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Determination of hemolysis, osmotic fragility and fluorescence anisotropy on irradiated red blood cells as a function of kV of medical diagnostic X-rays

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► ***Original article***

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ABSTRACT

Background*:** People occasionally undergo medical diagnostic X-ray examinations and expose their red blood cells to radiation. Radiation that is generated from medical diagnostic X-ray machines is widely used in medical diagnoses. One of the important parameters is kilo-voltage (kV) that is applied across the X-ray tube in medical diagnostic X-ray machines. Kilo-voltage influences the radiation dosage. The aim of this study is to determine the hemolysis, osmotic fragility, and fluorescence anisotropy value on irradiated red blood cells as a function of kV during medical diagnostic X-ray examinations. ***Materials and Methods*:** The kV, kilo-voltage that is applied across an X-ray tube, of a medical diagnostic X-ray machine was operated at 50, 70 and 100 kV. We determined the hemolysis, osmotic fragility, and fluorescence anisotropy value in red blood cells at 0.5 and 4 hours post-irradiation. In order to determine hemolysis and osmotic fragility, the release of hemoglobin was measured by spectrophotometry technique. 1,6-diphenyl-1,3,5-hexatriene (DPH) was used as a molecular probe for determining fluorescence anisotropy value by fluorescence anisotropy technique. Non-irradiated red blood cells served as the control. ***Results: For the 50, 70, and 100 kV of medical diagnostic X-rays, the hemolysis, osmotic fragility, and fluorescence anisotropy values of irradiated red blood cells at 0.5 and 4 hours post-irradiation did not significantly change when compared to the control. ***Conclusion:*** Our results suggested that 50, 70, and 100 kV of medical diagnostic X-ray did not influence hemolysis, osmotic fragility, and fluorescence anisotropy values of irradiated red blood cells.

Keywords: *Medical diagnostic X-ray, red blood cell, hemolysis, osmotic fragility, fluorescence anisotropy.*

INTRODUCTION

X-rays are widely used in medical diagnosis for diseases. After receiving an X-ray examination, blood cells are exposed to radiation. The most common type of blood cells are red blood cells, (RBCs) which delivers oxygen (O_2) to the body tissues by blood flow through the circulatory system. Red blood cells are biconcave anucleated cells containing hemoglobin molecules [1, 2]. Because RBCs are oxygen deliverers and have a high concentration of polyunsaturated fatty acids in cell membrane, RBCs are highly susceptible to oxidative stress that is implicated in the pathogenesis of diseases which can be induced by radiation [3, 4]. Consequently, the changing of RBCs properties is an indicator for predicting a disease or morbidity [3, 5]. The effects of radiation on RBCs have been reported in various studies. Most of these reports mainly studied by using gamma rays [6-12]. This may be due to the widespread use of gamma rays for preventing transfusion-associated-graft versus host diseases (TA-GvHD) in RBCs transfusion units. In addition, the effects of high dose X-ray on red

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blood cells have been documented on IAEA-TECDOC-934. However, those high doses X -ray are not used in medical diagnosis imaging [13]. X-rays that are used in medical diagnosis, particularly for tissue or organ imaging in diagnostic radiology in hospitals, are generated from medical X-ray machines. There are several parameters involved in operating a medical X-ray machine for generating X-rays. One of the important parameters is kilo-voltage (kV), the voltage that is applied across an X-ray tube, which influences the energy of the X-ray and radiation dosage [14]. To improve the quality of X -ray imaging, kV has to be increased which results in higher dosages of radiation exposure in patients. As mentioned earlier, RBCs are common cells that are exposed to X-rays during all X-ray examinations, thus the increments of kV may affect RBCs. This study was carried out in order to understand the influence of kV in medical X-ray machines on RBCs, as well as on hemolysis, which reflects a change in the RBCs membrane integrity; osmotic fragility, which reflects the capacity of RBCs to resist hemolysis; and on fluorescence anisotropy, which reflects the fluidity of RBCs membranes.

MATERIALS AND METHODS

Irradiation

Blood sample collected from two healthy male, age 20-30 years old who had no history of previous exposure to any clastogens. Blood sample collections were performed under the approved guidelines by the Institutional Committees on Research Involving Human Subjects approval of the Faculty of Associated Medical Sciences, Chiang Mai University.

