# Different responses of normal cells (red blood cells) and cancer cells (K562 and K562/Dox cells) to low-dose $^{137}$Cs gamma-rays

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**Abstract.** High-dose radiation is deleterious to cells or tissues. However, the health risks of exposure to low-dose radiation remain unclear. The present study aimed to investigate the biological responses of low-dose gamma-ray *in vitro* exposure to normal red blood cells (RBCs) and erythroleukemia (K562 and K562/Dox) cancer cells. Cells were given a low dose of 0.03, 0.05 and 0.1 mGy of $^{137}$Cs gamma-rays (at a dose rate of 0.001 Gy/min) under *in vitro* conditions. Cells exposed to 0 Gy served as controls. Hemolysis and reactive oxygen species (ROS) were measured in exposed RBCs following exposure to low-dose gamma-rays. In addition, complete blood count (CBC) parameters were determined in irradiated whole blood. For irradiated K562 and K562/Dox cancer cells, ROS and mitochondrial activity were measured at 0, 30, 60 and 120 post-irradiation times. The results showed no change in the percentage of ROS and hemolysis in irradiated RBCs. The data indicated no perturbation in the CBC parameters in irradiated whole blood. By contrast, statistically significant dose-dependent increases in the percentage of ROS and decreases in the mitochondrial activity in the K562 and K562/Dox cancer cells were observed from 0 min up to 120 min post-irradiation. These findings concluded that there were differences in biological responses in normal cells (RBCs) and cancer cells (K562 and K562/Dox) to low-dose gamma-rays when cells were irradiated under *in vitro* conditions.

## Introduction

It is well known that a high dose of radiation is deleterious to cells or tissue (1-4). However, the health risks of exposure to low-dose radiation remain unclear. Several researchers have studied the biological response of normal cells or tissue to low doses of radiation using various biological endpoints such as neoplastic transformation, chromosome or DNA damage and immune function (5-17). In addition, several studies investigated the biological response of cancer cells or tissue to low doses of radiation using biological endpoints such as cell cycle and cell death (18-20). These studies showed differences in the biological response of normal and cancer cells or tissues to low dose radiation. Nonetheless, the limitation of the previous data on the response of normal and cancer cell or tissue to low-doses radiation is radiation doses in the range of centi Gray (cGy). Hence, the present study investigated the different responses of normal cells (blood cells) and cancer cells to low dose gamma-rays in the milli Gray (mGy) range.

These current studies focused on the four endpoints of biological responses that are recognized to be associated with oxidative stress induced by radiation. These biological responses are reactive oxygen species (ROS) levels, mitochondrial activity (which represents mitochondrial function), hemolysis (which represents plasma membrane integrity in red blood cells (RBCs)] and complete blood count (CBC). The focus was on ROS levels since it radiation (both low- and high-dose radiation) generates free radicals including ROS, resulting in oxidative damage in cells or tissues (21,22). Oxidative stress is a disturbance in the balance between the yields of free radicals including ROS and antioxidant defenses (23). Typically, oxidative damage in cell or tissue induces mitochondria dysfunction or lipid peroxidation in plasma membranes (21,24-27). Mitochondrial dysfunction and plasma membrane damage is known to be involved in mitochondrial activity and red blood cell hemolysis, respectively. Moreover, the abnormal deformability of RBCs was observed in conditions linked to oxidative stress (28). In addition, radiation induced deleterious effects in blood cells in irradiated whole blood when compared with non-irradiated whole blood (29).

## Materials and methods

*Blood samples*. Blood samples (n=10) were collected from remaining normal blood test group (males; age range, 40-50 years)

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*Key words:* blood, cancer, low-dose radiation, gamma-ray, radiation

at the Associated Medical Sciences Clinical Service Center, Faculty of Associated Medical Sciences, Chiang Mai University, Thailand.

