Biological Effects of Interaction between Radiation and Chemicals

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1. Introduction

The organisms are exposed to natural radiations from cosmic or terrestrial origins. Radiation is known to cause cell death, mainly due to its ability to produce reactive oxygen species in cells. The combined action between radiation and various chemicals is a distinguishing feature of modern life. Mercury chloride is a widespread environmental pollutant that is known to have toxic effects. Synergistic effects of radiation and $HgCl_2$ on human cells was previously reported [1]. NAC is a well-known sulfhydryl-containing antioxidant whose role in radioprotection has been explored in several studies [2-4]. There has been an increasing interest of studying the role of NAC as a radioprotective substance.

The present study was designed not only to assess the synergistic effects between radiation and $HgCl_2$, but also to investigate protective effects of NAC on cells.

2. Methods and Results

2.1 Synergistic effects of Irradiation and $HgCl_2$-induced cell viability

PLHC-1 cells are derived from a hepatocellular carcinoma in an adult female (*Poeciliopsis Lucida)*, a topminnow from the Sonoran desert (ATCC® # CRL-2406). PLHC-1 cells are grown at 30°C in a humidified incubator containing 5% CO_2 and propagated in Eagle's Minimum Essential Medium (Hyclone, UT, USA) supplemented with 5% foetal bovine serum (Gibco™, Grand Island, NY), L-glutamine (Sigma-Aldrich, MO, USA), sodium pyruvate (Sigma-Aldrich), gentamicin (Hyclone). The cells routinely grow in 75 ㎠ flasks (Costar), and are subcultured every 3 to 5 days at a split ratio of 1:4.

PLHC-1 cells were seeded in 96-well plates at a density of 2 x 10^5 cells/㎖ and incubated for 24 hr. Non-adherent cells were removed by gently washing. Then cells were treated with 1～500 μM of $HgCl_2$ for 24hr. After the treatment with γ-rays from a ^{60}Co γ-ray source (Korea Atomic Energy Research Institute, Korea) with 10～300 Gy in the presence or absence of $HgCl_2$.

The MTT assay is based on the uptake of thiazolyl blue tetrazolium bromide (MTT) and its following reduction in mitochondria of living cells to MTT formazan, while dead cells are almost completely negative in this cleavage activity. To assess MTT assay, 100 ㎕ of MTT solution was added to each well after removal of 100 ㎕ supernatant and incubated for another 4 hr at 30 °C. The generated formazan crystal was dissolved and the absorbance was detected at 570nm using ELISA reader (Multiskan® EX, Forma Scientific, Inc.).

Simultaneous treatment of the cells with ionizing radiation and $HgCl_2$ resulted in a dramatic increase of cell death, while neither of them showed cytotoxic effects when treated alone. The cytotoxicity of ionizing radiation was enhanced in the presence of $HgCl_2$. Analysis of the extent of synergistic interaction enables to make quantitative estimation of irreversibly damaged cells after the combined exposure.

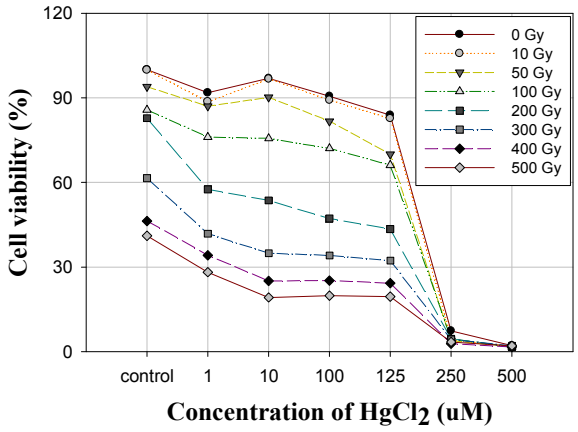

Fig. 1. The *in vitro* cytotoxicity assays of the irradiation and $HgCl_2$ treatment on PLHC-1 cells. Cytotoxicity was measured by MTT assay after 24 hr exposure, respectively (n=3). All the points showed a statistically significant difference from the control group according to Student's *t*-test ($p<0.005$).

2.2 Protective effects of NAC in radiation-induced cytotoxicity

The antioxidant property of NAC can be attributed to its ability to provide cysteine and other precursors of glutathione synthesis, as well as its ability to directly scaveng free radicals [5]. According to several studies, NAC on the one hand acts as an antioxidant, but on the other hand, it can also act as a prooxidant, resulting in cytotoxicity and oxidative stress [6, 7]. Wu et al. (2008) [8] conducted the initial *in vitro* studies using NAC as a cytoprotective agent for CHO cells exposed to radiation. The data indicated a significant prevention from loss of cell viability. The cell viability of 1 and 5 mM NAC treated groups was even lower than that of the radiation-induced group, indicating that, although NAC can provide some protection at lower concentrations, it is

cytotoxic at higher concentrations. The radioprotective effect of NAC was assessed also combined exposure between $HgCl_2$ and 4-nonylphenol, the result showed that cell death was prevented in the group pretreated with NAC. The potential utility of NAC in lower doses as a protector against radiation is worth considering here.

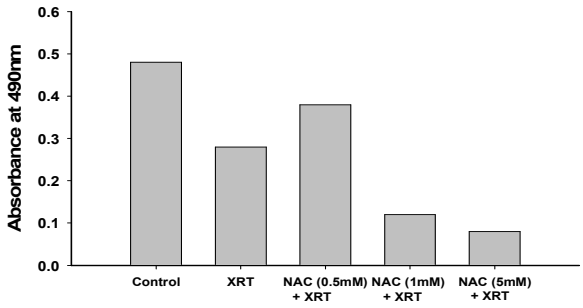

Fig. 2. Cytotoxicity of *in vitro* irradiated CHO cells pretreated with NAC. Data modified from Wu *et al.* (2008).

3. Conclusions

All organisms are continuously being exposed to harmful factors present in the environment. The biological effects due to the combined action of ionizing radiation with the other factor are hard to estimate and predict in advance. The synergistic effects observed after exposure of PLHC-1 cells to radiation and $HgCl_2$. On the other hand, NAC prevented cells from loss of viability. The investigations give a clue for establishment of fundamental theories associated with positive efficacy of radiation applications including synergistic or protective effects.

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