Journal of Associated Medical Sciences 2025; 58(2): 73-79

JAMS
Journal of Associated Medical Sciences

Scopus Indexed & Thai-Journal Citation Index Centre (TCI)

Journal of Associated Medical Sciences

Journal homepage: https://www.tci-thaijo.org/index.php/bulletinAMS/index

Effect of pre-exposure to low-dose radiation followed by H_2O_2 treatment on leukemic cells proliferation

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ARTICLE INFO

Article history:
Received 12 November 2024
Accepted as revised 18 December 2024
Available online 15 January 2025

Keywords:
Low-dose radiation, free radical, radiation, cancer, cell death.

ABSTRACT

Background: Leukemia is a blood cancer illness that causes morbidity and mortality around the world. A new approach to treatment is challenging.

Objective: The objective was to investigate the effects of pre-exposure to low-dose radiation (LDR) followed by H_2O_2 treatment on cell proliferation in leukemic cells.

Materials and methods: The human leukemic doxorubicin-sensitive K562 and doxorubicin-resistant K562/*adr* cells were exposed to 0.1 mGy of gamma radiation from radioactive ^{137}Cs at a dose rate of 0.001 Gy/min followed by treatments with various concentration of H_2O_2 at 4 hrs-post irradiation. The cell morphology and metaphase cells in treated groups were compared with a control group at 72 hrs post-irradiation. The cell proliferation was determined at days 1-8 post irradiation.

Results: The results showed that the number of cells in treated groups with 50, 100, and 200 µM of H_2O_2 was less than the control group (0 µM H_2O_2). The pattern of the cells rapidly increased at 24-48 hrs and then decreased until the 5th and 6th days. This was found in all treated groups, except those treated with 200 µM of H_2O_2 alone and with pre-exposure to LDR followed by H_2O_2 groups. The microscopic images showed rough cells, shrinking cells, irregular-shaped cells, and swelling cells in H_2O_2 alone and pre-exposure to LDR followed by H_2O_2 groups. The metaphase cells were significantly decreased in H_2O_2 alone groups in a concentration-dependent manner. In addition, the metaphase cells were also considerably reduced in pre-exposure to LDR, followed by H_2O_2 groups when compared with control and LDR alone groups.

Conclusion: This data provides a basis for additional studies to help clarify the potential use and benefits of pre-exposure to LDR followed by H_2O_2 treatment in cancer cells.

Introduction

Reactive oxygen species (ROS) are commonly used to define molecules with high oxidative reactivity. ROS are produced exogenously following exposure to xenobiotics like radiation, chemotherapy, or ultraviolet light. They are also physiologically produced due to aerobic metabolism and cellular respiration. Pathologically enhanced ROS are seen in disorders such as cancer and inflammation. ROS are a group of highly reactive oxygen-containing molecules such as hydroxyl radicals ($HO^{\bullet}$), superoxide radicals ($O_2^{\bullet-}$), and hydrogen peroxide (H_2O_2).[1,2] Under normal physiological conditions, intracellular ROS is scavenged

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doi: 10.12982/JAMS.2025.040
E-ISSN: 2539-6056

using antioxidants to control the dynamic redox balance in cells.[3] Evidence suggests that the high amounts of ROS typically detected in cancer cells are almost incompatible with cell viability, making these cells more sensitive to ROS-induced cell death than normal cells.[4,5]

Leukemia is a blood cancer illness that causes morbidity and mortality around the world.[6] Chemotherapy is routinely used to treat this condition. However, treatment with anticancer drugs for leukemia is frequently limited by the development of multidrug resistance (MDR) in cancer cells.[7,8] As a result, developing a new approach to overcoming MDR in cancer cells that involves MDR-reversing drugs that decrease undesired side effects is a formidable challenge.

Evidence shows several biological responses to low doses of low linear energy transfer radiation (i.e., gamma- and X-rays).[9-11] Chen Z. and Sakai K. found that LDR did not produce hormesis in human leukemic MOLT-4 cells.[12] However, pre-exposure to LDR could induce apoptosis when subjected to a high radiation dosage. These LDR effects were not observed in normal cells compared to tumor cells under identical circumstances. Furthermore, the LDR-induced cell growth has been shown in normal cells, but they did not cause the cell growth of cancer cells, including leukemic K562 cells.[13,14]

Based on these facts, we decided to evaluate the effect of pre-exposure to LDR followed by H_2O_2 treatment on human leukemic doxorubicin-sensitive K562 and doxorubicin-resistant K562/*adr* cells by investigating the cell proliferation, cell morphology, and metaphase cells. The objective was to investigate the effect of pre-exposure to LDR followed by H_2O_2 treatment on cell proliferation in leukemic cells.

