive oxygen species production

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Abstract

Ionizing radiation can induce oxidative stress through generation of reactive oxygen species (ROS). Flavonoids are a member of polyphenolic compounds that occur in fruits, vegetables, teas and red wines. 7, 8-dihydroxyflavone, a member of the flavonoid group, was elucidated the free radical scavenging effect against γ-ray radiation-induced ROS production. We found 7, 8-dihydroxyflavone to scavenge the intracellular ROS detected with fluorescence spectrometer and flow cytometry. (J Med Life Sci 2009;6:365-367)

Key Words : 7, 8-dihydroxyflavone, Reactive oxygen species, Flavonoids

Introduction

Radiation toxicity occurs either by direct attack on the genetic material and/or by generating reactive oxygen species (ROS) such as superoxide anion, hydroxyl radical, and hydrogen peroxide by radiolysis of water[1]. These free radicals react with cellular macromolecules such as DNA, protein, lipid membrane, and cause cell dysfunction and mortality[2]. ROS mediated bimolecular reactions and their relationship with radiation sickness is the current subject of scientific investigations in radiotherapy[3]. Antioxidants are capable of scavenging free-radicals from the radiolysis of water thereby protecting cell damage[4], thus supplementation of antioxidants to improve the efficacy of radiotherapy is today's proposed strategy[5].

Flavonoids are a member of polyphenolic compounds that occur in fruits, vegetables, teas and red wines[6]. Several reports have been demonstrated the relationship between structure and activity for antioxidant properties of flavonoids[7-9]. Recently we have reported the effect of 7, 8-dihydroxyflavone on hydrogen peroxide-induced DNA damage and cell death[10].

The present study investigated whether 7, 8-dihydroxyflavone is capable of reducing γ-ray radiation-induced ROS production in hamster lung fibroblast cells (V79-4).

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Materials and methods

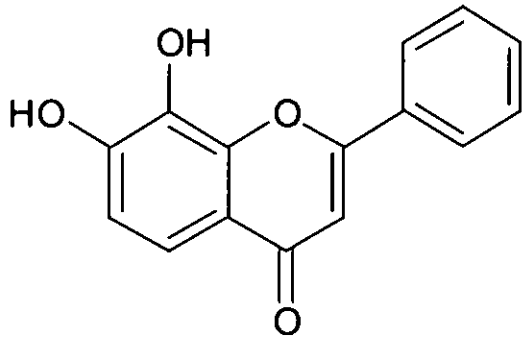
1. Reagents

The 7, 8-dihydroxyflavone (Fig. 1) was freshly dissolved in dimethyl sulfoxide (DMSO), yielding a final concentration, which did not exceed 0.1%. 2′, 7′-dichlorodihydrofluorescein diacetate (DCF-DA) was purchased from the Sigma Chemical Company (St. Louis, MO, USA).

2. Cell culture and irradiation

Chinese hamster lung fibroblasts (V79-4) cells from the American Type Culture Collection (Rockville, MD, USA) were maintained at 37℃ in an incubator with a humidified atmosphere of 5% CO_2 and cultured in Dulbecco's modified Eagle's medium, containing 10% heat-inactivated fetal calf serum, streptomycin (100 μg/ml) and penicillin (100 units/ml). The cells were exposed to γ-ray radation at 1.5 Gy/min from a ^{60}Co γ-ray source (MDS Nordion C-188

Figure 1. Chemical structure of 7,8-dihydroxyflavone.



standard source, Jeju National University, Jeju, Korea).

3. Intracellular reactive oxygen species (ROS) measurement

The V79-4 cells were treated with 7, 8-dihydroxyflavone at 10 g/ml and were exposed to γ-ray radiation an hour later. The cells were incubated for an additional 24 h at 37 ℃. After adding 25 M of DCF-DA solution, the fluorescence of 2',7'-dichlorofluorescein was detected using a Perkin Elmer LS-5B spectrofluorometer and a flow cytometer (Becton Dickinson, Mountain View, CA, USA), respectively[11].

4. Statistical analysis

All measurements were made in triplicate and all values were expressed as the means ± standard error of the mean (S.E.M.). The results were subjected to an analysis of variance (ANOVA) using the Tukey test to analyze the difference. $P < 0.05$ was considered significantly.

Results and Discussion

The γ-ray irradiation triggers a diverse array of functional changes in cells. The effects of γ-ray irradiation appear due to the ability of γ-ray radiation to interact with multiple cell organelles. To date, considerable evidences have been found that γ-ray radiation inducesROS generation, which plays an important role on the effect of γ-ray irradiation on cells[12, 13]. We have previously shown that 7, 8-dihydroxyflavone protected cells against H_2O_2 induced cell damage[10]. In the present study, we measured the radical scavenging effect of 7, 8-dihydroxyflavone on the ROS generated by γ-ray radiation and found that the level of ROS detected with a spectrofluorometer decreased in 7, 8-dihydroxyflavone treated irradiated cells, compared to ROS level in irradiated cells in a radiated dose-dependent manner (Fig. 2). This pattern was also confirmed by flow cytometry, showing 429 value of fluorescence intensity which was produced from ROS stained by DCF-DA fluorescence dye in 7, 8-dihydroxyflavone treated irradiated cells, compared to 456 value of fluorescence intensity in irradiated cells (Fig. 3).

Figure 2. Effect of 7,8-dihydroxyflavone on scavenging intracellular ROS generated by γ-ray irradiationof various radiation doses. The V79-4 cells were treated with 7,8-dihydroxyflavone at 10 μg/ml, followed by γ-ray irradiation at 5, 10, 15, 20 Gy an hour later. Next, the cells were incubated for 48h, the intracellular ROS was detected using fluorescence spectrophotometer after DCF-DA staining. *Significantly different from irradiated cells ($P < 0.05$).

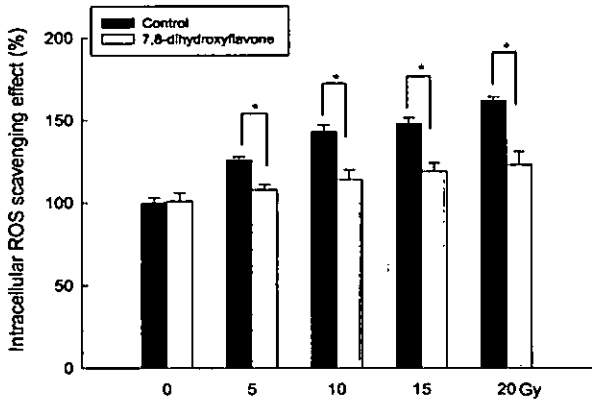
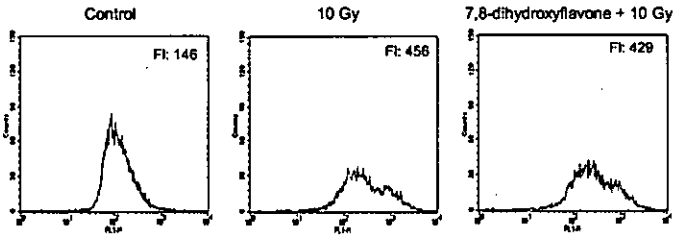


Figure 3. Effect of 7,8-dihydroxyflavone on scavenging intracellular ROS generated by γ-ray irradiation at 10 Gy. The V79-4 cells were treated with 7,8-dihydroxyflavone at 10 μg/ml, followed by γ-ray irradiation at 10 Gy an hour later. Next, the cells were incubated for 48h, the intracellular ROS was detected using flow cytometer after DCF-DA staining.



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