Assessment of Radiation-Equivalency of Favorite Foods Based on Apoptotic and Clonogenic Cell Death

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1. Introduction

People fear that ionizing radiation, despite of its usefulness, bears significant risk factor. In particular, after Fukushima nuclear accident in March of 2011, people become over-sensitive to radiation exposure and nuclear power [1]. Such fear by the public of nuclear power inevitably costs the nuclear industry a high expense for safety measures, not to mention brings the public uneasy life. It is important that the consequence of radiation exposure is conveyed to the public in a friendly way with actual data. In this study, the cytotoxicity of favorite foods was investigated to provide chemical doses causing bio-effects comparable with those by radiation exposure.

2. Methods and Results

2.1 Cell Preparation and Irradiation

Mouse endothelial cells (MECs) (ATCC, CRL-2161) were used in this study. MECs were incubated in a Dulbecco's Modified Eagle's Medium (GIBCO) supplemented with 10% fetal bovine serum (GIBCO) at 37 °C in a humidified 10% CO_2 atmosphere. The 10% FBS was heat-inactivated at 56°C.

Radiation exposure was made in the Radiation Bioengineering Laboratory at Seoul National University. The YXLON 450-D08 beam tube was operated at 200 kVp and 10 mA, delivering bremsstrahlung X-ray at 1.58 Gy/min.

2.2 Chemical Treatment

The cellular toxicity was assessed for alcohol, cigarette and coffee with their key extracts, i.e., ethanol, nicotine and caffeine, respectively. Ethanol was 79.15% in purity and 0.8g/ml in density (Hitech Pharm). Nicotine and caffeine extracts (Sigma Aldrich) were prepared as solutions with 100 µl of nicotine and 10 mg of caffeine per 1 ml distilled water. Preliminary test was performed to find the level of treatment dose causing comparable cytotoxicity to radiation.

With 260 µl ethanol added to 3 ml culture medium, the cell viability 24 h later was nearly zero so was it with 8 µl nicotine added. With caffeine, however, the cellular toxicity was observed only with high concentrations (more than 5 mM or 3 mg added to 3 ml culture medium). It has been known that low concentration of caffeine acts like antioxidant [2]. Doses of our treatment are listed in Table I. MECs were incubated in medium containing alcohol, nicotine or caffeine for 24 h.

Table I: Chemical treatment doses*

Ethanol(µl)	Nicotine(µl)	Caffeine(mg)
0	0	0
13	2	2
26	4	4
65	6	6
130		8
260		

*added to 3ml of cell culture medium

2.3 Apoptotic Cell Death

For apoptosis analysis, bindings of Annexin V to phosphatidylserine were counted by using Muse™ cell analyzer (Millipore Co). As shown in Fig. 1, all three chemicals and radiation, percentages of apoptotic death increase in dose dependent manner. Each set of experiment data was fitted (r^2 = 0.94~0.99) with a second order polynomial curve, as suggested in an earlier study [3]. Presented in Table II are 10% apoptosis induction doses, defined as doses causing additional 10% of apoptotic death to the control.

Table II: Chemical doses* and radiation dose causing comparable level of death with MECs in vitro.

	Radiation	Ethanol	Nicotine	Caffeine
10% apoptosis induction	8.45Gy	107µl	5.26µl	7.42mg
10% clonogenic cell survival	6.38Gy	135µl	6.53µl	10.7mg

*added to 3ml of cell culture medium

2.4 Clonogenic Cell Death

After chemical or radiation treatment, cells were washed with PBS and trypsinised. After 2 weeks of incubation, numbers of colony were counted. In Fig. 2,

each data set of surviving fraction measurements were fitted by employing the linear-quadratic model, $\ln(SF)=-\alpha D-\beta D^2$ [4]. Chemical and radiation doses leading to 10% of clonogenic cell survival (D_{10}) are list in Table II. Caffeine in amount less than 2 mg did not express cytotoxicity. Chemical doses equivalent to 1 Gy of radiation dose in apoptosis induction or clonogenic cell killing are summarized in Table III.