Material and methods

Chemicals

Fetal bovine serum (FBS) and penicillin/streptomycin were bought from Capricorn Scientific. RPMI-1640, hydrogen peroxide, and trypan blue were received from Caisson Labs Colcemid (GIBCO-BRL).

Cell line and cell culture

The human leukemic doxorubicin-sensitive K562 cell and doxorubicin-resistance K562/*adr* (P-glycoprotein-overexpression) cell were used in the study. The cells were grown in a complete RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum and 1% penicillin/streptomycin in a 5% CO_2, 37 °C incubator. The density of cells was initially at 1×10^5 cells/mL and increased to 8-10×10^5 cells/mL 72 hrs later, followed by sub-cultured cells.[15,16]

For the experiment, cells initially had a density of 5×10^5 cells/mL and increased to 8-10×10^5 cells/mL 24 hours later. Cells were in an exponential growth phase.

Irradiation

The irradiation was performed as previously described.[17] The ^{137}Cs radioactive standard source located at the Department of Radiologic Technology, Faculty of Associated Medical Sciences, Chiang Mai University, Thailand, was used as a gamma radiation source. Cells were given a dose of 0.1 mGy gamma rays at a dose rate of 0.001 Gy/min. The radiation dose was calculated by using the equation;

$$A_t = A_0 e^{-\lambda t}$$

And $$D = \frac{A_t \times \Gamma}{d^2}$$

When A_0 and A_t : activity of radioactive present at t=0 and time=t
λ : decay constant,
D : radiation dose
d : distance from radioactive
Γ : specific gamma ray constant.

Cell morphological observation

A number of cells at 1×10^5 cells/mL were irradiated to 0.1 mGy of gamma radiation from radioactive ^{137}Cs, followed by treatments with and without 50, 100, and 200 µM of H_2O_2 in a complete RPMI-1640 medium at 4 hrs post-irradiation. The cells were placed into a humidified atmosphere having 5% CO_2 at 37 °C incubation. After 72 hrs post-irradiation, the cells were observed under a light microscope without any cell disruption.

Cell proliferation

The cell proliferation was measured by using the trypan blue exclusion method. Cells (1×10^5 cells/mL) were irradiated to 0.1 mGy of gamma radiation from radioactive ^{137}Cs followed by treatments with and without 50, 100, and 200 µM of H_2O_2 in a complete RPMI-1640 medium at 4 hrs-post irradiation. They were placed into a humidified atmosphere with 5% CO_2 at 37 °C incubation. The number of cells was counted under a light microscope by staining with trypan blue solution at a ratio of 1:1 (volume: volume). The cell proliferation was determined at days 1-8 post irradiation.

Metaphase assay

Cells (1×10^6 cells/mL) were irradiated to 0.1 mGy of gamma radiation from radioactive ^{137}Cs, followed by treatments with and without 50, 100, and 200 µM of H_2O_2 in a complete RPMI-1640 medium at 4 hrs-post irradiation. They were placed into a humidified atmosphere with 5% CO_2 at 37 °C incubation. After 72 hrs-post irradiation, a colcemid (30 µL of 10 µg/mL) was added to each cell. After 45 min incubation in a 37 °C, 5% CO_2 humidified incubator, cells were centrifuged and washed twice in 1 mL of phosphate buffer saline (PBS). Five hundred µL of 0.075 M KCl was added to each cell, followed by 45 min of additional incubation. Afterward, cells were centrifuged and washed twice, then added 1 mL of fixatives (Carnoy's solution, 3:1 v/v methanol:acetic acid). Fixed cells were dropped gently on a clean microscope slide, air-dried, and stained with Wright Giemsa for 3 min. The slides were coded, and the codes were not disclosed until after the slides had been scored. The metaphase cells are shown in the Figure 1.