The red blood cells were separated from anticoagulated human whole blood using a ficoll hypaque solution (Lymphoprep™, Norway). The red blood cells were exposed to medical diagnostic X-rays generated by a medical diagnostic X-ray machine (Quantum Medical Imaging, Caresteam, Quest HF series) located in the Department of Radiologic Technology, Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai, Thailand. The medical diagnostic X-ray machine operated at 50, 70, and 100 kV (energy spectra showed in figure 1) with the current tube x times (milli-ampere x second, mAs) equaling 100 mAs. The red blood cells were placed 100 cm from the medical diagnostic X-ray tube. The field of view was 10 cm × 10 cm. The non-irradiated red blood cells served as the control.

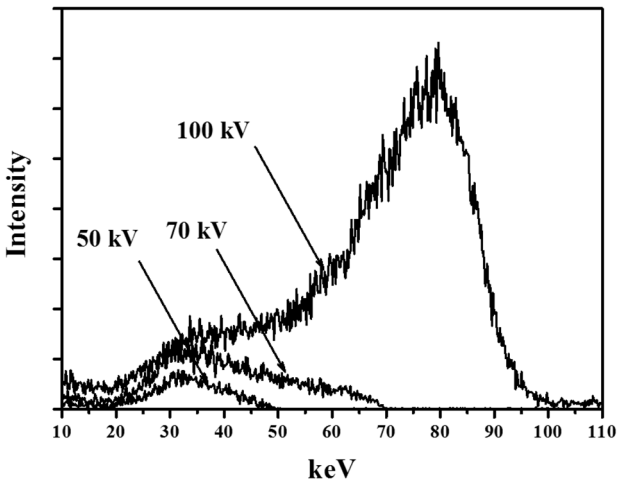

Figure 1. Energy spectra of X-ray from medical diagnostic X-ray machine that operated at 50, 70, and 100 kV.

Hemolysis

The hemoglobin released from the cells was used as an indicator of red blood cells hemolysis. 25 µL of irradiated red blood cells at 0.5 and 4 hours post-irradiation were incubated in 725 µL phosphate buffer saline (PBS), and in 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 spectrophotometer. The absorbance (Abs) at 415 nm was used to calculate the percentage of hemolysis as equation (1).

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 release of hemoglobin into PBS and H_2O, respectively.

Osmotic fragility (OF)

The osmotic fragility test was used to

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determine the degree of hemolysis. 25 µL of irradiated red blood cells at 0.5 and 4 hours post -irradiation were incubated in 1,000 µL of 0.9%, 0.7%, 0.5%, 0.3%, 0.1%, and 0.05% sodium chloride solutions for 3 minutes at 37°C. Afterward, samples were centrifuged at 7,000 rpm, for 1 minute. The release of hemoglobin into the supernatant was determined by spectrophotometer. The OF_{50} (the concentration of sodium chloride can induce hemolysis of red blood cells by 50%) was determined by plotting the relationship between absorbance at 415 nm versus the concentration of sodium chloride solution.

Fluorescence anisotropy

The fluidity of red blood cell membranes was determined by the fluorescence anisotropy technique. The DPH was used as a molecular probe. 5 µL of irradiated red blood cells at 0.5 and 4 hours post-irradiation were incubated in 2 mL PBS, pH 7.4, 37°C, containing 1 µM DPH for 30 minutes. The sample was excited with vertically polarized light (377 nm) and the vertical and horizontal emissions (460 nm) were measured. Fluorescence anisotropy value was defined by given equation (2).

$$\text{Fluorescence anisotropy} = (I_{VV} - GI_{VH}) / (Ivv + 2GI_{VH})$$
$$G = I_{HV} / I_{HH}$$

Where; I_{VV}: The intensity of sample that was excited with vertically polarized light and the vertical emission. I_{VH}: The intensity of sample that was excited with vertically polarized light and the horizontal emission. I_{HV}: The intensity of the sample that was excited with horizontally polarized light and the vertical emission. I_{HH}: The intensity of sample that was excited with horizontally polarized light and the horizontal emission.

Statistical analysis

The statistical analysis were performed on the Microsoft Excel. At each harvest time (0.5 and 4 hours post-irradiation), all assays (hemolysis, osmotic fragility, and fluorescence anisotropy) were performed in duplicate for each kV. Next, the average value for each subject was obtained. Subsequently, the average value and standard deviation (SD) for each kV were calculated from the means of the two subjects.