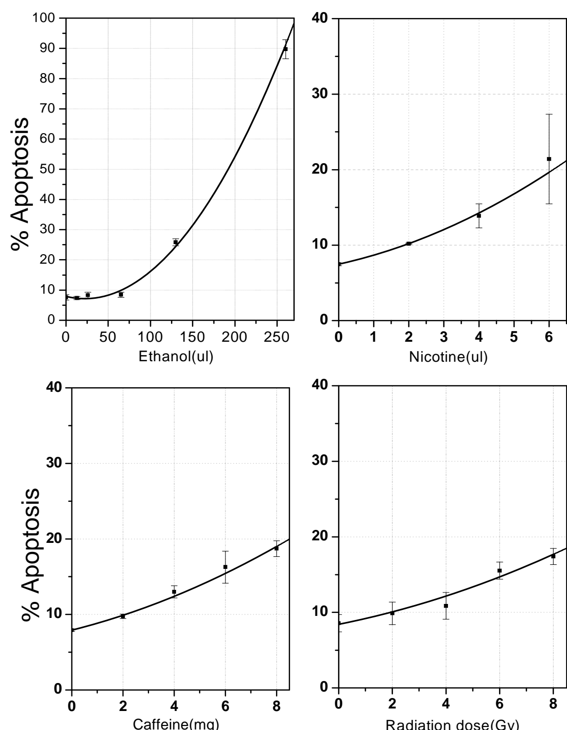

Fig. 1. Percent apoptosis of MECs observed after exposure to ethanol, nicotine, caffeine and radiation (dots). The solid lines are the second order polynomial curves fitting experimental data points. The error bars indicate one standard deviation.

Table III: Chemical doses* equivalent to 1 Gy of radiation dose in cytotoxicity with MECs in vitro.

	Radiation	Ethanol	Nicotine	Caffeine
0.8% apoptosis induction	1Gy	75μl	0.68μl	0.84mg
81% clonogenic cell survival	1Gy	73μl	3.6μl	3.0mg

*added to 3ml of cell culture medium

4. Conclusions

Extracts from alcohol, cigarette and coffee have been investigated for their cytotoxicity in terms of apoptosis induction and clonogenic cell killing. The comparison of familiar favorite food items with radiation in cellular toxicity might help the public have balanced judgment on the severity of radiation exposure. Future study pursues estimating dose-rate effect and the investigation will be continued with other cell lines.

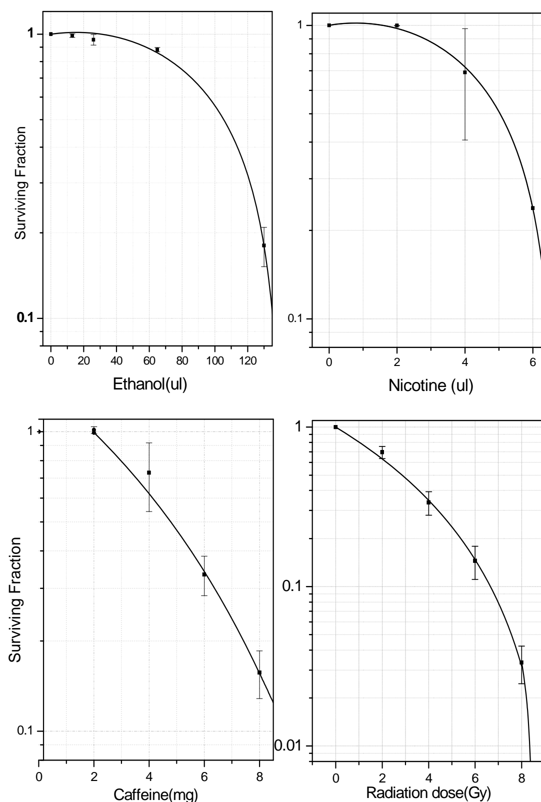

Fig. 2. Clonogenic survival fractions of MECs obseved after exposure to ethanol, nicotine, caffeine and radiation (dots). The solid lines are the linear-quadratic curves fitting experimental data. The error bars indicate one standard deviation.

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