Original article:

Effects of Medical Diagnostic X-rays Delivered at 0.01 or 0.05 mGy on Human Blood Cells

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Abstract:

Background: Low-dose X-rays are commonly used in medical imaging to help in the diagnosis of diseases. However, the deleterious effects of exposure to medical diagnostic low-dose X-rays remain a highly debated topic. The objective was to study the effects of medical diagnostic X-rays on human blood cells. ***Materials and Methods*:** We studied the effects of medical diagnostic low-dose X-rays (80 kVp), i.e.,0.01 or 0.05 mGy, after the *in vitro* exposure of human red blood cells (RBCs) and peripheral blood mononucleated cells (PBMCs).Cells with no irradiation served as the control group. The biological endpoints that were used to determine the effects of medical diagnostic low-dose X-rays were hemolysis for RBCs and mitochondrial membrane potential, lysosomes, and the cell cycle for PBMCs. ***Results*:** Our results showed no changes in the hemolysis of RBCs and mitochondrial membrane potential, lysosome, or cell cycle in cells exposed to these low doses of X-rays when compared to the corresponding nonirradiated cells at all harvest timepoints. ***Conclusion*:** These results suggested that there were no deleterious effects of diagnostic low-dose X-rays when human RBCs and PBMCs were exposed *in vitro*.

Keywords: medical diagnostic low-dose radiation, X-rays, hemolysis, mitochondrial membrane potential, lysosome, cell cycle

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Introduction

Exposure to high doses of radiation is known to have various harmful effects on cells or tissue [1-3]. There has been much discussion for many years on the relationship between these harmful effects and absorbed radiation at low doses. A linear extrapolation from a zero dose to high doses of radiation is usually applied for the estimation of health risks of exposure to low doses of radiation[4-6]. Currently, fears over low doses of radiation remain a concern despite evidence to the contrary. There are several previous works have shown no harmful effects after *in vitro* or *in vivo* receiving to low-dose radiation[7-18]. Hence, a better knowledge and understanding of the biological effects of low-dose radiation, in terms of the potentially harmful or beneficial effects, is needed. There is an increased use of low-dose radiation in the

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medical diagnosis of many diseases using imaging technology, such as computed tomography and mammography. The benefits of low-dose radiation in medical diagnosis outweigh the risks, but the health risks of exposure to diagnostic low-dose radiation are still an important consideration. There is evidence that suggests that chromosome aberrations can be induced by diagnostic low-dose radiation from a computed tomography scan[19-21] and mammography[22, 23].

Low-dose radiation from computed tomography and mammography is associated with absorbed doses higher than 1.0 mGy. However, evidence on the biological effects from exposure to diagnostic low-dose radiation for general radiography that is associated with absorbed dosages of less than 0.1 mGy[24, 25], such as skeletal extremity radiography, is lacking. As a beginning step to fill this information gap, we determined the effects of medical diagnostic low-dose radiation (0.01 or 0.05 mGy) after an *in vitro* exposure of human blood cells: red blood cells (RBCs) and peripheral blood mononucleated cells (PBMCs). RBCs and PBMCs were used as models in this study since both cell types received radiation in every medical radiography procedure and are circulated throughout the human body.

We focused on the biological endpoints associated with oxidative stress that is induced by radiation.It is well recognized that radiation can create reactive oxygen species (ROS), resulting in biomolecules and cell organelles demonstrating oxidative damage[26-28]. Thus, the biological endpoint of RBCs is hemolysis, which represents the integrity of the cell membrane. Other biological endpoints in PBMCs are mitochondrial membrane potential, which represents mitochondrial function, lysosome function, and cell cycle regulation.

Materials and Methods

Experimental design

The experimental design is shown in Figure 1. The hemolysis was determined at 0.5 and 4 hours post-irradiation. The cell cycle, mitochondrial membrane potential and lysosome function were determined at 0.5, 4, 8, 12, 24, 48, and 72 hours post-irradiation. Each assay for each dose of radiation was done in duplicate for each harvest time.

