Assessment of the Radioprotection Efficacy of Antioxidant Substances in Foods in regard to Clonogenic Cell Survival

Hyo-Sung Park†, Min-Ho Lee, Eun-Hee Kim*
Department of Nuclear Engineering, Seoul National Univ., Seoul 151-744, Korea (Republic of)
†*xserfc2@snu.ac.kr*, **Corresponding author: eunhee@snu.ac.kr*

1. Introduction

Radiation is perceived as a hazard in spite of its diverse uses. The public are more sensitive than ever to the activities from the nuclear power plant industry since Fukushima accident and the consequential environment contamination.[1] The never-dissolved fear from the public of the nuclear energy costs the nuclear industry too much for safety measures not to mention brings the public uneasy life. Beta carotene, oltipraz and luteolin, which are easily taken from various foods, are substances that may protect cells from damage caused by unstable molecules known as free radicals. The purpose of this study is to inform people of accessible radiation protection measures in everyday lives.

2. Methods

2.1 Cell culture and Irradiation

Mouse endothelial cells (MECs, ATCC, CRL-2161) were cultured in Dulbecco Modified Eagle's Medium (DMEM, GIBCO) supplemented with 10% Fetal Bovine Serum Albumin (FBS, GIBCO) inactivated at 56°C, and incubated at 37℃ in a humidified 10% CO2 atmosphere.

Cell irradiation was performed using the hard X-ray beam irradiator installed in Radiation Bioengineering Laboratory at Seoul National University. The X-ray beam tube (YXLON 450-D08) was operated at 200 kVp and 10 mA, delivering 4 Gy of radiation dose at 1.58 Gy/min.

2.2 Chemical treatments

Beta-carotene (Sigma Aldrich, C9750), oltipraz (Sigma Aldrich, O9389) and luteolin (Sigma Aldrich, L9283) were prepared as solutions with 10 μL DMSO (Sigma Aldrich) per 2 ml of culture medium. Treatment doses are listed in Table 1. MECs were treated with each antioxidant substance for 0.5 h prior to irradiation.

Beta-carotene is a red-orange pigment abundant in plants, fruits, vegetables and whole grains. Oltipraz is a synthetic dithiolthione ($C_8H_6N_2S_3$) that is structurally similar to the dithiolthiones found in cruciferous vegetables. Luteolin is found in celery, thyme, green peppers and chamomile tea. These substances are known to be relevant to decreased risk of colon cancer.[2]

Table 1: Antioxidant treatment doses*

Beta carotene (μg)	Oltipraz (μg)	Luteolin (μg)
0	0	0
0.54	0.054	0.028
1.08	0.108	0.057
2.7	0.27	0.14
5.4	0.54	0.28

*added to 2 ml of cell culture medium

2.3 Clonogenic assay

The radioprotection efficacy of antioxidant substances were assessed by comparing the clonogenic cell surviving fractions after radiation exposure with and without antioxidant substance in culture medium. The concentration of antioxidant was varied in certain range.

Beta carotene, oltipraz and luteolin were treated on MECs for 0.5 h prior to irradiation. After irradiation, MECs were washed and viable cells were collected after trypsinisation. After 2 weeks of incubation, colonies were fixed with ethanol and stained with 5% Giemsa solution (Sigma Aldrich, GS500) and then counted. Fig.1 is a schematic of our clonogenic assay.

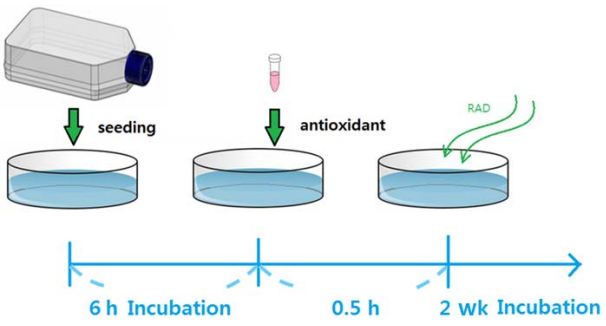

Fig. 1 A schematic of clonogenic assay.

3. Results

3.1 Cytotoxicity effect

Clonogenic assay was carried out to evaluate the cytotoxicity of three antioxidants after treating them on MECs for 0.5 h. As shown in Fig. 2, the clonogenic cell surviving fraction did not change much with beta

carotene and luteolin. With oltipraz, on the other hand, the clonogenic cell surviving fraction changed: it increased at oltipraz concentration of 1.08 μg/2 ml but decreased beyond the concentration.

