Protective Effect of Curcumin against Ionizing Radiation (IR)-induced Cytotoxicity and Genotoxicity in HepG2 Cells

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1. Introduction

Ionizing radiation (IR) has many practical applications such as medicine, foods, agricultures, industries, and research laboratories [1]. However, the increasing use of radiation is associated with radiation accidents threatening human health [2]. It is well known that exposure to IR gives rise to genomic alterations, mutagenesis, and cell death. IR is absorbed directly by DNA, leading to various DNA damages (single or double-strand breaks, base damage, and DNA-DNA or DNA-protein cross-linkages) in many living organisms [3]. Therefore, the development of effective and nontoxic radioprotective agents is of considerable interest.

Curcumin ($C_{12}H_{20}O_{6,}$ structure shown in Fig. 1) is the major yellow component of *Curcuma longa* with biological activities (antioxidant, anti-proliferative and anti-inflammatory properties) [4]. It has been widely used as food and medicine for a long time. The aim of our present study is to investigate the protective effects of curcumin against IR-induced cytotoxicity and genotoxicity in cultured HepG2 cells.

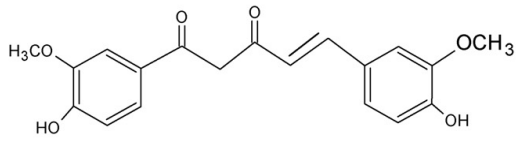

Fig. 1. Chemical structure of curcumin.

2. Materials and Methods

2.1 Cell culture and Treatment

HepG2 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (Gibco, Grand Isla, NY, USA) with 10% fetal bovine serum (FBS) (Hyclone, Logan, UT, USA), 100 U/mL penicillin and 100 U/mL streptomycin (Gibco, Grand Islan, NY, USA). Cells were incubated at 37°C with 5% CO_2. The protective effect of curcumin on IR-induced cytotoxicity and genotoxicity were examined by pretreating cells with or without 5 μM curcumin in FBS-free DMEM for 2 h before irradiation with 1, 5 and 10 Gy.

2.2 Trypan blue assay

Following irradiation, the cells were incubated for 0, 1, 2 and 3 days. After the incubation day, the cells isolated with trypsinization and examined for cell viability using the trypan blue assay previously described [5]. Cell suspensions were mixed with 0.4% trypan blue solution and counted by a hematocytometer. The percentage of cell viability (untreated control cells) was calculated as 100%.

2.3 Comet assay

Following irradiation, the cells were immediately isolated with trypsinization and examined for DNA damage using the alkaline comet assay previously described [6]. The comet assay was carried out under dark conditions. Cell suspensions (20 μl) mixed with 0.5% low-melting agarose (200 μl) were spreaded to slides pre-coated with 1% normal-melting agarose. The third layer of 0.5% low-melting agarose was added and solidified. After the solidification of gel layer, the slides were immersed in a lysis buffer (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 1% Triton X-100 and 10% DMSO, pH 10.0) at 4°C for 60 min. After lysis, slides were placed in electrophoresis buffer (0.3 M NaOH, 1 mM EDTA, pH 13) to allow DNA to unwind for 20 min. Subsequently, slides were subjected to electrophoresis (25 V, 300 mA, 25 min) in the same buffer. Slides were neutralized with Tris-HCl buffer, pH 7.5, and stained with proidium iodie (20 μg/mL) for 10 min. Slides were examined using an image analysis system (Komet 4.0 from Kinetic Imaging Ltd., Liverpoool, UKy).

2.4 Irradation

HepG2 cells were irradiated with 0, 1, 5 and 10 Gy. External gamma radiation was performed by a ^{60}CO source (7.4 PBq of capacity; AECL, Canada at the Korea Atomic Energy Research Institute).

3. Results and Discussion

2.1 Protective Effect of Curcumin against IR-induced Cytotoxicity

To determine the protective effect of curcumin against IR-induced cytotoxicity in HepG2 cells, cell viability was used as an indicator of cytotoxicity.

HepG2 cells were pretreated with or without 5 μM curcumin for 2 h, irradiated with 0, 1, 5 and 10 Gy. The cells were incubated for different time (0, 1, 2 and 3 days) to monitor cell cytotoxicity. After the different incubation days, cell viability was examined by the trypan blue assay (data not shown). As shown in Fig. 2, IR decreased the percentage of cell viability in a dose-dependent manner for 3 days after irradiation, with a lethal dose (LD_{50}) of 5 Gy. Pretreatment of 5 μM curcumin increased the percentage of cell viability as compared to that of IR alone. The results suggest that curcumin could have protective effect against cell cytotoxicity induced by IR.