We used Student's-t tests to compare results between controls and irradiated red blood cells. A p value of less than 0.05 was considered as statistically significant.

RESULTS

Hemolysis

At 0.5 hour post-irradiation, the percentage of hemolysis in the control was 1.99 ± 0.10. The percentage of hemolysis in the irradiated red blood cells were 2.03 ± 0.37, 2.06 ± 0.49 and 2.34 ± 0.54 for 50, 70, and 100 kV, respectively. These results did not show a significant difference when compared to the control (figure 2).

At 4 hours post-irradiation, the percentage of hemolysis in the control was 2.77 ± 0.14. The percentage of hemolysis in the irradiated red blood cells were 2.96 ± 0.29, 2.77 ± 0.33 and 3.66 ± 0.43 for 50, 70, and 100 kV, respectively. These results also did not show a significant difference when compared to the control (figure 2).

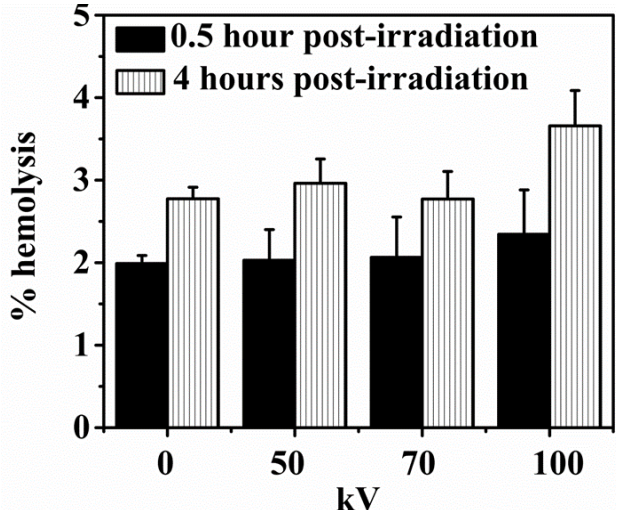

Figure 2. Effect of kV on % hemolysis of irradiated red blood cells at 0.5 hour post-irradiation and 4 hours post-irradiation.

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Osmotic fragility (OF)

At 0.5 hour post-irradiation, the OF_{50} of the control was 0.73 ± 0.04. The OF_{50} of the irradiated red blood cells were 0.75 ± 0.03, 0.75 ± 0.04 and 0.76 ± 0.07 for 50, 70, and 100 kV, respectively, which did not show a significant difference as compared to the control (figure 3).

At 4 hours post-irradiation, the OF_{50} of the control was 0.69 ± 0.03. The OF_{50} of the irradiated red blood cells were 0.76 ± 0.02, 0.78 ± 0.02 and 0.80 ± 0.02 for 50, 70, and 100 kV, respectively, These results also did not show a significant difference compared to the control, except in the red blood cells that were exposed to 100 kV of medical diagnostic X-rays (figure 3).

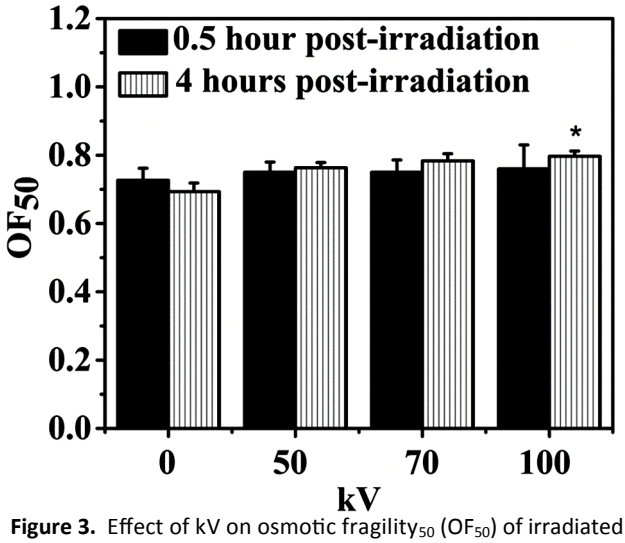

Figure 3. Effect of kV on osmotic fragility$_{50}$ (OF_{50}) of irradiated red blood cells at 0.5 hour post-irradiation and 4 hours post-irradiation * : $p < 0.05$ versus control.