Cell preparation

Peripheral blood samples(approximately 10 mL)were collected by venipuncture into heparinized syringes from a non smoking healthy male volunteer (23 years old) who had no history of previous exposure to any clastogens. Blood sample collection was performed under the approved guidelines by the Institutional Committees on Research Involving Human Subjects, and approval was obtained from the Faculty of Associated Medical Sciences, Chiang Mai University. Informed consent was documented. Red blood cells (RBCs) and peripheral blood mononucleated cells (PBMCs) were isolated using Ficoll-Hypaque solution (Lymphoprep™, Oslo, Norway).Freshly isolated PBMCs were cultured at a concentration of 10^6 cells·mL^{-1} in a tissue-culture flask containing 10 mL of RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum, 1% glutamine, and 1% penicillin/streptomycin. Cells were incubated at 37 °C in a humidified 5% CO_2 atmosphere. All treatment protocols were performed in replicate.

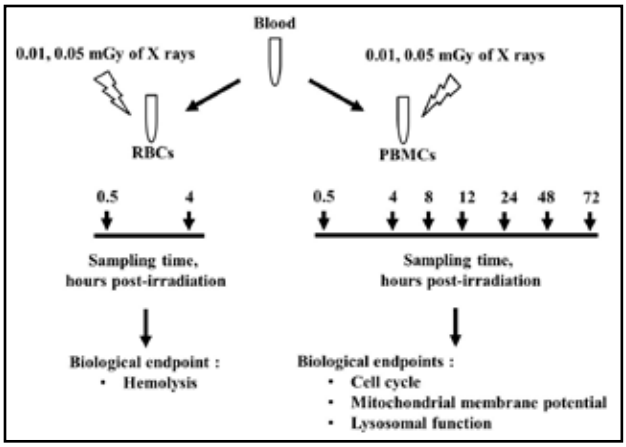

Figure 1. Diagram of experimental design.

Irradiation

For irradiation, the tissue culture flasks were placed in the center of an X-ray beam 100 cm away from the X-ray tube. A medical diagnostic X-ray machine (Quantum Medical imaging, Quest HF series, Carestream, NY, USA) operated at 80 kVp, 100 mA, and 0.01 second for 0.01 mGy, and 80 kVp, 200 mA, and 0.01 second for 0.05 mGy was used. The half value layer(HVL)was 3.1 mmAl.The target/filter was tungsten/aluminum.

Hemolysis

The hemoglobin released from the cells was used as an indicator of red blood cell hemolysis. The hemolysis method was performed following a previously published work[29, 30].Briefly, 25 µL of irradiated red blood cells at 0.5 and 4 hours post-irradiation were incubated in 725 µL phosphate buffered saline (PBS) and 725 µL distilled H_2O for 30 minutes at 37°C. Next, samples were centrifuged at 7,000 rpm for 1 minute.

The release of hemoglobin into the supernatant was determined by a spectrophotometer. The absorbance (Abs) at 415 nm was used to calculate the percentage of hemolysis as follows:

Percentage of hemolysis = (Abs $_{(415\ nm)}$ in PBS / Abs $_{(415\ nm)}$ in H_2O) × 100

where Abs $_{(415\ nm)}$ in PBS and Abs $_{(415\ nm)}$ in H_2O were the absorbance of the released hemoglobin into PBS and H_2O, respectively.

Cell cycle

Cell cycle analysis and DNA content measurement were performed using a flow cytometer (Beckman Coulter, CA, USA). The staining method was modified from a previously published work[31]. Briefly, a total of 10^6 cells·mL^{-1} were fixed with ice-cold ethanol at 4°C overnight, followed by washing with PBS buffer, incubation with 0.1% Triton X-100, and 0.2 mg·mL^{-1} RNase A for 1 minute at room temperature. Finally, the cells were stained with the DNA dye propidium iodide at 37°C for 30 minutes in the dark. The cell cycle distribution was determined by flow cytometry. The assay was performed in duplicate for each X-ray dose.

Mitochondrial membrane potential

The alteration of mitochondrial membrane potential was characterized using a flow cytometer. The staining method was modified from previously published work[32]. Briefly, 10^6 cells were suspended in 2 mL PBS buffer, pH 7.25 at 37°C in the presence of 1mM rhodamine B for 20 minutes in the dark. The accumulation of rhodamine B in mitochondria was determined by using a flow cytometer. The assay was performed in duplicate for each X-ray dose.