*Irradiation*. Blood samples were given a dose of 0.03, 0.05 and 0.1 mGy gamma-rays (at a dose rate of 0.001 Gy/min) using a $^{137}Cs$ radioactive standard source (located at the Department of Radiologic Technology, Faculty of Associated Medical Sciences, Chiang Mai University, Thailand). Samples exposed to 0 Gy served as controls. The equations were used to calculate radiation dose as following; i) $At = A_0e^{-\lambda t}$; ii) $D = A_t \times \Gamma/d^2$.

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

*Cancer cells and culture*. Doxorubicin-sensitive erythroleukemia K562 cells (K562) and doxorubicin-resistant erythroleukemia K562 cells (K562/Dox, overexpressing P-glycoprotein) were provided by Dr Udomtanakunchai C. The cells were grown in RPMI-1640 medium supplemented with 10% fetal calf serum and 1% penicillin/streptomycin at 37˚C, 95% humidity and 5% $CO_2$. The cells were seeded at a density of $1x10^5$ cells/ml then exponentially grown to $8\text{-}10x10^5$ cells/ml in 3 days. To obtain cells in the exponential growth phase for the experiments, cells were initiated at a density of $5x10^5$ cells/ml. Cells were used for experiments 24 h later after reaching a density of $8\text{-}10x10^5$ cells/ml.

*Measurement of intracellular ROS levels*. Cells ($5x10^5$ cells/ml) were incubated with 10 μM 2',7'-dichlorofluorescein (DCF) diacetate for 30 min in the dark. Subsequently, intracellular ROS levels were measured using fluorescence intensity at an emission wavelength of 523 nm (excitation wavelength, 502 nm) using a fluorescence spectrometer (PerkinElmer, Inc.).

*Mitochondrial activity*. Living cells are able to reduce the nonfluorescent dye resazurin into the fluorescent dye resorufin via mitochondrial reductase. Hence, resazurin sodium salt (Sigma-Aldrich; Merck KGaA) was used to determine mitochondrial activity. Cells ($5x10^5$) were incubated with 100 μl resazurin solution (0.1 mg/ml) in 1 ml PBS at 37˚C and were humidified with 5% $CO_2$ for 2 h. Subsequently, resazurin fluorescence intensity at a wavelength of 590 nm (excitation wavelength, 570 nm) which is an indicator of mitochondrial activity in living cells was measured on a spectrofluorometer using a well-plate reader.

*Hemolysis in normal RBCs*. The hemolysis assay was performed based on previously published studies (30,31). Briefly, 25 μl of blood sample was incubated in 725 μl PBS and in 725 μl distilled $H_2O$ for 30 min at 37˚C. Next, blood samples were centrifuged at 7,000 rpm for 1 min. The absorbance at wavelength 415 nm was recorded using a spectrophotometer (Agilent 8453 UV-vis spectrophotometer; Agilent Technologies, Inc.). The percentage of hemolysis was then calculated.

*Determination of CBC parameters in whole blood*. CBC parameters were measured at the AMS Clinical Service Center, Faculty of Associated Medical Sciences, Chiang Mai University, Thailand. CBC parameters considered for the current included red blood cell count, hematocrit (HCT), mean corpuscular volume (MCV), red cell distribution width standard deviation (RDW-SD), white blood cell (WBC) count, neutrophil (NEUT) count, lymphocyte (LYMPH) count, monocyte (MONO) count, eosinophil (EO) count, basophil (BASO) count, platelets (PLT) count, platelet distribution width (PDW), pateletcrit (PCT) and mean platelet volume (MPV).

*Statistical analysis*. The data were expressed as the mean ± SEM. An analysis of variance (ANOVA) method appropriate for a one-factor experiment (radiation dose) was used to assess the significance of radiation dose. Further, the post hoc test (Tukey test) was used to evaluate statistical differences in the mean values between each group. Student's t-test was used independently to evaluate statistical differences in the mean values between each test group and the corresponding control group. $P<0.05$ was considered to indicate a statistically significant difference.