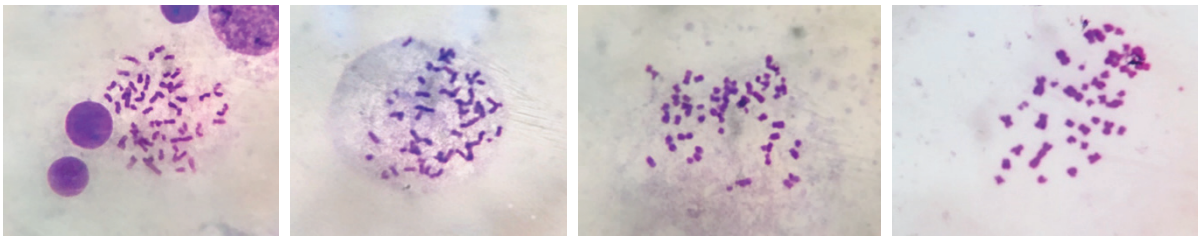

***Figure 1**. Metaphase cells under a light microscope (40X magnification).*

Statistical analysis

The results were shown as the mean ± SEM. Student's t-test was used independently to evaluate statistical differences in the mean values between the treated and non-treated groups. The $p<0.05$ was considered to indicate a statistically significant difference.

Results

Cell morphology

Figure 2 shows the morphology of K562 and K562/*adr* cells under a light microscope. The microscopic images showed rough cells, shrinking cells, irregular-shaped cells, swelling cells, and decreases in cell density in K562 and K562/*adr* cells treated with H_2O_2 alone and having pre-exposure to LDR followed by H_2O_2 groups. These phenomena in treated cells were linked to characteristics of cell death.

