The combined effect of alpha particles and cigarette smoke on human lung epithelial cells in terms of DNA double strand breakage induction

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1. Introduction

Internal exposure due to radon inhalation is an issue in radiation protection of the workers handling the uranium- and thorium-containing materials. The major risk of cancer incidence in human respiratory system is attributed not only to internal radiation exposure but also to smoking. Radiation exposure and smoking were reported by *in vitro* studies to show additive health effect [1, 2]. However, previous epidemiological studies showed no consistent results. They reported various results such as additive, sub-multiplicative and multiplicative [3].

In this study, we investigated the response of cells to radiation, specifically alpha particles, and to the cigarette smoke, either in separate or together in terms of DNA double strand breaks.

2. Methods

2.1 Exposure to alpha particles

Normal human lung epithelial cells (Nuli-1, CRL-4011, ATCC, VA, USA) were cultured in airway epithelial cell basal medium (PCS-300-030, ATCC) containing bronchial epithelial cell growth kit (PCS-300-040, ATCC). Cells were incubated at 37 °C under humidified 5 % CO_2 environment.

Cells were irradiated in the alpha particle irradiation chamber of the Radiation Bio-engineering Laboratory at Seoul National University. The alpha dose rate was 0.064 Gy/min. Doses of up to 0.2 Gy were delivered to the cells.

2.2 Exposure to cigarette smoke

The extract of cigarette smoke was obtained by employing a method modified based on the previous studies [1, 4]. Smoking does harm to the smoker via the mainstream smoke. Cigarettes containing 0.45 mg of nicotine and 4.5 mg of tar per piece were used in this study. The mainstream smoke from four cigarettes with its own filter was bubbled through 40 ml of DPBS (14040-117, Gibco, NY, USA). Their mixture called cigarette smoke extract (CSE) was filtered through a 0.2 μm-thick rapid-flow bottle top filter (596-3320, Nalge Nunc International, NY, USA). The CSE was then diluted with airway epithelial cell basal medium at concentrations of 0.1 to 10 %. Cells were cultured in this CSE-containing media for 24 hours.

2.3 Gamma-H2AX assay

After irradiation or treatment with CSE, cells were fixed with 4 % paraformaldehyde (163-20145, Wako) for 15 minutes. After fixation, cells were permeabilized with 1 % Triton X-100 (T9500-010, GenDEPOT) solution for 15 minutes. Then, 10 % bovine serum albumin (BSA) solution was added to block undesirable protein binding. Anti-gamma-H2AX phosphor S139 antibody (Ab2893, Abcam. primary antibody) in 1 % BSA was added to the samples. Samples were kept for an hour to facilitate immune reaction. The cells were treated with goat anti-rabbit IgG H&L (Ab6717, Abcam. secondary antibody) in 1 % BSA. Cells were stained with DAPI in fluoroshield mounting medium.

The foci images were taken by using a fluorescence microscope (BX53F, Olympus). The image-analyzing software 'CellProfiler' (Broad Institute's Imaging Platform) was used to count the number of gamma-H2AX foci per cell (FPC). The FPC values at varying radiation and chemical doses were normalized to the background FPC value.

3. Results

3.1 DNA damage induced by alpha particles or cigarette smoke in separate

Fig. 1 shows the numbers of FPC induced by alpha particles and CSE. The normalized FPC values increased linearly with radiation dose, and a similar tendency was observed from CSE treatment. The concentration of CSE was chosen to induce the FPC comparable to that induced by radiation.

3.2 Combined effect by alpha particles and cigarette smoke

Fig. 2 presents the number of FPC induced by alpha particle exposure in CSE-treated medium. For comparison, the sums of the FPC values either by alpha particle exposure or by CSE treatment are also shown. The FPC values of experimental data increased in proportional to dose. The simple sum was slightly higher at low doses and the combined effect became greater as radiation dose increases. Nevertheless, the difference was not significant.

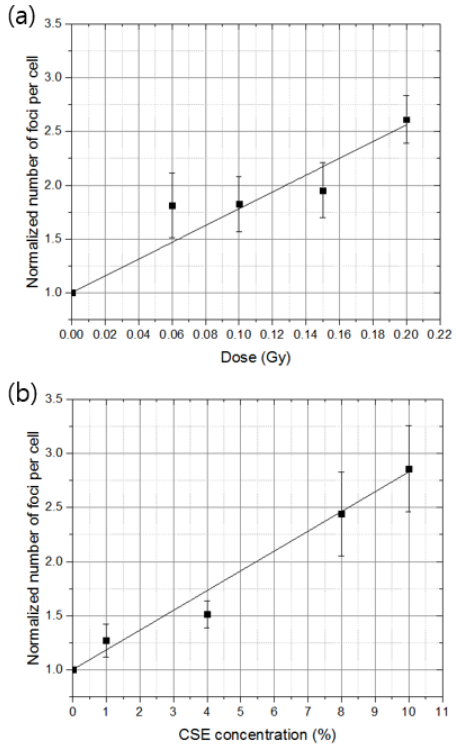

Fig. 1. The numbers of gamma-H2AX foci per cell in normal human lung epithelial cells normalized to the background value after exposure to: (a) alpha particles and (b) extract of cigarette smoke. Error bars show the standard errors.

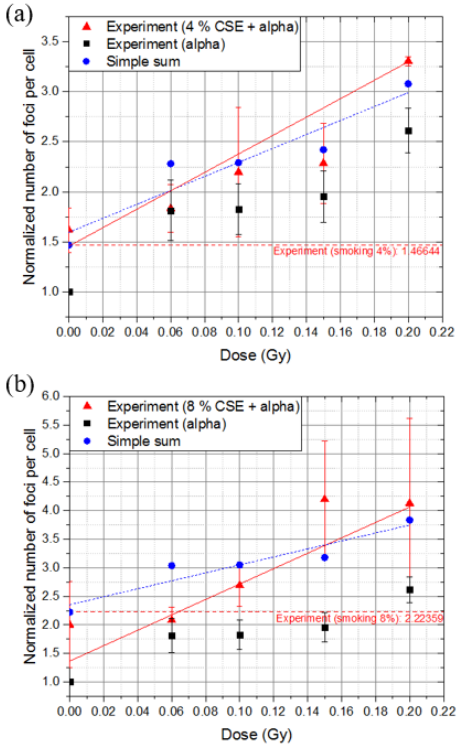

Fig. 2. The mean numbers of gamma-H2AX foci per cell in normal human lung epithelial cells as combined effect by both alpha particle exposure and CSE treatment (triangle). CSE concentrations were (a) 4 % and (b) 8 %. Those FPC values were compared with the sum of the FPC by alpha particle exposure and that by CSE treatment (circle). Error bars show the standard errors.

4. Conclusion

According to the observations regarding the induction of DNA double strand breakage, we conclude that alpha particles and cigarette smoke influence on the normal human lung epithelial cells in an additive mode rather than in a synergistic mode.

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