Effect of Pulse Electromagnetic Field Exposure on the Expression of Lipo Protein Lipase (LPL) on the Differentiation of Mesenchymal Stem Cell

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Abstract. Pulsed electromagnetic fields (PEMFs) have an important role in cell differentiation. Previous study reported that PEMFs had positive and negative effect towards cell differentiation that depends on their frequencies applied to the cells. Human adipose-derived stem cells (ASCs) are mesenchymal stem cells that have an ability to differentiate into several types of cell including adipocytes, chondrocytes and osteocytes. This study aimed to evaluate the effect of human ASCs towards their adipogenic differentiation during PEMFs exposure. Human ASCs were isolated from adipose tissue. The cells then cultured in specific medium of adipocyte that induced ASCs differentiation along with PEMFs exposure. The maximum magnetic field used is 2 mT with a frequency of 75 Hz. To confirm the effect of PEMFs exposure towards adipogenic differentiation, mRNA expression of lipo protein lipase (LPL) was measured in mRNA expression level. The results showed that ASCs cultured on adipogenic differentiation without PEMFs exposure gradually increased LPL expression until day 14 of observation, while ASCs with PEMFs exposure significantly decreased LPL expression from day 2 to day 14. Based on the results, we concluded that PEMFs exposure can inhibit LPL expression that suppressed adipogenic differentiation.

1. Introduction
Adipose-derived stem cells (ASCs) are mesenchymal stem cells that exist in adipose tissue [1]. ASCs have ability to multi-directional differentiation into bone cells, cartilage cells, or fat cells. Their differentiation ability depends on the supplementation in the microenvironment [1,2]. However, recent study showed that electromagnetic also promote stem cells differentiation. Pulsed electromagnetic fields (PEMFs) enabled to generate magnetic field effects with pulsed intervals that changes microenvironment of physiological activities [3]. Due to the change microenvironment of cells, PEMFs can promote bone marrow mesenchymal stem cells (BMSCs) into osteogenic and inhibit other lineage of differentiation. The technique is safe, environmentally friendly, non-invasive, and simple [4].
Obese mice lose their weight and fat after treated by electromagnetic fields. Extremely low frequency magnetic fields (ELF-MF) also is capable to inhibit the effect of obesity. Inhibitory effect of ELF-MF on obesity may be related to the differentiation of MSCs to adipocytes [5]. Adipogenesis of MSCs expressed lipoprotein lipase (LPL). LPL is an enzyme that hydrolyzes triglycerides and releases free fatty acids in cardiac muscle as an energy source [6]. Furthermore, previous study showed that PEMFs can promote the expression of ALP and bone morphogenetic protein-2 (BMP-2) in BMSCs, and have the effect of promoting the differentiation of BMSCs into osteogenic tissue [7] suggesting the PEMF suppressed adipogenesis due to their negative feedback mechanism.

In this study, we manage to induce ASCs to differentiate into adipocytes using specific media. The induced cells then exposed using PEMFs. To evaluate the adipogenesis, the expression of LPL was measured. We suggest that PEMFs regulate the expression of adipogenesis.

2. Material and Method

2.1. Culture of Mesenchymal Stem Cell

MSCs were obtained from Stem Cell and Tissue Engineering Research Center (SCTE-RC) Indonesian Medical Education and Research Institute (IMERI), Faculty of Medicine, University of Indonesia. MSCs were isolated from adipose tissue [8]. The cells were cultured in the complete medium containing of 1% antibiotic, 1% anti-mycotic, 1% glutamine, 1% heparin, 10% serum platelet-rich plasma (PRP), and αMEM. The expansion of cells was conducted using flasks 25 and incubated at 37°C, 5% CO₂. To maintain the cells, the medium was changed every three days. After the cells reach 100% confluency, the cells were harvested using Triple Select and seeded in the six well culture flask.

To induce MSCs differentiation, the complete medium was replaced to specific medium of adipogenesis (StemPro® Adipogenesis Differentiation Kit). This induction medium contains 1% antibiotic, 1% anti-mycotic, 10% supplement, and basal medium. The adipogenic induction medium was replaced every three days for 14 days.

2.2. PEMFs Exposure

The construction of PEMFs generator has been described previously [9-11]. This PEMFs generator consisted of a signal generator and a pair of 40-cm diameter Helmholtz coil. The coil were connected to a signal generator that delivered repetitive square-wave pulses with pulse duration 1 s and frequency 75 Hz. (Figure 1).

The maximum magnetic field used is 2 mT with a frequency of 75 Hz. The cells were placed in flask between the coil. MSCs from the experimental group were exposed to PEMFs for 10 minutes daily for 14 days. The control group was not exposed to PEMF. On days 2, 4 and 14, most of the MSCs were harvested for qRT PCR analysis.