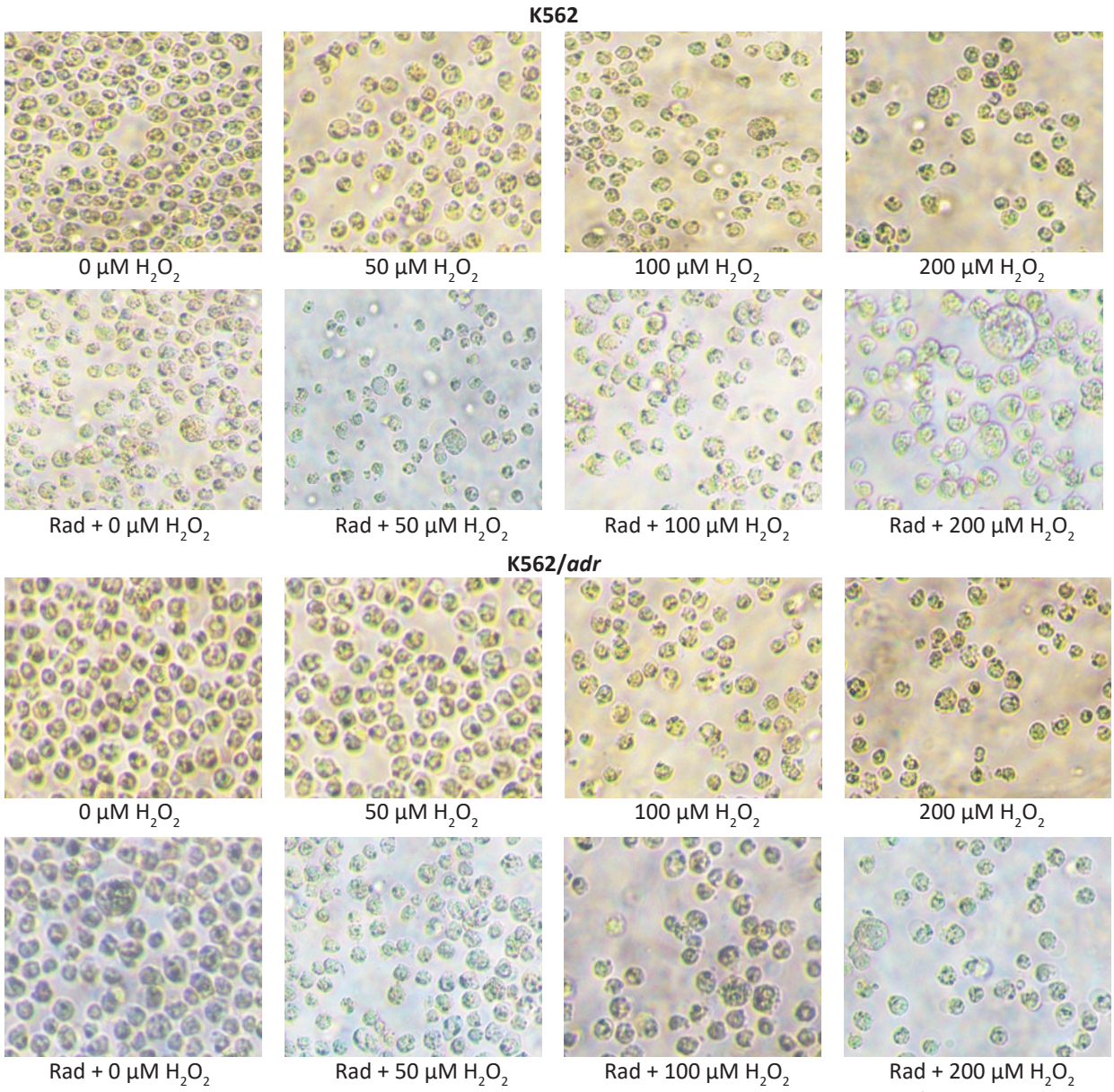

***Figure 2**. Effect of H_2O_2 and pre-exposure to LDR followed by H_2O_2 on K562 and K562/adr cells, light microscope, 20X magnification. Rad: radiation.*

Cell proliferation

The cells rapidly increased at 24-48 hrs and then decreased until 5-6 days. This pattern was found in all treated groups, except cells treated with 200 µM of H_2O_2 alone and with pre-exposure to LDR followed by 200 µM of H_2O_2 groups, as indicated in Figure 3. In addition, the number of cells in treated cells with 50, 100, and 200 µM of H_2O_2 was less than in non-treated cells (0 µM H_2O_2).

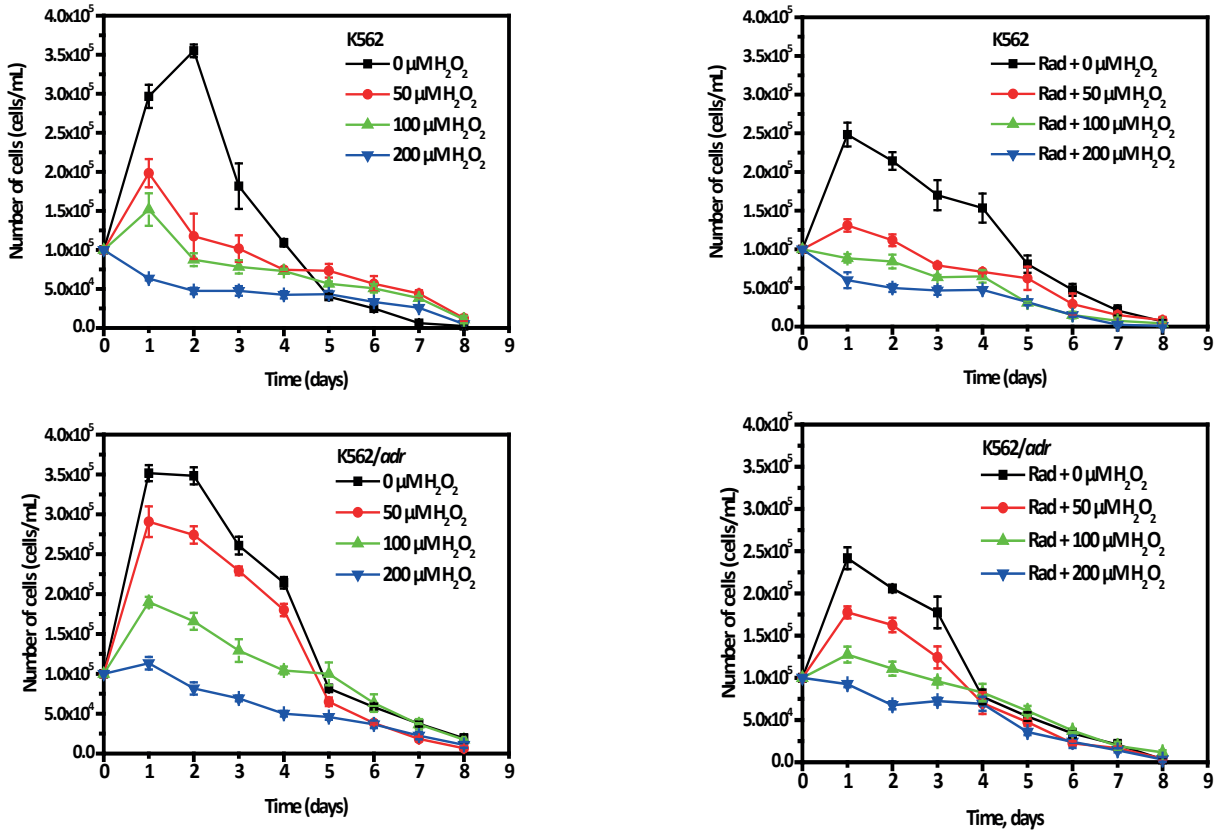

Figure 3*. Effect of H_2O_2 alone and pre-exposure to LDR followed by H_2O_2 on cell viability of K562 and K562/adr cells. Rad: radiation.*