## Results

*Effect of low-doses gamma-rays on blood cells*

*Effect on ROS in normal RBCs*. Fig. 1 shows the percentage of ROS in RBCs following *in vitro* exposure to various low doses of gamma-rays and in the corresponding non-irradiated control groups. The data showed no change in the percentage of ROS in irradiated RBCs relative to the corresponding non-irradiated RBCs (ANOVA test; P-value =0.11).

*Effect on the percentage of hemolysis in normal RBCs*. Fig. 2 shows the percentage of hemolysis in RBCs following *in vitro* exposure to various low doses of gamma-rays and in the corresponding non-irradiated control groups. The results showed that the percentage of hemolysis did not change in irradiated RBCs compared with corresponding non-irradiated RBCs (Student's t-test; P-value range, 0.61-0.87).

*Effect on CBC parameters in whole blood*. Table I shows the CBC parameters in whole blood following *in vitro* exposure to various low doses of gamma-rays. Similar to the percentage of ROS and hemolysis, this data indicated no alteration in the complete blood count in irradiated whole blood compared with the corresponding non-irradiated whole blood.

*Effect of low-doses gamma-rays on K562 and K562/Dox cancer cells*

*Effect on ROS in cancer cells*. Fig. 3 shows the percentage of ROS in K562 and K562/Dox cancer cells collected at 0, 30, 60 and 120 min after exposure to various low doses of gamma-rays. The data showed statistically significant dose-dependent increases in the percentage of ROS in the K562 and K562/Dox cancer cells from 0 min up to 120 min post-irradiation.

In K562 cancer cells, the increases were 1.04-, 1.08- and 1.10-fold higher compared with the control at 0 min post-irradiation; 1.06-, 1.11- and 1.20-fold higher compared with the control at 30 min post-irradiation and 1.10-, 1.17- and 1.28-fold higher compared with the control at 60 min post-irradiation. Likewise, the increase in ROS levels in exposed cells at 120 min post-irradiation were 1.17-, 1.22- and 1.29-fold higher compared with the control.

In K562/Dox cancer cells, at 0 min post-irradiation, the increases were 1.02-, 1.05- and 1.09-fold higher compared

Table I. Complete blood count parameters in whole blood following *in vitro* exposure to various low doses of gamma-rays.

| | Radiation dose | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 mGy | 0.03 mGy | | 0.05 mGy | | 0.1 mGy | |
| Parameters | Mean ± SE | Mean ± SE | P-value | Mean ± SE | P-value | Mean ± SE | P-value |
| RBC ($10^6/\mu$l) | 2.86±0.36 | 2.40±0.05 | 0.29 | 2.30±0.14 | 0.23 | 2.20±0.14 | 0.17 |
| HCT (%) | 24.58±3.22 | 20.90±0.55 | 0.34 | 20.02±1.16 | 0.26 | 19.14±1.28 | 0.19 |
| MCV (fl) | 85.93±2.88 | 87.20±2.53 | 0.75 | 87.20±2.58 | 0.75 | 86.98±2.51 | 0.79 |
| RDW-SD (fl) | 40.05±1.32 | 41.76±2.08 | 0.51 | 41.68±2.02 | 0.52 | 41.58±1.87 | 0.53 |
| PLT ($10^3/\mu$l) | 77.75±11.24 | 105.00±21.10 | 0.30 | 106.40±20.60 | 0.27 | 107.00±21.34 | 0.27 |
| PDW (fl) | 11.88±0.83 | 11.52±0.99 | 0.79 | 11.34±0.71 | 0.64 | 11.08±0.81 | 0.51 |
| MPV (fl) | 10.65±0.29 | 10.04±0.44 | 0.29 | 10.10±0.39 | 0.30 | 10.08±0.36 | 0.26 |
| PCT (%) | 0.08±0.01 | 0.10±0.02 | 0.39 | 0.11±0.02 | 0.32 | 0.10±0.02 | 0.39 |
| WBC ($10^3/\mu$l) | 4.03±0.32 | 4.12±0.24 | 0.83 | 4.15±0.37 | 0.82 | 4.05±0.37 | 0.98 |
| NEUT (%) | 62.63±3.95 | 59.42±3.63 | 0.57 | 59.06±3.79 | 0.54 | 58.14±3.45 | 0.42 |
| LYMPH (%) | 28.75±3.82 | 30.32±3.36 | 0.77 | 30.86±3.38 | 0.69 | 31.60±3.31 | 0.59 |
| MONO (%) | 5.73±0.60 | 6.84±0.16 | 0.16 | 6.48±0.25 | 0.31 | 6.86±0.24 | 0.16 |
| EO (%) | 2.73±0.36 | 3.28±0.97 | 0.61 | 3.46±1.10 | 0.55 | 3.36±1.02 | 0.58 |
| BASO (%) | 0.18±0.06 | 0.14±0.06 | 0.70 | 0.14±0.06 | 0.70 | 0.04±0.04 | 0.13 |