Lysosome

Lysosomal function was studied by altering the accumulation of acridine orange (AO) in lysosomes. The staining method was modified from a previously published work[33, 34]. Briefly, 10^6 cells were suspended in 2 mL PBS buffer, pH 7.25 at 37°C in the presence of 1μM AO for 5 minutes in the dark. The accumulation of AO in lysosomes was determined by using a flow cytometer. The assay was performed in duplicate for each X-ray dose.

Statistical analyses

The authors expressed the data as the mean ± standard error of the mean (S.E.). Student's t-test was used independently to evaluate significant differences in the mean values between each exposed group and the corresponding sham-control group. A p-value of less than 0.05 was considered statistically significant.

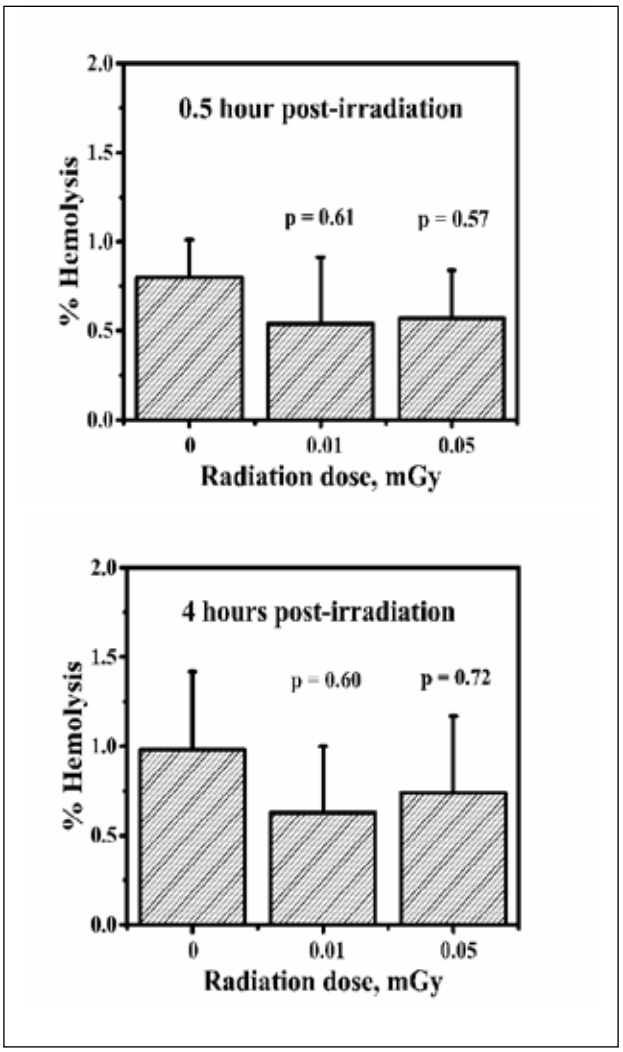

Figure 2. The percentage hemolysis of RBCs collected at 0.5 and 4 hours after *in vitro* exposure to 0.01 or 0.05 mGy of X-rays. The p-values (Student's t-test) indicate that there are no significant differences in the %hemolysis between the irradiated and corresponding nonirradiated control groups.

Results

Hemolysis

Figure 2 shows the percentage of hemolysis values in RBCs collected at 0.5 and 4 hours after an *in vitro* exposure to a 0.01 or 0.05 mGy dose of X-rays and the corresponding nonirradiated control groups. These data show no change in the percentage of hemolysis values in irradiated RBCs when compared to the corresponding nonirradiated RBCs at all harvest times.

Mitochondrial membrane potential

Figure 3 shows the mean rhodamine B fluorescence values in PBMCs collected at 0.5 and 4 hours after *in vitro* exposure to a 0.01 or 0.05 mGy dose of X-rays and the corresponding nonirradiated control groups. These data show no change in the mean rhodamine B fluorescence values in irradiated PBMCs when compared to the corresponding non irradiated PBMCs at all harvest times. This finding suggests that these radiation doses had no effect on the mitochondrial membrane potential of PBMCs.