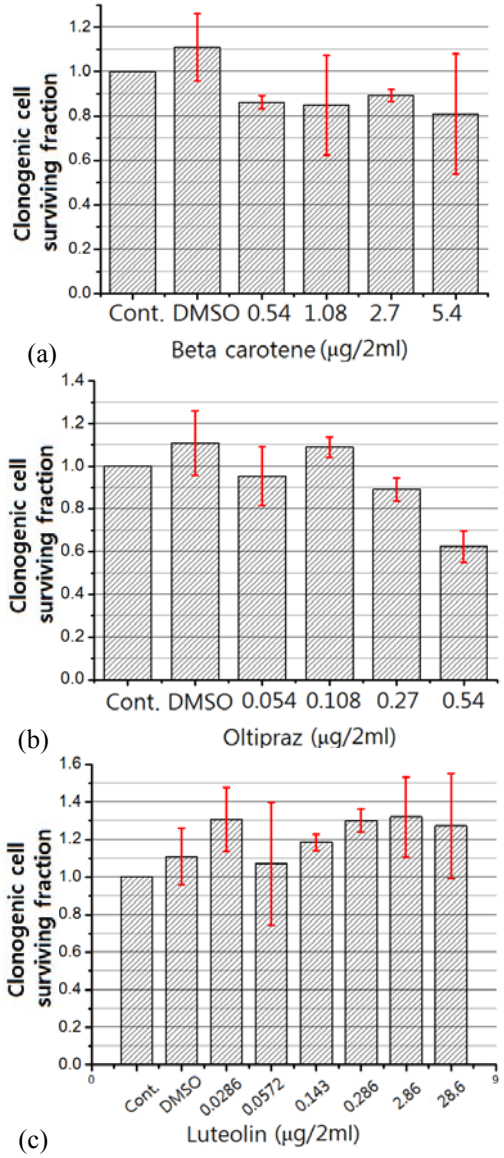

Fig. 2 Clonogenic surviving fractions of MECs after 0.5 h treatment of beta carotene(a), oltipraz(b) and luteolin(c). The error bars indicate one standard deviation.

3.2 Radioprotection effect

Significant ($p < 0.05$) increase in clonogenic surviving fraction was observed with beta carotene of 1.08 μg/2 ml, oltipraz of 0.108 μg/2 ml, and luteolin of 0.286 μg/2 ml treated on MECs for 0.5 h prior to irradiation as compared to control group (see Fig. 3). Data in Fig. 3 inform that cells are protected from radiation damage with some amount of antioxidant substances, but the protection efficacy becomes offset by cytotoxicity from too much antioxidant substances.

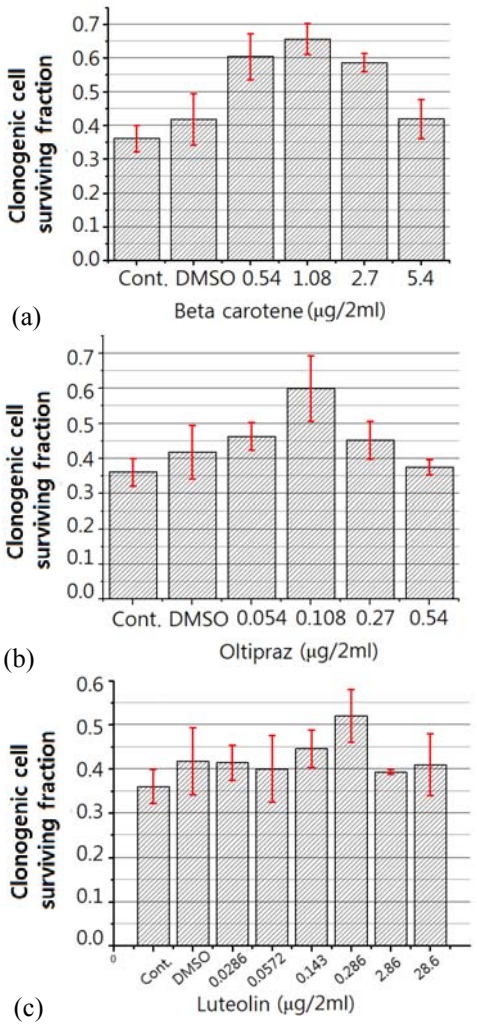

Fig. 3 Clonogenic surviving fractions of MECs observed after 4 Gy of radiation exposure with or without beta carotene(a), oltipraz(b) and luteolin(c) in culture medium. The error bars indicate one standard deviation.

3. Conclusion

Three antioxidant substances were investigated regarding their radioprotection effects counted in terms of clonogenic cell surviving fraction in vitro. The radioprotection efficacy was observed with those substances at concentrations below certain levels. At concentrations beyond those levels, the efficacy was canceled out by their inherent cytotoxicity.

REFERENCES

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