

Brief communications:

Lysosomes of Cancerous and Normal cells in Response to Low-energy/low-dose Medical Diagnostic X-rays

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Abstract:

Background: Low-energy/low-dose ionizing radiation is increasingly being used in medical diagnosis, yet the effects of low-energy/low-dose medical diagnostic X-rays on biological systems remains a mystery. Thus, the aim of this present study is to determine the characteristics of lysosomes of cancerous and normal cells in response to low-energy/low-dose medical diagnostic X-rays. **Methods and materials:** Three cell lines composed of peripheral blood mononuclear cells (PBMCs), adriamycin-sensitive erythroleukemia cells (K562), and adriamycin-resistant erythroleukemia cells (K562/*adr*), were all exposed to low-energy/low-dose medical diagnostic X-rays that operated at 50, 70, and 100 kV, and 100 mAs for obtaining radiation doses at 0.03, 0.07, and 0.10 mGy, respectively. Accumulation of acridine orange in the lysosomes of each cell line was determined using flow cytometry completed at 4 and 6 hours-post irradiation. **Results:** The data showed an increase in fluorescence intensity of acridine orange in three irradiated cells line at 4 hours post-irradiation, but this intensity did not change at 6 hours post-irradiation when compared to that of corresponding non-irradiated cells. **Conclusion:** This finding suggests that changes in lysosomes might be a response to low-energy/low-dose medical diagnostic X-rays.

Keywords: Lysosome; Medical diagnostic X-ray; Radiation

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Introduction

It is well known that high doses of ionizing radiation can induce harmful health effects. However, a low dose of ionizing radiation is still not fully recognized as a potential health risk. There are several studies that have indicated that the effects of low dose and high dose ionizing radiation is dissimilar with regard to biological responses. Regardless of this evidence, the health risks of exposure to low dose ionizing radiation are still being examined, as low dose ionizing radiation is increasingly being used in medical diagnosis¹. Ionizing radiation not only induces nuclear DNA damage, but intracellular organelles such as lysosomes, can also be affected². Lysosomes are acidic intracellular organelles that contain several enzymes that can break down many

kinds of intracellular molecules such as DNA, lipids, and proteins. It also acts as a waste elimination system for the cell by digesting unwanted molecules from extracellular and intracellular compartments in the cytoplasm. Extracellular molecules are up taken through a process of endocytosis, while intracellular molecules are digested through autophagy³. Some evidence suggests that ionizing radiation induces autophagy in several types cancer cells such as bladder⁴ and, colorectal cancer cells⁵ However, this evidence was demonstrated only by exposure to high doses of ionizing radiation to induce these damaging effects. Thus, the aim of present study was to determine the characteristics of lysosomes in both cancerous and normal cells in response to low-energy/low-dose medical diagnostic X-rays.

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Materials and Methods

Cell culture

This present study used peripheral blood mononuclear cells as a model for normal cells. Cells were isolated and cultured following procedures listed in our previous reports⁶. Peripheral blood mononuclear cells were isolated using ficoll hypaque solution (Lymphoprep™, Oslo, Norway). The number of viable cells was determined by a trypan blue exclusion assay. The number of cells was determined by haemocytometer. Freshly isolated peripheral blood mononuclear cells were cultured to obtain a total number equaling 10^6 cells/mL in a tissue-culture flask containing RPMI 1640 medium, supplemented with 10% heat-inactivated fetal bovine serum and 1% penicillin/streptomycin. Cells were incubated at 37 °C in a humidified 5% CO₂ atmosphere.

Cancerous cells line was cultured following the procedure listed in our previous report⁷. Adriamycin-sensitive erythroleukemia cells line (K562) and adriamycin-resistant erythroleukemia cells line (K562/*adr*, overexpressing P-gp), were also cultured in a tissue-culture flask containing RPMI 1640 medium, supplemented with 10% heat-inactivated fetal bovine serum and 1% penicillin/streptomycin. As was the case for normal cells, cancerous cells were incubated at 37 °C in a humidified 5% CO₂ atmosphere. Both cancer cells lines cultures initiated a total of 10^5 cells/mL before being exponentially proliferating to a total of $8-10 \times 10^5$ cells/mL over 3 days. For the experiment, cultures were initiated at 5×10^5 cells/mL to obtain cells in exponential growth phase to a total of about $8-10 \times 10^5$ cells/mL over 24 hours. The total number of viable cells was determined by a trypan blue exclusion assay. The total number of cells was determined by haemocytometer.

