Assessment of Mobile Lipids with ^{1}H-NMR Spectroscopy in Enriched CD34$^+$ Human Peripheral Blood Stem Cells/Progenitor Cells after Exposure to Polyenergetic Medical Diagnostic X-Rays

Montree Tungjai[1] and **Nathupakorn Dechsupa**[1],

ABSTRACT

Polyenergetic medical diagnostic x-rays are widely used across the world for the diagnosis of many diseases by means of a variety of imaging technologies. In this study, the effect of polyenergetic medical diagnostic x-rays, ranging from 30-50, 50-70 and 70-100 keV, on enriched CD34$^+$ human peripheral blood stem cells/progenitor cells was studied. The mobile lipids (apoptosis biomarkers) in enriched CD34$^+$ human peripheral blood stem cells/progenitor cells after exposure to polyenergetic medical diagnostic x-rays were measured by ^{1}H-NMR spectroscopy. The cells without irradiation served as sham controls. ^{1}H-NMR signals at a chemical shift of 1.3 and 0.9 ppm, corresponding to methylene and methyl groups, respectively. These were called mobile lipid signals. The result showed no significant mobile lipid signal change in irradiated enriched CD34$^+$ human peripheral blood stem cells/progenitor cells as compared to sham controls. It was suggestive that polyenergetic medical diagnostic x-rays ranging 30-50, 50-70, and 70-100 keV did not affect mobile lipid levels in enriched CD34$^+$ human peripheral blood stem cells/progenitor cells.

Keywords: mobile lipid, ^{1}H-NMR, stem cells/progenitor cells, medical diagnostic x-rays

1. INTRODUCTION

There were many publications indicated that radiation effected to living cells by inducing apoptosis [1-4], cell cycle arrest [5-7], DNA damage [8-10], NF-κB and cytokine expression [9,11], metabolite and protein profile changing [12-14]. The energies of radiation that used in those publications were monoenergetic radiation. Recently, x-ray is widely used in medical examination for diagnosis of various diseases by means of a variety of examination such as x-ray examination, mammogram examination, and computed tomography scans [15,16]. The x-ray that used in medical examination is polyenergetic x-ray. There is little information on the effect of polyenergetic medical diagnostic x-rays on living cells. Of note, blood cells are always exposed to polyenergetic x-ray during diagnostic x-ray examination. Therefore, we interest the effect of polyenergetic medical diagnostic x-rays on blood cells.

In this study, the mobile lipids in enriched CD34$^+$ human peripheral blood stem cells/progenitor cells after exposure to polyenergetic medical diagnostic x-rays were measured by ^{1}H-NMR spectroscopy at 24 hours post-irradiation. The mobile lipids consisted mainly of methyl and methylene groups (originating from the mobile acyl chains in triacylglycerides), corresponding to cell death via apoptosis pathway [17-19].

2. MATERIALS AND METHODS

2.1 Separation of human peripheral blood mononucleated cells (PBMCs) and expansion of enriched CD34$^+$ human peripheral blood stem cells/progenitor cells (PBSCs/progenitor cells)

PBMCs were separated from anticoagulated whole blood samples using a ficoll hypaque solution (Lymphoprep^{TM}, Norway). The ficoll hypaque was layered under whole blood in a 15 mL tube. This tube was centrifuged at 300 x g for 5 minutes. Then, the PBMCs were collected and washed with phosphate buffer saline (PBS), pH 7.4. Jaruchainiwat et al. (2009) has previously described the expansion of enriched CD34$^+$ human PBSCs/progenitor cells [20]. Briefly, PBMCs (10^6cells/mL) were cultured in RPMI1640 (Gibco,USA) media supplemented with 1% penicillin-streptomycin (Gibco, USA) and 10% fetal bovine serum (PAA, Austria) at 37°C in a humidified incubator at 95% humidity. The number of enriched CD34$^+$ human PBSCs/progenitor cells reached a plateau at 3-4 days after culture initiation.

