Combined Effects of Radiation and Mercury on PLHC-1 Cells

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1. Introduction

It is inevitable for living objects to expose themselves to multiple factors present in the environment. The combined effect of multi-factors is hard to estimate and predict in advance. Expecially factors harmful to organisms can synergistically interact with each other. When the effect of the combined action is greater than expected additivity, it is called synergism or supra-additivity [1].

Ionizing radiation can cause cell death, mainly due to its ability to produce reactive oxygen species in cells. Mercury is one of widespread environmental pollutants which is known to have toxic effects on organisms. There are many reports indicating its genotoxic potential in a variety of aquatic species. Synergistic effects of radiation and mercury on human cells was previously reported [2]. Aerobically growing organisms suffer from exposure to oxidative stress, caused by partially reduced forms of molecular oxygen, known as reactive oxygen species. These are highly reactive and capable of damaging cellular constituents such as DNA, lipids and proteins. Consequently, cells from many different organisms have evolved mechanisms to protect their components against reactive oxygen species. Reactive oxygen species can also be formed by exposure of cells either to ionizing radiation or redox-cycling chemicals present in the environment like heavy metals [3].

PLHC-1 hepatoma cell line derived from topminnow (*Poeciliopsis lucida*) is the most commonly used cell line in toxicology. The PLHC-1 cells are easy to cultivate, and can be used for screening the toxicity of chemicals. The present study was done to evaluate the combined effects of radiation with mercury chloride on the PLHC-1 cells.

2. Materials and Methods

2.1 Cell culture

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℃ 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.

2.2 Treatment of radiation and mercury chloride

PLHC-1 cells were seeded in 96-well plates at a density of 2 x 10^5 cells/㎖ and incubated for 24 hours. Then cells were irradiated with γ -rays from a ^{60}Co isotopic source of KAERI (source strength; 7.4 PBq) with doses of 10～500 Gy in the presence or absence of 1～500 μM of $HgCl_2$ which were added 24, and 48 hours before.

2.3 Measurement of cell viability

After the treatment of radiation and mercury, cell viability was analyzed by the MTT assay. For the 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 570 nm using ELISA reader (Multiskan® EX, Forma Scientific, Inc.).

3. Results

Effect of ionizing radiation on the viability of PLHC-1 cells was assessed *in vitro* by MTT assays after 24 and 48 hours. The cell viability was linearly reduced in a dose- and time-dependent manner by radiation exposure. All the data showed a statistically significant difference from those of the control group. The half lethal dose (LD_{50}) was calculated as 298 and 255 Gy for 24 and 48 hr treatment, respectively.

Effect of the mercury chloride on viability of PLHC-1 cells was also assessed *in vitro* by MTT assays after 24 and 48 hours. The cell viability was greatly reduced in a dose- and time-dependent manner by $HgCl_2$ exposure. Upto 100 μM of $HgCl_2$, the cell viability did not change. However, it drastically decreased, and most of cells were died at 250 μM $HgCl_2$. All the points

showed a statistically significant difference from the control group according to Student's *t*-test ($p<0.005$).

The *in vitro* cytotoxicity assay of PLHC-1 cells was performed after simultaneous treatment of ionizing radiation and $HgCl_2$. The simultaneous treatment of the cells resulted in a dramatic increase of cell death even at the low intensity of both factors.

A simple addition of the effect of separate action was tabulated in Table 1. The experimental data on the simultaneous treatment of ionizing radiation and mercury chloride resulted in much higher values in cell killing than estimated from a simple additive summation (Table 2). Big differences between the additive sum of the effect of separate treatment and simultaneously treated effects is a clear proof of synergistic interaction between two inactivation factors, ionizing radiation and mercury chloride.

This kind of synergistic interaction in the cells was thought to be due to an increase of irreversibly damaged cells after the combined exposure of cells to harmful factors.

Table 1. Additivity of the effect of separate treatment

Hg IR	0 mM	1 mM	10 mM	100 mM	125 mM
0 Gy	100	8.19	3.12	9.37	16.14
10 Gy	0.15	8.35	3.28	9.53	16.30
50 Gy	0.07	14.26	9.19	15.44	22.21
100 Gy	14.32	22.51	17.44	23.69	30.46
200 Gy	17.22	25.41	20.35	26.59	33.36
300 Gy	38.50	46.69	41.62	47.87	54.64
400 Gy	53.66	61.85	56.79	63.03	69.80
500 Gy	58.94	67.13	62.06	68.31	75.08

Table 2. Effects of simultaneous treatment of radiation and $HgCl_2$

Hg IR	0 mM	1 mM	10 mM	100 mM	125 mM
0 Gy	100	8.19	3.12	9.37	16.14
10 Gy	0.15	11.58	3.27	10.82	17.36
50 Gy	0.07	12.97	9.85	18.29	30.04
100 Gy	14.32	23.90	24.40	27.96	33.90
200 Gy	17.22	42.49	46.38	52.80	56.54
300 Gy	38.50	58.20	65.10	65.88	67.76
400 Gy	53.66	65.82	74.91	74.75	75.73
500 Gy	58.94	71.83	80.80	80.13	80.46

4. Conclusions

The cytotoxicity of ionizing radiation on a fish hepatoma cell line (PLHC-1) was assessed. In the cytotoxicity tests using on MTT assay, radiation treatment elicited a toxicity in the PLHC-1 cells in a dose dependent pattern with on LD50 values of 298.7 (24 hr) and 254.9 (48 hr) Gy. 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. According to these results, we suggested that PLHC-1 cells can serve as rapid, screening test tools for detecting the toxicity of radiation. Also, this study may be useful for elucidating the toxicity levels of ionizing radiation which would display in the aquatic organisms.

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