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2.3. RNA Extraction and qRT-PCR Analysis

The total RNA was isolated from harvested MSCs on day 0 (as calibrator), 2, 4 and 14 using TRIzol TM LS Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer’s instruction. The RNA concentration and purity was performed using a spectrophotometer. Primer sequences are described in Table 1.

| No. | Gene  | Nucleotides (5′-3′) |
|-----|-------|---------------------|
| 1   | LPL   | F: CAGCCCTACCCCTTGTTAGTTATT  
        |       | R: ACGTTGGAGGAGTGCTATTTTT |
| 2   | GAPDH | F: CCCTTCATTGACCTCAACTACA  
        |       | R: ATGACAAGCTTCCCCGTTC |

LPL: Lipoprotein Lipase

RT-qPCR was performed using a standard protocol from the MIQE (minimum information for publication of quantitative real-time PCR experiments) guide [12]. The mRNA expression of GAPDH and LDL was analyzed using Sensi FAST™ SYBR® Lo-ROX One-Step Kit material, which allowed detection of the PCR products by measuring the increase in SYBR green fluorescence caused by the binding of SYBR green to double-stranded DNA. The qRT-PCR thermal cyling program was as follow: 10 min at 45°C for activation of the reverse transcription enzyme, 2 min at 95°C for activation of the polymerase enzyme. Then followed by 40 cycles of 15 s at 95°C, 30 s at 60°C and 30 s at 72°C. The level of expression was calculated based on the PCR cycle number (Ct) and the relative gene expression level was determined using the Livak method [13].

2.4. Statistical Analysis

Statistical analysis was performed using SPSS 21 for Windows. Statistical analysis was began with normality and homogeneity testing using the Shapiro Wilk and Lavene test. The data presented as means ± SEM. One-way analysis of variance (ANOVA) with a post hoc Tukey test was used to compare means. The differences were analyzed (p < 0.05).

3. Results and Discussion

Mesenchymal stem cells (MSCs) are multi-potent cells that can be differentiated into several types of cells in the specific microenvironment including adipocytes, chondrocytes and osteocytes [2]. Their abilities lead MSCs to be used as a model for cell differentiation study [14]. Differentiation of MSCs depends on signaling pathway, transcription factor, growth factors and physical factors as well [15]. Furthermore, recent study showed that the use of electromagnetic field also promotes osteogenesis and increases proliferation rate of stem cells in vitro [7-16].

Those studies provided an insight to break down the effect of electromagnetic field toward stem cells differentiation. Hu et al., reported that during adipogenic differentiation of hASCs, LPL is gradually up-regulated but tended to become stable 14 days after induction [17]. In this study, we investigate the effect of PEMFs on the adipogenic differentiation of MSCs towards LDL expression of adipogenesis for 14 days. MSCs were cultured in an adipogenic medium that also has an effect to induced the cells to undergo adipogenesis. LPL that regulate human adipose-derived mesenchymal stem cells (hASCs) into adipogenic differentiation was measured by qRT-PCR. The data obtained on day 0 (calibrator), 2, 4, and 14 from each group. We observed that the control group expressed LDL and gradually increased until day 14. However, the MSCs that exposured by PEMF tended to become stable on day 2 and 4 and significantly decreased on day 14 (Figure 2).

Our previous study showed that PEMF exposure may have potent effect to inhibit adipogenic differentiation indicated by PPARγ and ADIPOQ mRNA expression [9-10]. Extremely low frequency magnetic fields (ELF-MF) also inhibited adipogenic differentiation of ASCs showed by decreasing
proliferator-activated receptor 2 (PPARγ2) expression but had no effect on osteogenic differentiation [5]. ELF-MF regulates JNK-dependent intracellular signaling in MSCs [18]. JNK signaling is essential in the bone formation [5]. However, no data of osteogenesis were recorded in this study. Moderate intensity Static Magnetic Field (SMF) also inhibited adipogenesis of MSCs, which was indicated by reducing LPL and PPAR-γ and by decreasing the number of lipid droplets [19].

Figure 2. Expression level of LPL indices stimulated by pulsed electromagnetic field (PEMF).

Based on these results there are some limitations to our study. First, to evaluate adipogenesis, osteogenesis marker should be measured as well because their negative feedback mechanism. It means that if adipogenesis was promoted, osteogenesis will be inhibited. In another study we have found that PEMF exposure can promote fracture healing. This was indicated by an increase in the expression of osteoblast activity marker genes including Wnt 5a, Wnt10b and β-catenin [20]. Second, the mechanism of LPL inhibition after PEMF exposure was not clear yet. Further study should be conducted to evaluate and confirm our results.

4. Conclusion
Adipogenesis of MSCs could be inhibited by PEMF suggesting the exposure of PEMF may provide a potential approach for the treatment of obesity.

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