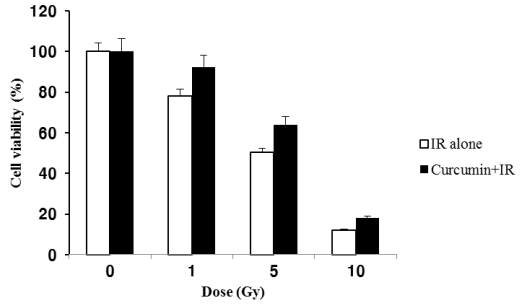

Fig. 2. Protective effect of curcumin against IR-induced cytotoxicity in HepG2 cells.

2.2 Protective Effect of Curcumin against IR-Induced Genotoxicity

To determine the protective effect of curcumin against IR-induced genotoxicity in HepG2 cells, DNA damage was used as an indicator of genotoxicity. HepG2 cells were pretreated with or without 5 μM curcumin, irradiated, and immediately isolated to avoid DNA repair. As shown in Fig. 3, IR increased DNA damage of HepG2 cells in a dose-dependent manner. After irradation, the fragmented DNA formed a typical comet tail-like pattern. The higher dose led to a longer DNA tail length, indicating that IR induced DNA damage in HepG2 cells. In contrast, pretreatment of 5 μM curcumin decreased DNA tail length. The results suggest that curcumin could protect cell genotoxicity induced by IR.

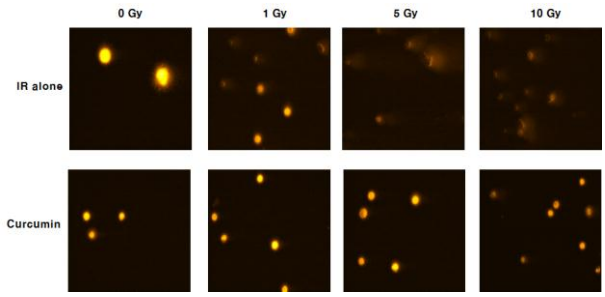

Fig. 3. Protective effect of curcumin against IR-induced genotoxicity in HepG2 cells.

In summary, we investigated the protective effect of curcumin against IR-induced cytotoxicity and genotoxicity in HepG2 cells by using the trypan blue and the comet assay, respectively. Pretreatment of 5 μM curcumin attenuated IR-induced cell viability loss and DNA damage in HepG2 cells. The results suggest that curcumin could be usable as a radioprotective agent.

REFERENCES

[1] A. G. Chmielewski and M. Haji-Saeid, Radiation technologies: past, present and future, Radiation Physics and Chemistry, Vol. 71, pp. 16-20, 2004.
[2] J. E. Moulder, Post-irradiation approaches to treatment of radiation injuries in the context of radiological terrorism and radiation accidents: a review, International Journal of Radiation Biology, Vol. 80, pp. 3-10, 2004.
[3] K. D. Held, Radiation-induced apoptosis and its relationship to loss of clonogenic survival, Apoptosis, Vol.2, pp. 265-282, 1997.
[4] R. K. Maheshwari, A. K. Singh, J. Gaddipati, and R. C. Srimal, Multiple biological activities of curcumin: A short review, Life Science, Vol. 78, pp. 2081-2087, 2006.
[5] H. O. Jauregui, N. T. Hayner, J. L. Driscoll, R. Williams-Holland, M. H. Lipsky, and P. M. Galletti. Trypan blue dye uptake and lactate dehydrogenase in adult rat hepatocytes-freshly isolated cells, cell suspensions, and primary monolayer cultures, In Vitro, Vol. 17, pp. 1100-1110, 1981.
[6] R. R. Tice, E. Agurell, D. Anderson, B. Burlinson, A. Hartmann, H. Kobayashi, Y. Miyamae, E. Rojas, J. C. Ryu, and Y. F. Sasaki, Single cell gel/comet assay: guidelines for in in vitro and in vivo genetic toxicology testing, Environmental and Molecular Mutagenesis, Vol. 35, pp. 206-221, 2000.