2.2 Polyenergetic x-ray beam design

A medical diagnostic x-ray machine (Quantum medical imaging, Caresteam, Quest HF series) was used to produce x-rays. These x-rays have a continuous spectrum (Figure 1A). The medical diagnostic

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[1] Department of Radiologic Technology, Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai, 50200 Thailand. Email: mtungjai@gmail.com, nathupakorn@gmail.com, and nathupakorn.d@cmu.ac.th

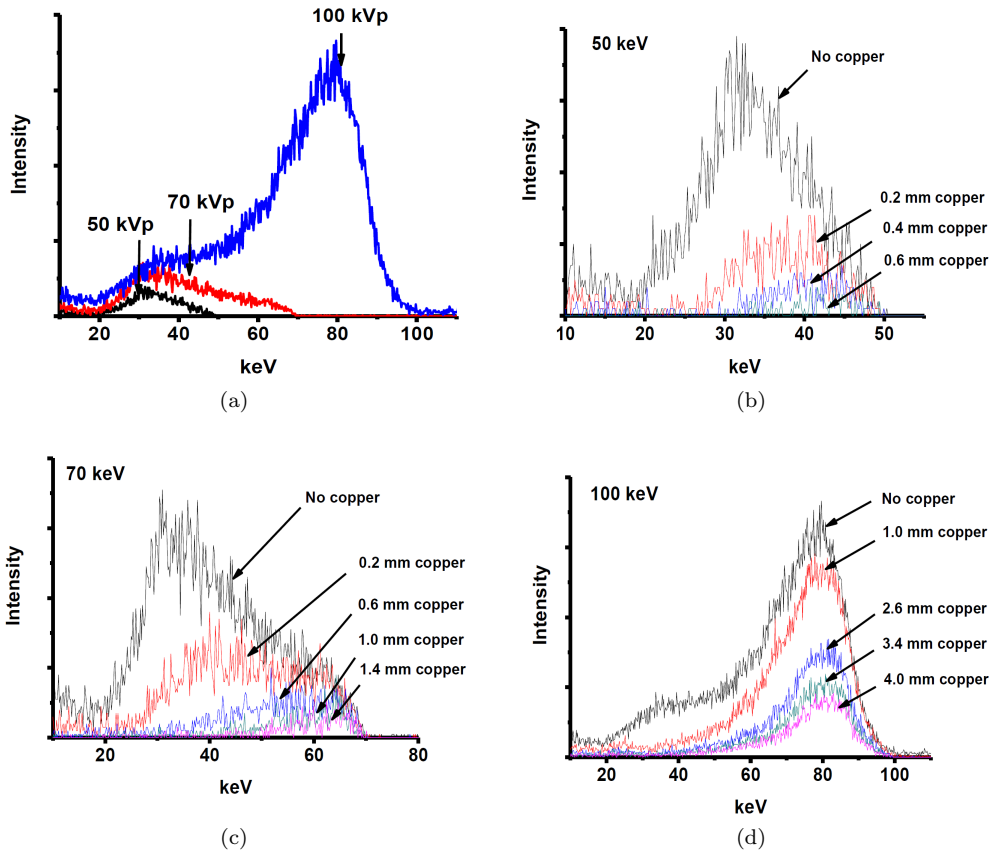

Fig.1:: X-ray spectra: (a) Spectra of 50, 70 and 100 keV of x-rays, (b) Spectra of 50 keV of x- ray with copper sheet filter, (c) Spectra of 70 keV of x-ray with copper sheet filter and (d) Spectra of 100 keV of x-ray with copper sheet filter.

Table 1:: ^{1}H-NMR peak ratios measured on enriched CD34^{+} human peripheral blood stem cells/progenitor cells with and without irradiation. Results are expressed as means ± SD.