Metaphase cells

Figure 4 shows the number of metaphase cells in treated and non-treated K562 and K562/*adr* cells. This data showed that the metaphase cells significantly decreased in both treated K562 and treated K562/*adr* cells with 50, 100, and 200 µM of H_2O_2 compared to a control group.

Similarly, the metaphase cells in K562 and K562/*adr* cells decreased in pre-exposure to LDR, followed by treatments with 50, 100, and 200 µM of H_2O_2 groups compared to a control group. Of note, the number of metaphase cells in irradiated K562 and irradiated K562/*adr* cells significantly decreased compared to the control group.

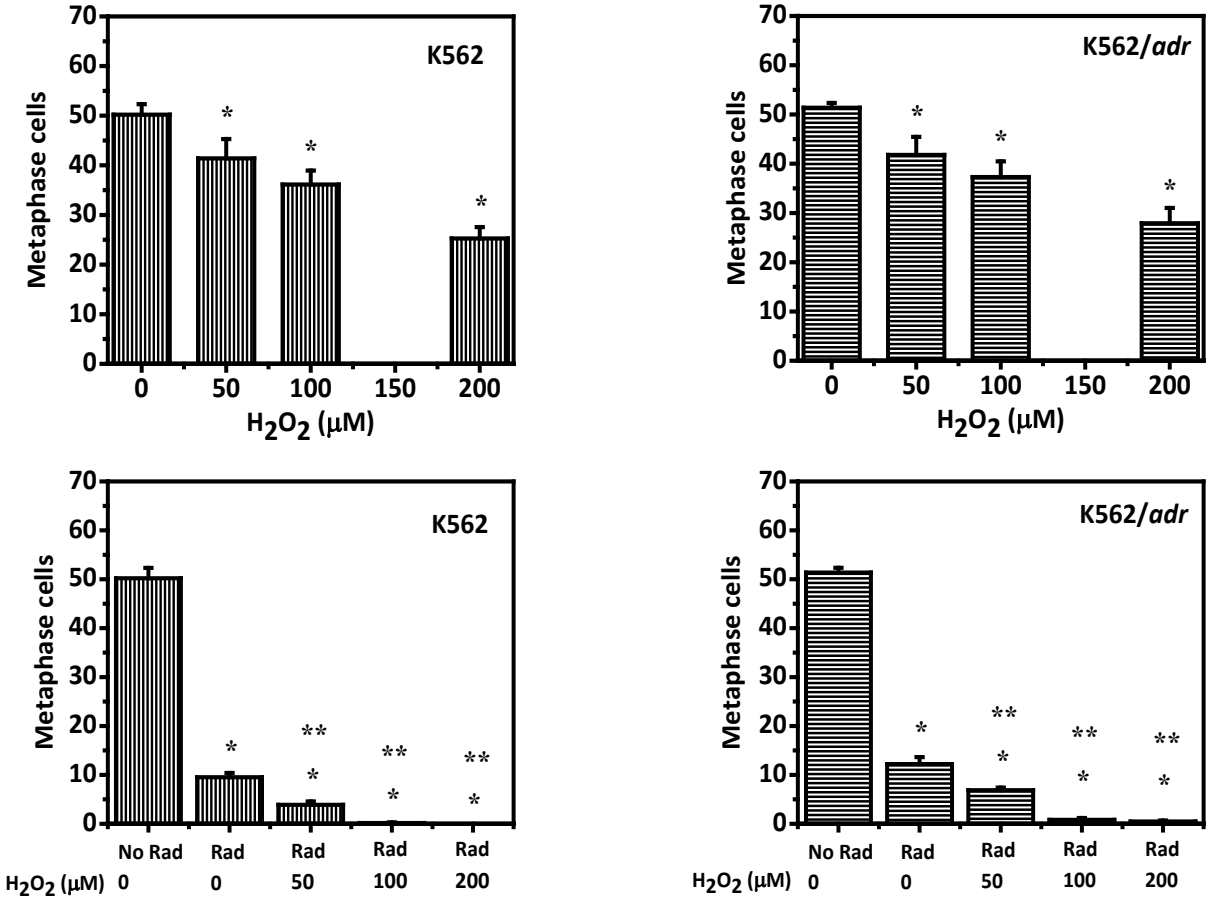

Figure 4*. The number of metaphase cells in 1,000 treated and non-treated K562 and K562/adr cells. *p<0.05 as compared with No Rad, H_2O_2 0 µM group. **p<0.05 as compared to Rad, H_2O_2 0 µM group, Rad: radiation.*

Discussion

Our study provides additional evidence regarding the effect of pre-exposure to LDR followed by H_2O_2 on the morphology, proliferation, and metaphase cells of the K562 and K562/*adr* cells. The microscopic images showed rough cells, shrinking cells, irregular-shaped cells, and swelling cells in pre-exposure to LDR, followed by the H_2O_2 groups and H_2O_2 alone group. These morphological changes and reduction of the number of metaphase cells are the typical signs of cell death. Therefore, this data suggests pre-exposure to LDR followed by H2O2-induced K562 and K562/*adr* cells deaths. Consequently, the number of cells in pre-exposure to LDR followed by H_2O_2 groups and in 50, 100, and 200 µM of H_2O_2 groups were less than non-treated cells (0 µM H_2O_2).

We have not examined the mechanisms by which pre-exposure to LDR followed by H_2O_2 induced cell death in the cells. However, it is well known that hydrogen peroxide is a potent pro-oxidant that can generate and accumulate intracellular ROS, resulting in cell death and apoptosis or necrosis.[18-22] Kohshour MO *et al*. studied the antiproliferative effects of H_2O_2 against human acute myelogenous leukemia KG1 cell lines.[23] The authors showed that H_2O_2 significantly decreased cell proliferation and led to the induction of apoptosis via activation of caspase-3, affecting the expression of Bcl-2 and Bax. Datta K. *et al*. investigated the induction of cell death by H_2O_2 and its relation to p53 in human glioma cells.