RBC, red blood cell; HCT, hematocrit; MCV, mean corpuscular volume; RDW-SD, red cell distribution width standard deviation; PLT, platelets; PDW, platelet distribution width; MPV, mean platelet volume; PCT, pateletcrit; WBC, white blood cell; NEUT, neutrophil; LYMPH, lymphocyte; MONO, monocyte; EO, eosinophil; BASO, basophil; SE, standard error.

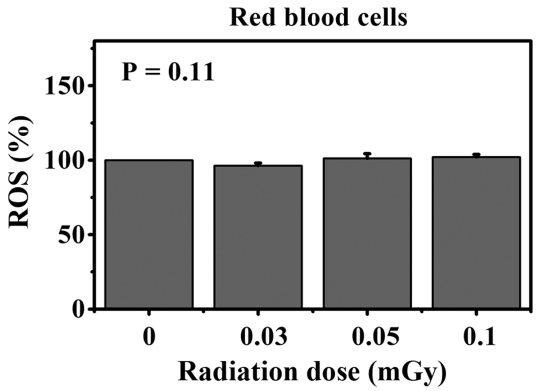


Figure 1. Reactive oxygen species percentage in red blood cells following *in vitro* exposure to various low doses of gamma-rays and in the corresponding non-irradiated control groups. ROS, reactive oxygen species.

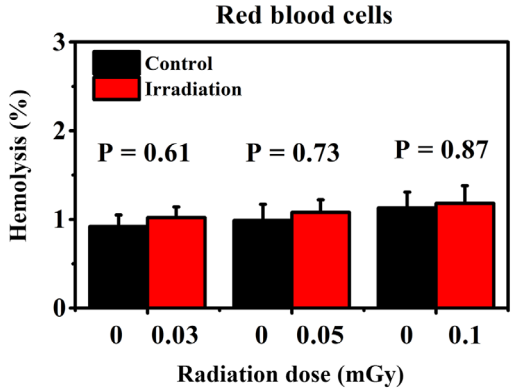


Figure 2. Hemolysis percentage in red blood cells following *in vitro* exposure to various low doses of gamma-rays and in the corresponding non-irradiated control groups.

with the control; at 30 min post-irradiation, the increases were 1.03-, 1.05- and 1.11-fold higher compared with the control and at 60 min post-irradiation, the increases were 1.04-, 1.05- and 1.11-fold higher compared with the control. Likewise, the increase in ROS levels in exposed cells at 120 min post-irradiation were 1.03-, 1.06- and 1.11-fold higher compared with the control.

*Effect on mitochondrial activity in cancer cells*. Fig. 4 shows the mitochondrial activity in K562 and K562/Dox cancer cells, collected at 0, 30, 60, and 120 min following exposure to various low doses of gamma-rays. The results showed statistically significant dose-dependent decreases in the mitochondrial activity of K562 and K562/Dox cancer cells from 0 min up to 120 min post-irradiation.

In K562 cancer cells, at 0 min post-irradiation, the decreases were 0.98-, 0.89- and 0.83-fold lower compared with the control; at 30 min post-irradiation, the decreases were 0.96-, 0.87- and 0.81-fold lower compared with the control, and at 60 min post-irradiation, the decreases were 0.94-, 0.84- and 0.79-fold lower compared with the control. Likewise, the decreases in the mitochondrial activity of exposed cells at 120 min post-irradiation were 0.89-, 0.83- and 0.75-fold lower compared with the control.