Energy, keV	Mobile lipids		
	Methyl/Ct	Methylene/Ct	Methylene/Methyl
0	2.25 ± 0.29	3.27 ± 1.95	1.49 ± 0.91
30-50	2.19 ± 0.65	3.63 ± 1.96	1.59 ± 0.53
50-70	2.29 ± 0.97	3.68 ± 2.47	1.53 ± 0.63
70-100	2.39 ± 0.62	3.95 ± 2.65	1.54 ± 0.60

x-ray machine can generate several energies of x-rays by the kilovoltage peak (kVp) setting (Figure 1A). In this study, the kVp was set to 50, 70 and 100 kVp for generating polyenergetic x-ray beams of 0-50, 0-70 and 0-100 kiloelectron volts (keV) of x-rays, respectively. The x-ray tube current was 25 milliamperes (mA) and time was 4 seconds (s). In order to design the x-ray beam, the x-ray beam was filtered using copper sheets for generating ranges of energy from 30-50, 50-70, and 70-100 keV. A multichannel analyzer (Amptek) was used to measure x-ray spectrum from medical diagnostic x-ray machine. The multichannel analyzer was placed in the middle of x-ray beam, at a distance of 100 cm from the x-ray tube of the medical diagnostic x-ray machine.

2.3 Irradiation

A number of enriched CD34^{+} human PBSCs/progenitor cells (10^{7} cells/mL) were centrifuged at 1,400×g for 1 minute and then were washed with PBS. The enriched CD34^{+} human PBSCs/progenitor cells were placed in the center of the x-ray beam, at a distance of 100 cm from the x-ray tube. Field of view was 10 cm x10 cm. The energies of x-ray were 30-50, 50-70, and 70-100 keV. The enriched CD34^{+} human PBSCs/progenitor cells without irradiation served as the sham controls. The x-irradiated enriched CD34^{+} human PBSCs/progenitor cells were cultured for 24 hours before assessment of mobile lipids with ^{1}H-NMR spectroscopy.

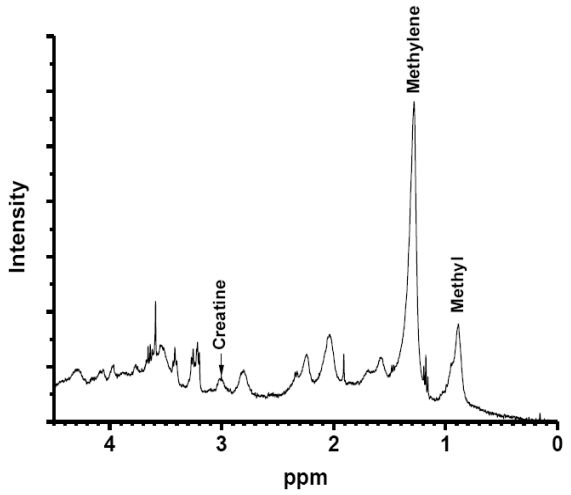

Fig.2:: Typical ^{1}H-NMR spectrum of enriched CD34$^+$ human peripheral blood stem cells/progenitor cells. Peak assignment: methyl group (peak at 0.9 ppm), methylene group (peak at 1.3 ppm) and creatine (peak at 3.0 ppm).

2.4 Assessment of mobile lipids with 1H-NMR spectroscopy

The irradiated and non-irradiated enriched CD34$^+$ human PBSCs/progenitor cells were washed with PBS and phosphate buffer saline/deuterium oxide (PBS/D_2O). Then, enriched CD34$^+$ human PBSCs/progenitor cells were suspended with PBS/D_2O in a shigemi NMR tube. ^{1}H-NMR spectra using 500 μL of this solution were acquired on a Bruker Avance III spectrometer operating at 400 MHz proton resonance frequency. The enriched CD34$^+$ human PBSCs/progenitor cells in tube that were located in coil volume did not rotate during NMR operation. The following resonances were incorporated: methyl group (peak at 0.9 ppm), methylene group (peak at 1.3 ppm), and creatine (Ct) (peak at 3.0 ppm). The peak areas of ^{1}H-NMR spectrum were measured.

2.5 Statistical analysis

We used Students-t tests to compare mobile lipid signals between controls and irradiated enriched CD34$^+$ human peripheral blood stem cells/progenitor cells. A p value of less than 0.05 was considered as statistically significant.