[24] The authors showed that H_2O_2 induced p53-dependent apoptosis in glioma cells in a dose-dependent manner. Moreover, H_2O_2 could cause an increase in the level of DNA damage, resulting in apoptotic cell death in Jurkat T-cells.[25] Dumont A. *et al*. showed H_2O_2-induced T-cell death that involved the formation of mitochondrial permeability transition pores, resulting in a rapid decrease of the mitochondrial transmembrane potential and the release of cytochrome C.[26] In addition, it has been well established that biological effects of radiation result from energy

deposition in irradiated cells in which ROS, including H_2O_2, are produced.[27] We have reported that ROS statistically significantly increased.

In contrast, mitochondrial activity decreased,[17] and lysosomal pH changed [28] in the K562 and K562/*adr* cells after exposure to LDR from ^{137}Cs in a dose-dependent manner.

Taken together, for these reasons, it is plausible to hypothesize that the effect of pre-exposure to LDR from ^{137}Cs followed by H_2O_2 treatment observed in our *in vitro* study is due to its pro-oxidative activity. However, the limitation of our study was that only one dose of LDR (0.1 mGy) was used in our research. Therefore, it remains unknown to what extent LDR can sustain the effect of pre-exposure to LDR followed by H_2O_2 in K562 and K562/*adr* cells. Consequently, further investigation is required to determine the magnitude of the impact of pre-exposure to LDR followed by H_2O_2 in K562 and K562/*adr* cells, using different treatment protocols.

Conclusion

These findings suggest that pre-exposure to LDR followed by H_2O_2 treatment significantly decreased cell proliferation in leukemic doxorubicin-sensitive K562 and doxorubicin-resistant K562/*adr* cells in a H_2O_2 concentration-dependent manner. Therefore, this data provides a basis for additional studies to help clarify the potential use and benefits of pre-exposure to LDR followed by H_2O_2 treatment in cancer cells.

Acknowledgements

This research was partially supported by Chiang Mai University. The authors would like to thank the Faculty of Associated Medical Sciences, Chiang Mai University, for their support. We also want to thank all staff and students who participated in this study.

Ethical approval

This article contains no studies with human subjects or animals performed by any authors.

Conflict of interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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