Fluorescence anisotropy

At 0.5 hour post-irradiation, the fluorescence anisotropy value of the control was 0.26 ± 0.03. The fluorescence anisotropy values of the irradiated red blood cells were 0.36 ± 0.18, 0.21 ± 0.02 and 0.22 ± 0.01 for 50, 70, and 100 kV, respectively; which did not show a significant difference compared to the control (figure 4).

At 4 hours post-irradiation, the fluorescence anisotropy value of the control was 0.22 ± 0.01. The fluorescence anisotropy values of the irradiated red blood cells were 0.15 ± 0.09, 0.22 ± 0.03 and 0.22 ± 0.02 for 50, 70, and 100 kV, respectively; which also did not show a significant difference as compared to the control (figure 4).

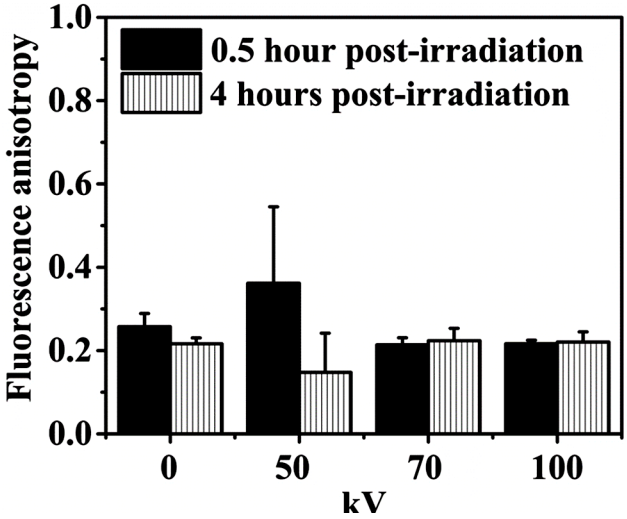

Figure 4. Effect of kV on fluorescence anisotropy of irradiated red blood cells at 0.5 hour post-irradiation and 4 hours post-irradiation.

DISCUSSION

The effect of gamma and X-rays on blood and blood components for sterilization, for inactivation of a particular blood component such as, for example, lymphocytes in preventing graft versus host disease have been documented on IAEA-TECDOC-934 [13]. In diagnostic radiology, X-ray images are created by X-rays that are generated from X-ray machines in hospitals. When generating X-rays, the kV of the X-ray machine is an important parameter since kV influences the energy of the X-rays (figure 1). Moreover, kV is associated with radiation dosage [14]. It is known that radiation can induce the formation of free radicals which causes a variety of cell membrane changes. Membranes of RBCs have an abundance of polyunsaturated lipids and hemoglobin that react to free radicals initiating lipid peroxidation [15], resulting in induction of oxidative stress conditions. It is widely known that oxidative stress condition causes disease. Hence, the possible effects of red blood cells concerning exposure to kV of X-rays is being evaluated. Our results suggests that red blood cells exposed to 50, 70, and 100 kV of X-rays at 0.5 and 4 hours post-irradiation did not change

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the percentages of hemolysis and OF_{50}, except in the OF_{50} of red blood cells exposed to 100 kV of X-rays at 4 hours post-irradiation. In addition, red blood cells exposed to 50, 70, and 100 kV of X-ray at 0.5 and 4 hours post-irradiation also did not change in fluorescence anisotropy. In contrast, Mestres et al. studied the effects of X-rays of 30, 80 and, 120 kV on chromosome aberrations using cytogenetic fluorescence *in situ* hybridization (FISH). The induction of complex chromosome aberrations evidenced by three or more breaks in two or more chromosomes, by 30, 80, and 120 kV of X-rays were compared. The results indicated that the percentage of complex chromosome aberrations were 14.1 ± 1.9, 9.8 ± 1.6, and 7.8 ± 1.19 for 30, 80, and 120 kV, respectively. This suggests that complex chromosome aberrations increased as kV deceased [16]. In addition, our results were dissimilar to other studies previously reported in the literature [17-20]. Hence, differences in cell types, radiation energy, radiation doses, and biological endpoints may contribute to the dissimilar findings regarding the effects of kV result based on the results of our study. In conclusion, the results obtained in the present study suggest that 50, 70, and 100 kV medical diagnostic X-rays did not show any effects on hemolysis, osmotic fragility, and fluorescence anisotropy values of irradiated red blood cells at 0.5 and 4 hours post-irradiation.

***Conflicts of interest*:** *Declared none.*

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