Irradiation system

An irradiation system was designed based on a procedure indicated in one of our previous reports⁶. Tissue culture flasks were placed in the center of an X-ray beam at a distance of 100 cm from the X-ray tube. A medical diagnostic X-ray machine (Quantum medical imaging, Quest HF series, Caresteam, NY, USA) operating at 50, 70, and 100 kV, and 100 mAs was used. Table 1 shows the parameters obtained by this medical X-ray machine. Energy spectra of X-ray from medical diagnostic X-ray machine that operated at 50, 70, and 100 kV was also shown in one of our previous studies⁸

Table 1 The parameters obtained by the medical X-ray machine used in this study.

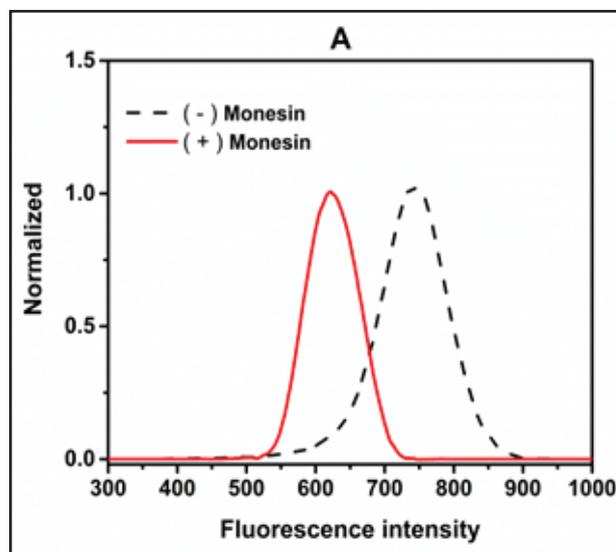
Setting		Measurement		Calculation		HVL, mmAl	Target /filter
kV	mAs	Exposure, nC	Exposure, R	Total dose, mGy			
50	100	0.03	0.0036	0.03	3.1	W/Al	
70	100	0.06	0.0073	0.07	3.1	W/Al	
100	100	0.09	0.0109	0.10	3.1	W/Al	

kV = kilovoltage, nC = nanocoulomb R = roentgen, W = tungsten, Al = aluminum

Accumulation of acridine orange in lysosomes

The accumulation of acridine orange in various intracellular organelles such as in the cytoplasm, nucleus, and lysosome expressed an emission spectrum of light that can be determined by flow cytometer based on the following: 1) Acridine orange in cytoplasm emits bright green fluorescence, 2) Acridine orange in the nucleus emits dim red fluorescence, and 3) Acridine orange in lysosomes emits bright red fluorescence⁹. Figure 1 shows the typical fluorescence intensity of acridine orange in cells taken from a flow cytometer. In the presence of 3 μM monensin, bright red fluorescence intensity decreased (Figure 1A), whereas bright green fluorescence intensity increased (Figure 1B). It is suggested that acridine orange is released from the lysosomes into the cytoplasm.

The authors determined the accumulation of acridine orange in lysosomes occurring at 0, 4, and 6 hours post-irradiation using a flow cytometer. For the assays, 10^6 cells were suspended in 1 mL phosphate buffer saline at a pH of 7.25 in the presence of 1 μM acridine orange for 30 minutes prior to determination of fluorescence intensity with flow cytometer (Coulter Epics XL-MCL).