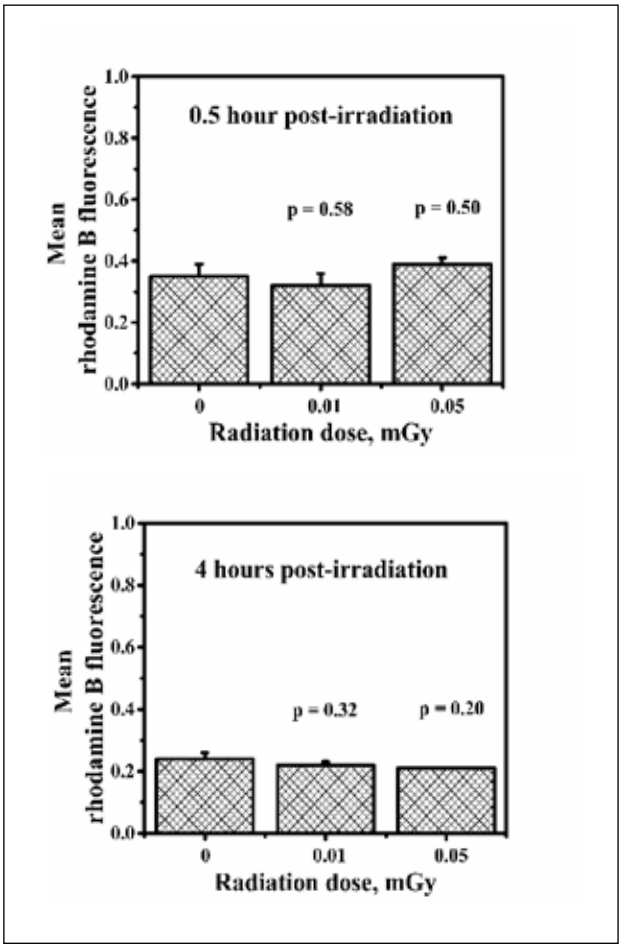

Figure 3. The mean rhodamine B fluorescence of PBMCs collected at 0.5 and 4 hours after *in vitro* exposure to 0.01 or 0.05 mGy of X-rays. The p-values (Student's t-test) indicate that there are no significant differences in the mean fluorescence between the irradiated and corresponding nonirradiated control groups.

Lysosomes

Figure 4 shows the mean AO fluorescence values in PBMCs collected at 0.5, 4, 6, and 8 hours after *in vitro* exposure to a 0.01 or 0.05 mGy dose of X-rays and the corresponding nonirradiated control groups. Similar to the mitochondrial membrane potential values, these data show no change in the mean AO fluorescence values in irradiated PBMCs when compared to the corresponding nonirradiated PBMCs at all harvest times. This finding suggests that these radiation doses had no effect on the lysosome function of PBMCs.

Cell cycle

Figure 5 shows the numbers of cells in different stages of the cell cycle in PBMCs collected at 4, 8, 12, 24, 48,and 72 hours after *in vitro* exposure to a 0.01 or 0.05 mGy dose of X-rays. Similar to the mitochondrial membrane potential values and lysosomes, these data indicate that there was no perturbation in the cell cycle in irradiated PBMCs when compared to the corresponding nonirradiated PBMCs at all harvest times.This finding suggests that these radiation dosages did not affect the cell cycle of PBMCs.

Discussion

Although it is believed that a high dose of ionizing radiation contributes to cell damage,less is known about the cellular response to low doses of ionizing radiation, such as medical diagnostic X-rays.The present study showed the effects of 80 kVp medical diagnostic X-rays on human blood cells (RBCs and PBMCs).

The present study also showed the effects of medical diagnostic X-rays delivered at 0.01 or 0.05 mGy on the percentage of hemolysis in normal human RBCs. These current results showed the percentage of hemolysis values in irradiated RBCs were not statistically significant changes when compared to the corresponding nonirradiated RBCs at all collecting time after exposure to low-dose X-ray.