In K562/Dox cancer cells, the fold decrease in the mitochondrial activity were dose-dependent at all four timepoints relative to the corresponding controls: 0.99, 0.92 and 0.83 at 0 min post-irradiation; 0.98, 0.88 and 0.82 at 30 min post-irradiation; 0.96, 0.85 and 0.79 at 60 min post-irradiation and 0.94, 0.83 and 0.76 at 120 min post-irradiation.

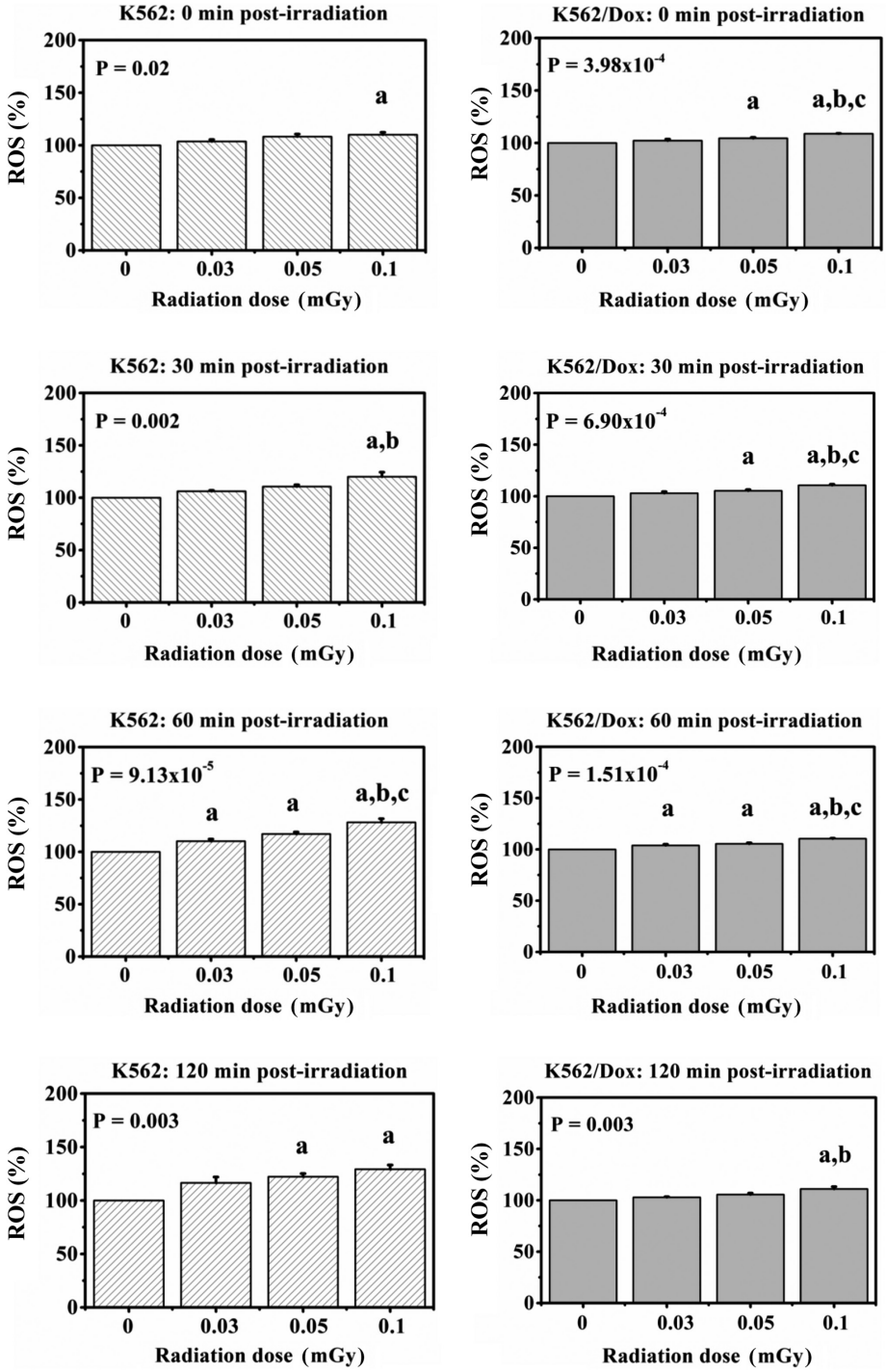


Figure 3. ROS percentage in K562 and K562/Dox cancer cells collected at 0, 30, 60 and 120 min after exposure to various low doses of gamma-rays. [a]P<0.05 vs. 0 mGy; [b]P<0.05 vs. 0.03 mGy; and [c]P<0.05 vs. 0.05 mGy. ROS, reactive oxygen species.

## Discussion

The dose ranges of the gamma-rays damage to normal RBCs *in vitro* were reported in IAEA-TECDOC-934 document. This document reported that examining the nature of the membrane injury in gamma irradiated RBCs in the dose range 2 to 200 Gy, It was concluded that the sulphydryl group was the major target in radiation-induced alteration of sodium and potassium ion permeability. In addition, an *in vitro* study on the effect of X-rays on movement of sodium in human RBCs, showed a loss of sodium/potassium ion balance in RBCs, following radiation doses in the range of 8.9 to 89 Gy. This phenomenon was due in part to discontinuation of membrane integrity (32). However, those radiation dose ranges are rather highly and most that dose find in radiation accident or radiotherapy. Whereas radiation dose in low-dose range that find in diagnostic radiology or nuclear medicine examination is still challenges. Our previous studies investigated biological responses to radiation after blood tissue was exposed to low dose X-rays in an *in vitro* system. The results showed that hemolysis and osmotic fragility in irradiated human RBCs did not significantly differ from non-irradiated RBCs. The results also showed that low-dose