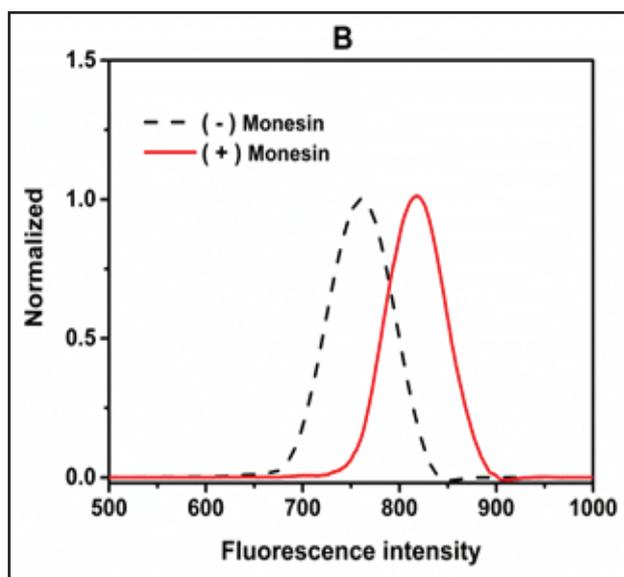


Figure 1. Typical histograms of fluorescence intensity of acridine orange accumulated in lysosomes (A) and cytoplasm (B). Cells were incubated with 1 μ M acridine orange for 30 minutes. The fluorescence intensity was recorded by flow cytometer without or after immediately adding 3 μ M of monensin. The total number of cells in each histogram was 10,000 cells.

Results and Discussion

Previously, we indicated that low energy/low-doses of medical diagnostic X-rays did not significantly change various biological end points such as hemolysis, osmotic fragility, fluorescence anisotropy, mitochondrial membrane potential, the cell cycle, and the number of apoptotic cells of irradiated cells as compared to corresponding non-irradiated groups at each time end point⁸⁻¹⁰.

Figure 2 summarizes the results as histograms of the accumulation of acridine orange in lysosomes. The number of cells in each histogram was 10,000 cells. For the peripheral blood mononuclear cells, the histograms of accumulation of acridine orange in lysosome of cells were collected at 4 and 6 hours after an *in vitro* exposure to various low-energy/low-doses of medical diagnostic X-rays. This also included the results of corresponding non-irradiated control groups. This data shows an increase in fluorescence intensity for irradiated cells, relative to that of the corresponding non-irradiated cells at 4 hours post-irradiation (Figure 2A - C). Fluorescence intensity shows no change in irradiated cells, as compared to the corresponding non-irradiated cells at 6 hours post-irradiation (Figure 2A - C). The histograms for adriamycin-sensitive erythroleukemia cells (K562) and adriamycin-resistant erythroleukemia

cells (K562/*adr*, overexpressing P-gp), show the accumulation of acridine orange in lysosomes of cells collected at 4 and 6 hours after an *in vitro* exposure to various low-energy/low-dose of medical diagnostic X-rays, and that of the corresponding non-irradiated groups. This data shows that an increased fluorescence intensity of irradiated cells had taken place, in relation to the corresponding non-irradiated cells at 4 hours post-irradiation (Figure 2 D - I). Fluorescence intensity showed no change in irradiated cells, as compared to the corresponding non-irradiated cells at 6 hours post-irradiation time (Figure 2 D - I).

The present results suggest that lysosomes had responded to radiation within 4 hours post-irradiation time and then regulated to a resting stage at 6 hours post-irradiation. Acridine orange, an intrinsic fluorescent probe, is often used to determine the luminal pH of intracellular acidic compartments, like lysosomes. The accumulation of acridine orange in the lysosome depends on the pH gradient across the lysosome, so a collapse of the pH gradient results in acridine orange being released from the lysosome¹¹. This present result shows an increase in the accumulation of acridine orange in lysosomes occurring at 4 hours post-irradiation. It could be possible that the lysosomes had responded to radiation by increments of pH gradient. However, since this was a preliminary study, the effects of low-energy/low-dose medical diagnostic X-ray on lysosomes needs to be better understood based on further research.

Conclusions

These findings suggest that the lysosomes may have responded to low-energy/low-dose medical diagnostic X-rays.

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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.

