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# Protective Activity of *N*-acetyl-*L*-cysteine (NAC) against Cellular Oxidative Stress Induced by Radiation

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## 1. Introduction

Oxidative stress occurs due to numerous factors such as irradiation, redox decomposition by ions of hydroperoxides or hydrogen peroxide, and thermal decomposition of free radical initiators including peroxides and hyponitrites.

The antioxidant and free-radical scavenger *N*-acetyl-*L*-cysteine (NAC) is used extensively as a conditional nutrient. NAC acts as a cysteine donor and maintains or even increases the intracellular levels of glutathione (GSH), a tripeptide which protects cells from toxins such as free-radicals. With regard to the radioprotective effects of NAC, the majority of studies have been performed *in vitro* [1-2]. NAC were used to protect the Chinese hamster ovary (CHO) cells from radiation-induced apoptosis by controlling the enzyme that triggers programmed cell death [3]. Some studies have successfully demonstrated sporadic radioprotection following low-level chronic administration of NAC, though the mode and optimal dose of NAC are yet to be fully determined.

This study was designed to evaluate the effects of NAC in different doses on the activity levels of GSH and the cell viability in the fish cell line against ionizing radiation.

## 2. Methods and Results

### *2.1 Cell culture conditions and NAC treatments of cells*

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 were cultured in EMEM supplemented with 5 % fetal bovine serum (FBS) and incubated at 30 ℃ in a humidified atmosphere with 5 % $CO_2$. PLHC-1 cells were seeded in 96-well plates at a density of $2 \times 10^5$ cells/㎖ and incubated for 24 hr. Then cells were treated γ-rays from a $^{60}$Co γ-ray source (Korea Atomic Energy Research Institute, Korea) with 100 ~ 500 Gy in the presence or absence of 0.05 ~ 10 mM of NAC which were added 1 hr before.

### *2.2 Protective effects of NAC against radiation cytotoxicity*

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.).

The cell viability in the NAC pretreated groups was higher than that of the 50 and 100 Gy radiation-treated groups without NAC. The results showed that NAC prevented cells from radiation-induced death, but it caused cytotoxicity in the 300 Gy radiation-treated groups as its concentration increases.

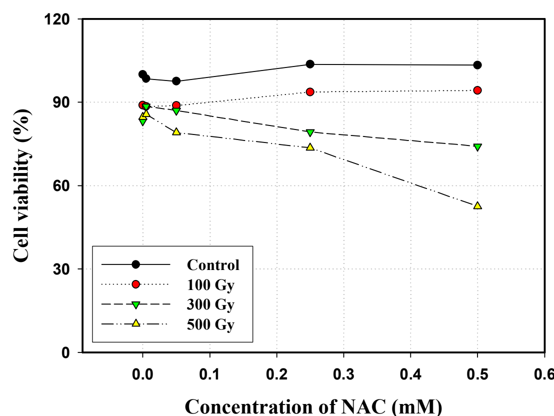


Fig. 1. Protective effects of NAC in radiation-induced cytotoxicity. Cytotoxicity was measured by MTT assay after 24 hr exposure, respectively. All the points showed a statistically significant difference from the control group according to Student's *t*-test ($p$<0.005).

### *2.3 The combined effects of radiation and NAC: Change of GSH levels*

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

scavenge free radicals [4]. According to several studies, NAC on one hand acts as an antioxidant, but on the other hand, it can also act as a pro-oxidant, resulting in cytotoxicity and oxidative stress [5-7]. 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 cells were collected in MES buffer. GSH (μ mol/mg protein) were assayed with Cayman Kits according to the manufacturer's instructions and determined by colorimetric method. Protein content was determined by the method of Bradford, using bovine serum albumin as standard. This study showed that intracellular GSH levels significantly decreased after treatment with radiation alone while the combined treatment of NAC and radiation prevented the decrease in GSH levels. These results suggest that NAC prevents radiation-induced cell damage including cell death by increasing the level of GSH.

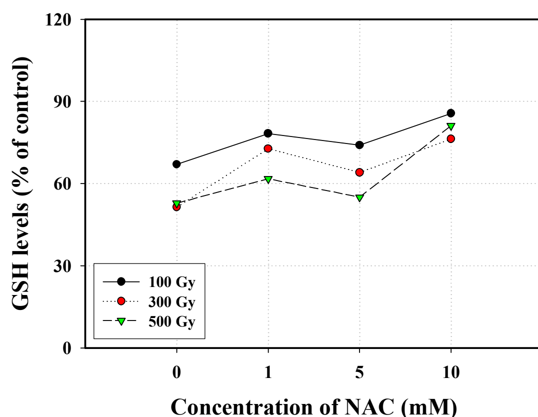


Fig. 2. Intracellular GSH levels measured by treating cells with radiation only and in combination with NAC. All the points showed a statistically significant difference from the control group according to Student's *t*-test ($p<0.005$).

## 3. Conclusions

Effects of NAC were assessed in PLHC-1 cells irradiated with 100 ~ 500 Gy. The data showed that NAC in lower concentrations prevented cell death after irradiation of lower doses. But NAC didn't prevent cells from radiation-induced death in higher doses of radiation (300, 500 Gy). The intracellular GSH levels significantly decreased after treatment with radiation alone while the combined treatment of NAC and radiation alleviated the decrease in GSH levels. These results suggest that radiation-induced oxidative stress and NAC prevents radiation-induced cell damage including cell death by increasing the level of GSH. Based on these results, it is suggested that PLHC-1 cells can serve as rapid, screening test tools for detecting the response of radiation. Also, this study may be useful for elucidating the effects of radiation on aquatic organisms.

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