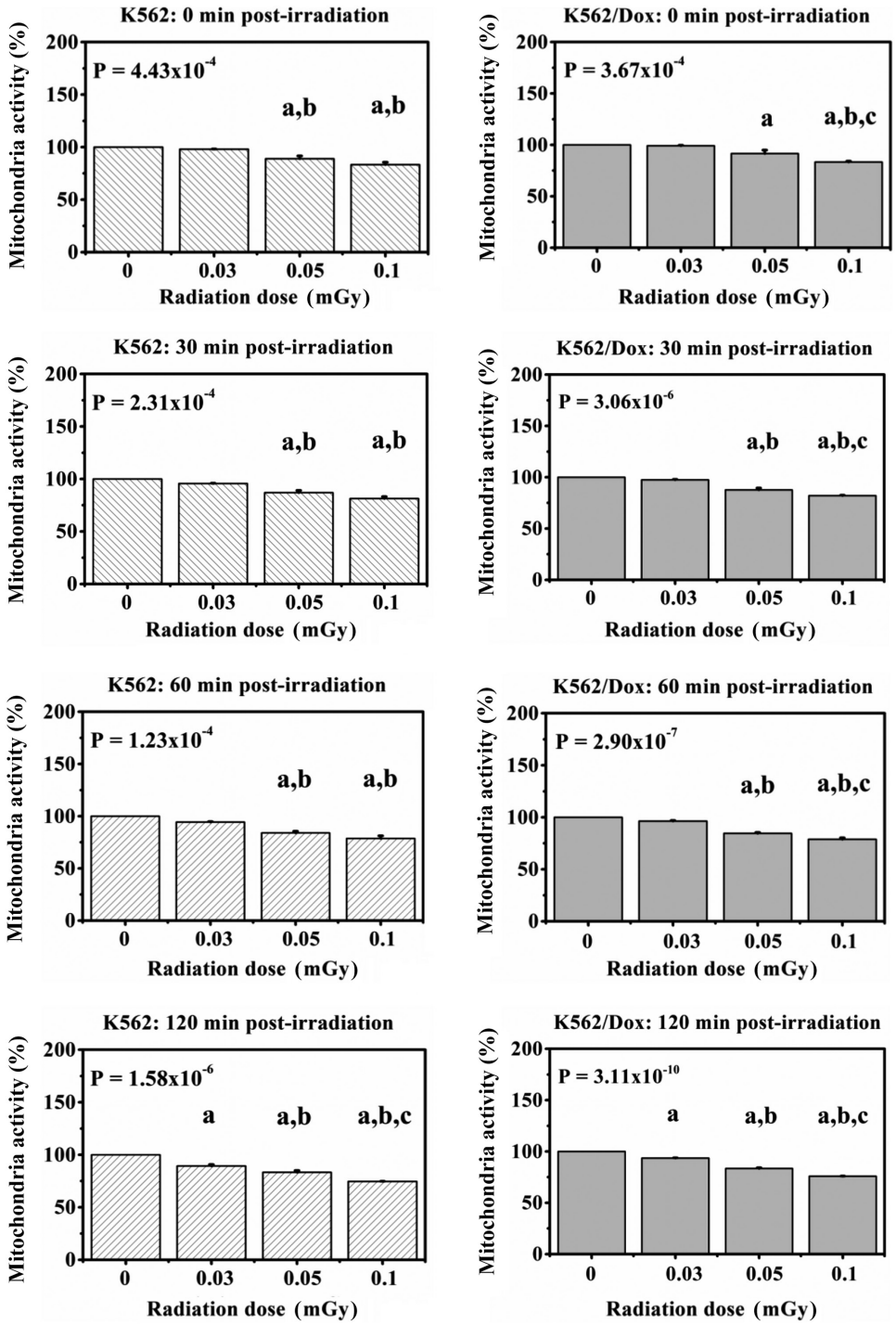


Figure 4. Mitochondrial activity in K562 and K562/Dox cancer cells collected at 0, 30, 60 and 120 min after exposure to various low doses of gamma-rays. [a]P<0.05 vs. 0 mGy; [b]P<0.05 vs. 0.03 mGy; and [c]P<0.05 vs. 0.05 mGy.

X-rays did not induce a change in mitochondrial membrane potential, number of apoptotic cells and perturbation of the cell cycle in irradiated human lymphocytes compared with non-irradiated lymphocytes. The authors suggested that there were no deleterious effects of low-dose X-rays when blood tissues were exposed in an *in vitro* system (30,31,33).

The present data demonstrated no changes in ROS levels and percentage of hemolysis of RBCs in irradiated whole blood when compared to the non-irradiated control groups. In addition, the CBC values in whole blood following *in vitro* exposure to low-dose gamma-ray groups have not differed compared with the non-irradiated control groups. The current findings suggested that low-dose gamma-ray do not induce any harmful effects to human blood cells. It should be noted that the current results are in agreement with our previous studies (30,31,33) and El-Shanshoury *et al* (34). These authors showed that statistically significant alteration in white blood cell, red blood cell and platelet count did not occur in rats after exposure to low-dose gamma radiation when compared with non-irradiated groups (34). Conversely, studies have demonstrated radiation-induced red blood cell damage such as increment of hemolysis and lipid peroxidation in RBCs. However,

those studies on irradiated RBCs involved high dose gamma radiation (27,35-37). It could be suggested that, depending on radiation dose, there are different responses in normal cells (red blood cells) between low- and high-dose radiation.