There is other research that also studied the effects of low-dose ionizing radiation on RBC damage.Krylov et al. 2015 showed that β-ionizing radiation (^{90}Sr–^{90}Y source) in the dose range from 0.08 to 0.16 mGy contributed to the deceleration of hemolysis and the stabilization of rat red blood cells[35]. Kaczmarska et al. 2011 studied the effects of γ-ionizing radiation (^{137}Cs source) in the dose range from 0.04 to 0.18 mGy on the percentage of hemolysis in RBCs isolated from healthy people and people with diabetes. The authors showed that exposure of healthy human RBCs to γ-ionizing radiation leads to nonlinear changes in the percentage of hemolysis of RBCs as well as in people with diabetes. However, the changes in the percentage

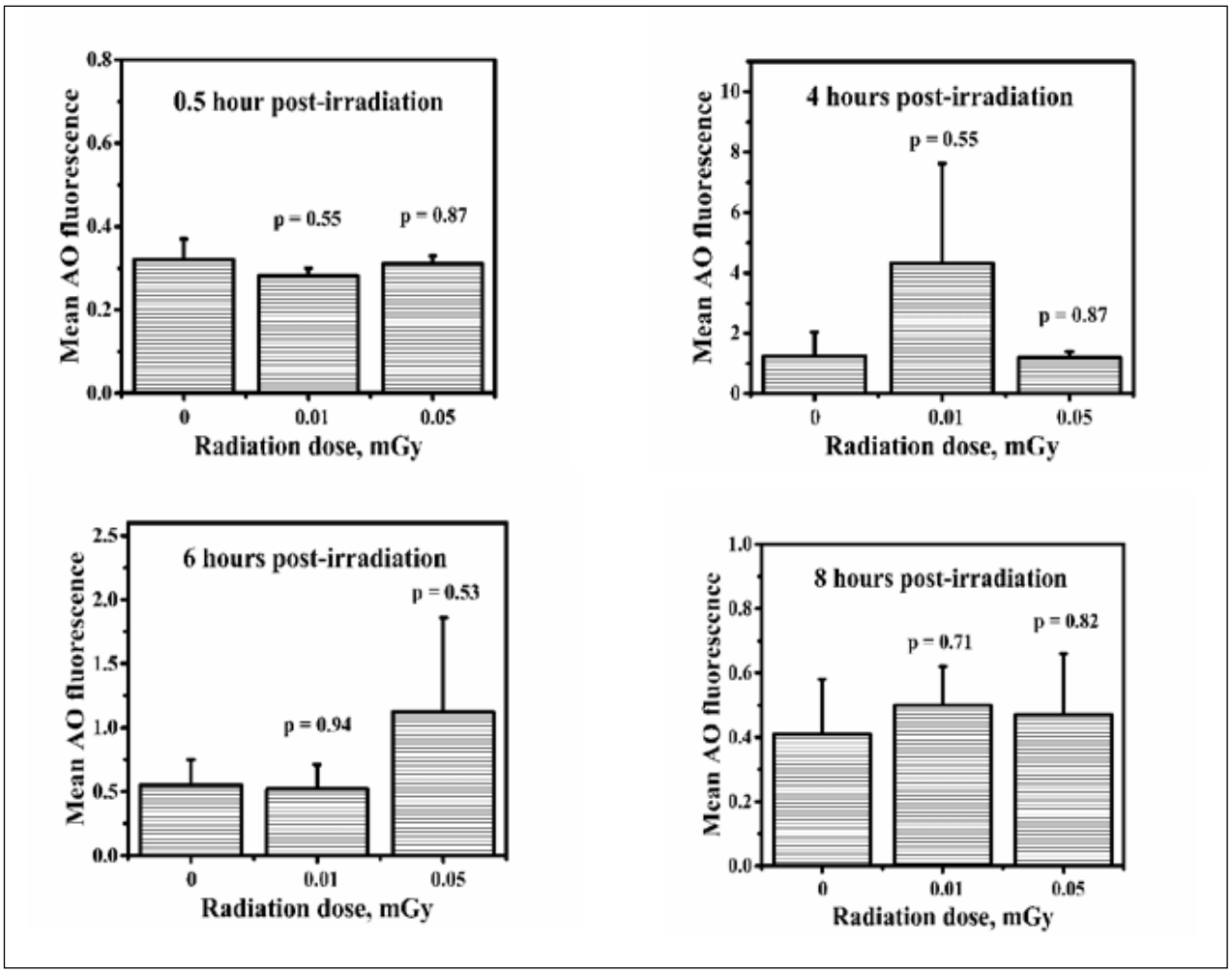

Figure 4. The mean acridine orange (AO) fluorescence of PBMCs collected at 0.5, 4, 6, and 8 hours after *in vitro* exposure to 0.01 or 0.05 mGy of X-rays. The p-values (Student's t-test) indicate that there are no significant differences in the mean fluorescence between the irradiated and corresponding nonirradiated control groups.