3. RESULTS AND DISCUSSIONS

3.1 X-ray beam design

The x-rays are produced within the x-ray machine. The x-rays occur by two main mechanisms, resulting characteristic and bremsstrahlung x-rays. Thereby, x-rays form a polyenergetic beam which can be manipulated by changing the x-ray tube current or voltage settings or by adding filters to cut off low energy x-rays [21].

Continuous spectra of x-rays are shown in Figure 1. Figure 1A shows energy spectra of x-rays ranging from 0-50, 0-70, and 0-100 keV for 50, 70 and 100 kVp settings, respectively. We designed x-ray beam using copper sheets to filter out unwanted energy. Figure 1B indicates that a 0.2 mm copper sheet was needed to receive the energy spectrum of 30-50 keV. Figure 1C indicates that a 0.4 mm copper sheet was needed to receive the energy spectrum of 50-70 keV. Figure 1D indicates that a 3.4 mm copper sheet was needed to receive the energy spectrum of 70-100 keV. It was clearly shown that the thickness of the copper sheet affects the energy spectrum.

3.2 Mobile lipids in enriched CD34+ human PBSCs/progenitor cells with and without irradiation

The typical ^{1}H-NMR spectrum of enriched CD34$^+$ human PBSCs/progenitor cells without irradiation is shown in Figure 2. The ^{1}H-NMR spectrum indicated well resolved signals of methyl group, methylene group, and creatine.

Table 1 shows the peak ratio of mobile lipid to creatine including methyl/Ct, methylene/Ct and ratio of methylene to methyl group. These ratios were measured from mobile lipid signals in enriched CD34$^+$ human PBSCs/progenitor cells. The baselines of methyl/Ct, methylene/Ct and methylene/methyl were 2.25 ± 0.29, 3.27 ± 1.95 and 1.49 ± 0.91, respectively. The mobile lipid signals did not significantly change between irradiated enriched CD34$^+$ human PBSCs/progenitor cells and sham controls.

In contrast, there were publications reported that the human hematopoietic stem/progenitor cells exposed to x-rays, resulting increased frequencies of apoptotic cells and chromosome aberrations that were both dose- and time-dependent [22]. X-ray could induce intracellular reactive oxygen species expression in human hematopoietic stem/progenitor cells [23] and human hematopoietic stem cells [24]. However, the energies that used in those publications were monoenergetic x-ray while the energies that used in this study were polyenergetic x-ray. A limitation of our study was dose rate. The dose rates of polyenergetic medical diagnostic x-rays, ranging 30-50, 50-70 and 70-100 keV, were not similar.

In conclusion, this finding was suggestive that polyenergetic medical diagnostic x-rays ranging from 30-50, 50-70 and 70-100 keV did not significantly change the mobile lipids in enriched CD34$^+$ human PBSCs/progenitor cells.

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Montree Tungjai received his B.Sc. degree in Radiologic Technology from Chiang Mai University, Chiang Mai, Thailand, in 2000 and his M.Sc. degree in Biological Sciences from Burapha University, Chonburi, Thailand, in 2004. He holds a Ph.D. degree in Biomedical Sciences from Chiang Mai University, Chiang Mai, Thailand, in 2008. He is currently working at the Center of Excellence for Molecular Imaging (CEMI) and the Department of Radiologic Technology, Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai, Thailand. His research emphasized on radiation biology, radiosensitizer and radioprotector.

Nathupakorn Dechsupa received his B.Sc. degree in Physics and his M.Sc. degree in Biological Sciences from Burapha University, Chonburi, Thailand, in 1998 and 2001, respectively. He holds a Ph.D. degree in Biomedical Sciences from Chiang Mai University, Chiang Mai, Thailand, in 2006. He is currently working at the Center of Excellence for Molecular Imaging (CEMI) and the Department of Radiologic Technology, Faculty of Associated Medical Sciences, Chiang Mai University, Chiang Mai, Thailand. His research emphasized on development of a molecular imaging probe especially for medical imaging modality such MRI, PET, SPECT and Optical imaging. He is interested also in natural products and drug discovery.