By contrast, normal cells (red blood cells) with low dose gamma irradiation caused significant increase in ROS levels in both irradiated K562 and K562/Dox cancer cells at all harvest time points, whereas the mitochondrial activity was decreased in both irradiated K562 and K562/Dox cancer cells at all harvest time points relative to non-irradiated cells. ROS and cell type [normal cells (RBCs) vs. cancer cells (K562 and K562/Dox)] were also compared. In the present study, K562 and K562/Dox exhibited sensitivity to low dose gamma radiation more than RBCs. Cancer cells show a wide range of sensitivity to radiation with different radiosensitivities. Low-dose hypersensitivity is found in various cancer cell lines upon receiving radiation (38-41). In addition, Dai *et al* investigated low dose hyper-radiosensitivity in the cancer cell line A549 irradiated with $^{60}$Co gamma-rays at doses of 0-2 Gy. The results showed that A549 cells exhibited low dose hyper-radiosensitivity. The type of death observed in cells was mainly apoptosis (18). Enns *et al* studied the response of three cancer cell lines, A549, T98G and MCF7, exposed to 0-200 cGy radiation doses from $^{137}$Cs source gamma-rays. The authors found that hypersensitivity occurred in the A549 and T98G cancer cells, but not in MCF7 cancer cells at radiation doses <50 cGy. The authors also suggested that hyper-radiosensitivity was involved in p53-dependent apoptosis (19). Short *et al* (20) investigated low dose hyper-radiosensitivity in the cancer cell lines T98G and U373 irradiated with X-rays. The results showed that hyper-radiosensitivity was observed in both T98G and U373 cancer cells. The authors also demonstrated that low-dose hyper-radiosensitivity depended on the cell cycle phase (20). Therefore, the present results agree with the hypothesis that cancer cell lines exhibit low-dose hypersensitivity to radiation.

ROS have been shown to play important roles in cell proliferation and cell death (42,43). Typically, ROS are produced in cells upon cells that are exposed to radiation in which ROS is mediated from the indirect effects of low linear energy transfer radiation as gamma-rays (44,45). A study has demonstrated that radiation potently induced cancer cell death via generation of ROS and oxidative response in cell organelles such as the mitochondria (46). In addition, Walsh *et al* performed mitochondrial staining with tetramethyl rhodamine ethyl ester in live MCF-7 and A549 cancer cells after exposure to 55 MeV carbon ions or 3 MeV proton radiation. The results showed that tetramethyl rhodamine ethyl ester levels were decreased in the mitochondria. The authors suggested that there was an induction of mitochondrial membrane depolarization after cancer cells received either protons or carbon ions (47). Leach *et al* had shown increased DCF fluorescence in A431 cancer cells. It was found that radiation stimulated ROS production in cells after exposure to 3 Gy of 90Sr radiation source. The authors also showed transient depolarizing effects of radiation on the mitochondrial membrane potential in A431 cancer cells (48). However, ROS is not only generated in cells by high dose radiation, but also by low-dose radiation, resulting in a number of deleterious effects on cells (21). Hence, the present study hypothesized that low-dose gamma-rays might induce increments of ROS in K562 and K562/Dox cancer cells, resulting in occurrence of oxidative stress that plays a role in decreasing mitochondria activity.

The current study showed the biological responses in K562 and K562/Dox cancer cells to low-dose gamma-rays but did not show that in RBCs. These findings suggested that erythroleukemia was more sensitive to low-dose gamma-rays compared with normal RBCs. In addition, the results of the current study suggested the possibility of using low-dose gamma radiation to treat erythroleukemia.

In conclusion, the current study showed the difference in biological responses in normal cells (RBCs) and cancer cells (K562 and K562/Dox) to low-dose gamma-rays when cells were exposed under *in vitro* conditions.

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**Availability of data and materials**

All data generated or analyzed during this study are included in this published article.

**Authors' contributions**

BS, PH, NK and NS performed the experiments. ST, SK and CU interpreted the data and revised the manuscript. MT conceived and designed the current study, performed the experiments, analyzed and interpreted the data, and drafted and revised the manuscript. All authors have read and approved the final manuscript.

**Ethics approval and consent to participate**

Blood sample collections were performed under the approved guidelines by the Institutional Committees on Research Involving Human Subjects and approval of the Faculty of Associated Medical Sciences, Chiang Mai University (approval no. AMSEC-62EM-002).

**Patient consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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