of hemolysis could be distinguished in five and three characteristic periods for RBCs isolated from healthy people and people with diabetes, respectively[36].It is obvious that there are several factors contributing to the RBC response to ionizing radiation:the type of radiation (β-, γ-, X-rays), the radiation dose, and the characteristics of the cells (animal or human cells, normal or abnormal cells). Therefore, our *in vitro* data using hemolysis as the biological endpoint suggested that the medical diagnostic low-dose X-rays (energy of 80 kVp) did not induce harmful effects on human RBCs. In addition, medical diagnostic X-rays with an energy of 50, 70, or 100 kVp did not influence the hemolysis, osmotic fragility, and fluorescence anisotropy values of irradiated human red blood cells[29].In contrast, Taqi et al. 2018 studied the hematological parameters of diagnostic technicians exposed to medical X-rays. These authors suggested that chronic exposure to X-rays can significantly alter some hematological parameters such as neutrophils, monocytes, basophils, mean cell volume, red cell distribution width, platelets, lymphocytes, red blood cells, hemoglobin, and hematocrit[37]. However, the present study used a single exposure of X-rays,while the studies that were conducted by Taqi et al. 2018 were associated with chronic exposure.

The present study also showed the effects of medical diagnostic X-rays delivered at 0.01 or 0.05 mGy in terms of how these levels change mitochondrial membrane potential, lysosome function, and the cell cycle phases in PBMCs.We were interested in three biological endpoints; mitochondrial membrane potential, lysosome function, and cell cycle, since mitochondria play an important role in the cellular response to radiation.Mitochondrial damage is