Conflict of interest: The authors declared no potential conflicts of interest

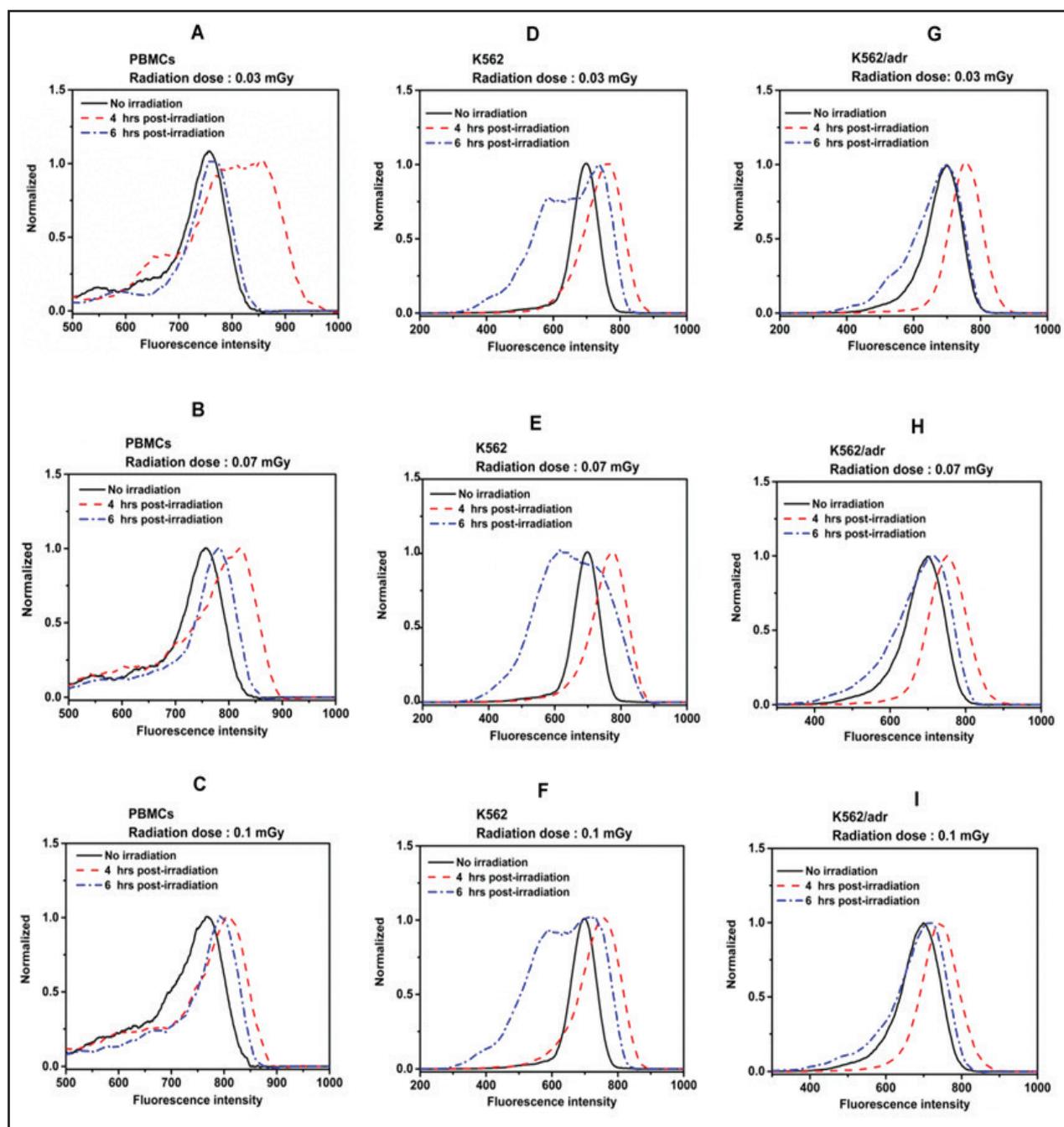


Figure 2. Histograms of accumulation of acridine orange in lysosomes of peripheral blood mononuclear cells (PBMCs), adriamycin-sensitive erythroleukemia cells (K562), and adriamycin-resistant erythroleukemia cells (K562/adr, overexpressing P-gp) after irradiation. The number of cells in each histogram was 10,000 cells.

Individual Contribution of the Authors:

Conceptual work: Montree Tungjai, Nuttakan Tubthaing, Suchart Kothan

Data collection: Montree Tungjai, Nuttakan Tubthaing, Suchart Kothan

Manuscript writing: Montree Tungjai, Nuttakan Tubthaing, Suchart Kothan

Editing of final manuscript: Montree Tungjai, Nuttakan Tubthaing, Suchart Kothan

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