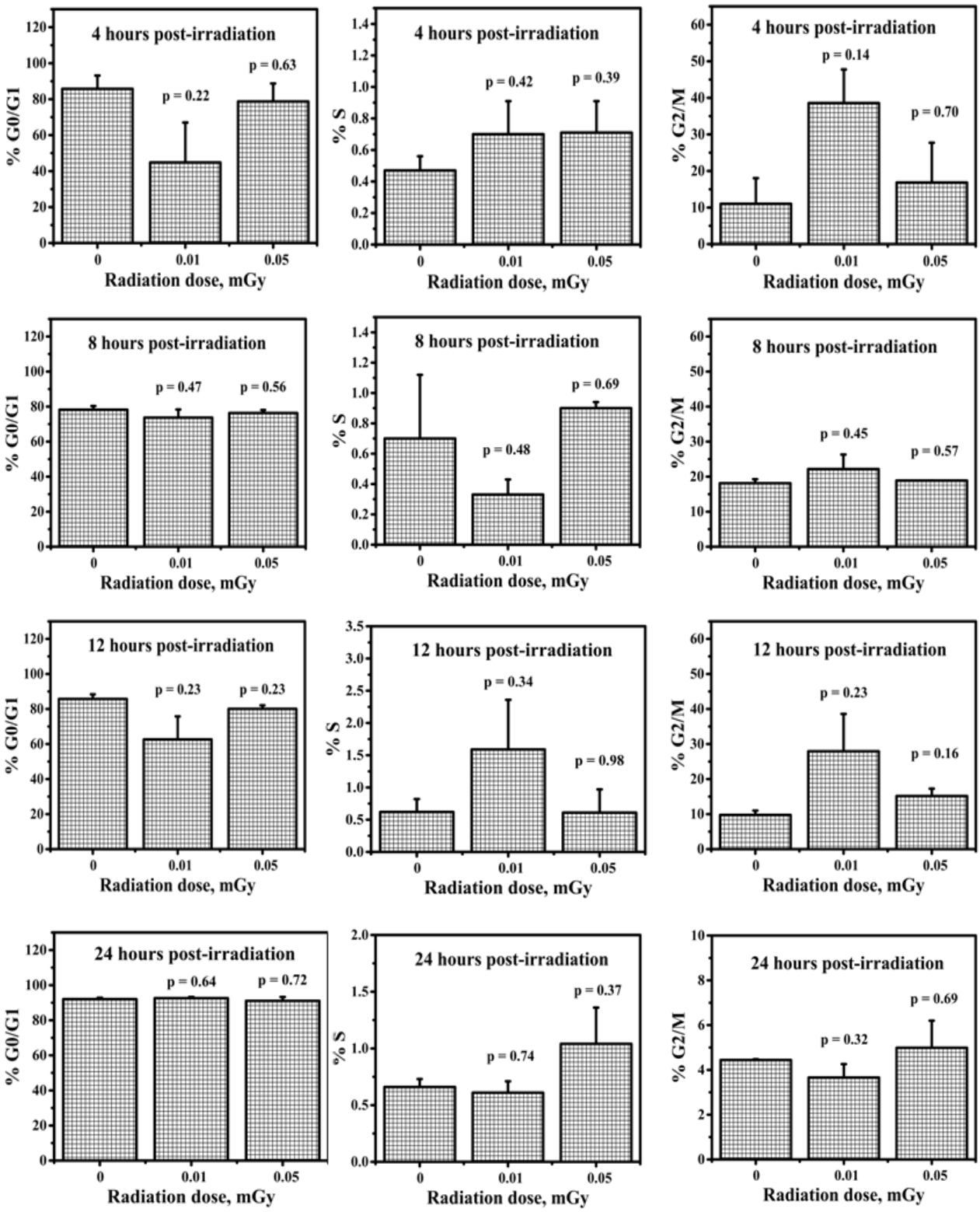

Figure 5.Alterations of the cell cycle in PBMCs collected at 4, 8,12, 24, 48, and 72 hours after *in vitro* exposure to 0.01 or 0.05 mGy of X-rays. The p-values (Student's t-test) indicate that there were no significant differences in the alteration of cell cycles between the irradiated and corresponding nonirradiated control groups.

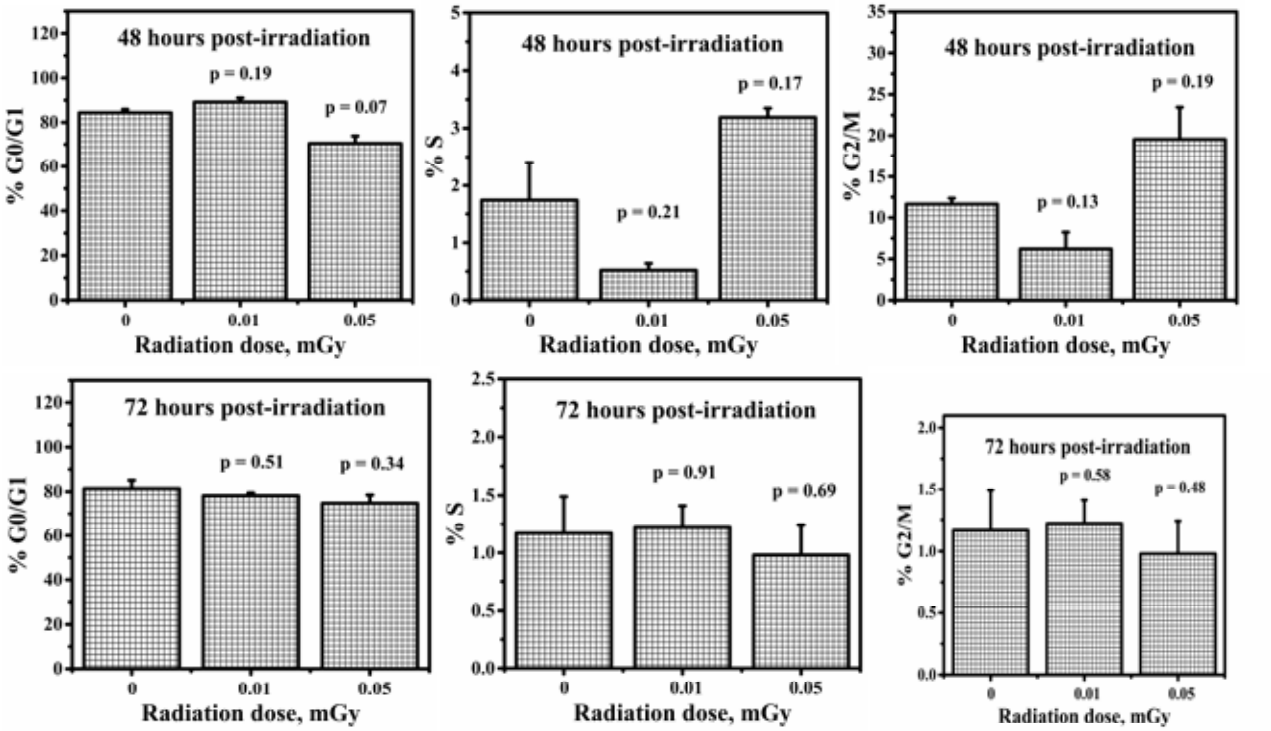

Figure 5. (continue)Alterations of the cell cycle in PBMCs collected at 4, 8,12, 24, 48, and 72 hours after *in vitro* exposure to 0.01 or 0.05 mGy of X-rays. The p-values (Student's t-test) indicate that there were no significant differences in the alteration of cell cycles between the irradiated and corresponding nonirradiated control groups.

associated with lysosomal rupture[38, 39] and the cell cycle[40]. High doses of acute single exposure to X-rays (> 5 Gy) induced mitochondrial damage that led to G2-phase cell cycle arrest and cellular senescence in irradiated human neural progenitor stem cells and human fibroblasts[41]. Chronic low-dose α-radiation from ^{226}Ra induced the depolarization of the mitochondrial membrane, resulting in an alteration of cell survival in human cell lines[42, 43].Our results showed that no statistically significant changes in mitochondrial membrane potential, lysosome function, and the cell cycle phases in PBMCs occurred after *in vitro* exposure to medical diagnostic low-dose X-rays compared to the corresponding nonirradiated cells at all harvest timepoints. Thus, our *in vitro* data using these three biological endpoints suggests that the medical diagnostic low-dose X-rays do not induce any detrimental effects on human PBMCs.This is consistent with our previous studies that showed no changes the mitochondrial membrane potential, number of apoptotic cells, or cell cycle in lymphocytes exposed to radiation at 0.03, 0.05, or 0.1 mGy of (70 kV) medical diagnostic X-rays when compared to the corresponding nonirradiated cells at all collecting timepoints[31]. However, low-dose medical diagnostic X-rays might affect to the function of lysosome of cancer cells [34].

The strengths of this study were that these data are highly relevant to humans because primary human blood cells were used rather than established human cell lines. In addition, there were no differences that could be due to interindividual variability. However, we suggest that further studies consider the number of exposures to diagnostic low-dose radiation because patients may receive diagnostic low-dose radiation exposure more than once. In addition, the age of the subject needs to be taken into account when obtaining blood samples because older subjects are less sensitive to radiation than younger subjects.

Conclusion

These findings suggested that there were no

deleterious effects of the diagnostic low-dose X-rays when human RBCs and PBMCs were exposed *in vitro*. Nonetheless, these data will help improve the understanding of diagnostic low-dose X-rays in terms of fundamental radiation biology. Moreover, these data may also help to decrease the fears of diagnostic low-dose radiation.

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Author's contribution

Data gathering and idea owner of this study: MontreeTungjai

Study design: Montree Tungjai

Data gathering: Benjamaporn Supawat, Jongchai Tinlapat, Rusleena Wongmahamad, Chuleekorn Silpmuang, Montree Tungjai

Writing and submitting manuscript: Montree Tungjai

Editing and approval of final draft: Benjamaporn Supawat, Suchart Kothan, Montree Tungjai

Ethical clearance

Blood sample collections were performed under the approved guidelines set by The Institutional Committees on Research Involving Human Subjects, and approval for testing was obtained by The Faculty of Associated Medical Sciences, Chiang Mai University, Thailand.

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Department of Radiologic Technology, Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai, Thailand.

Conflicts of interest: None

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