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RESEARCH ARTICLE

Expression of Adipocyte Binding Protein-2 (AP-2) on the Adipogenic Differentiation of Mesenchymal Stem Cell after Pulsed Electromagnetic Field (PEMF) Exposure

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Abstract

Obesity is a health condition that can cause serious health problems in developing countries such as Indonesia and still a major health concern worldwide. Obesity increasing the risk of a number of pathological disorders including certain cancers, heart disease, stroke, and diabetes. It has been reported that Pulsed Electromagnetic Field (PEMF) have an inhibitory effect on adipogenesis of MSCs. However, it is not clear if this effect is direct or indirect. Various genes are involved in the development of obesity include adipocyte binding protein 2 (AP-2) which is associated with inflammation and the metabolic syndrome. In the present study, we investigated the effect of PEMF exposure with frequency 75 Hz and magnetic field 2 mT on the adipogenic differentiation of mesenchymal stem cells (MSCs). In the present study we used the MSCs second passage. The cells were stimulated by PEMF of 75 Hz/2mT for 14 days 10 in/day. The relative expression of AP-2 was measured on days 2, 4, 7 and 14 by using quantitative reverse transcription polymerase chain reaction (qRT-PCR) method. The PEMF significantly inhibited adipogenic differentiation by down regulating the expression of AP-2 on days 2, 4 and 7. This result indicated that PEMF exposure may have potent effect to inhibit adipogenic differentiation.

Keywords: *Pulsed electromagnetic field, Adipogenic, Mesenchymal Stem Cells, AP-2*

Introduction

Obesity is a common condition that is often neglected in public health management in several countries. Even in some groups of society, obesity is considered a symbol of social status. Currently obesity is a global epidemic in all age ranges in both developed and developing countries [1]. The imbalance between energy intake and energy expenditure primarily causes obesity.

Obesity is a condition in which body has a body mass index ≥ 30 kg/m2. The body has accumulated excess body fat that detrimental to health. Several chronic health disorders such as type 2 diabetes mellitus, hypertension, coronary heart disease, dyslipidemia, and some cancers were associated with an obesity problem. The direct and indirect medical cost of obesity in the United States is about ~$100 billion annually [2]. Indonesian adults have a high prevalence of obesity and central obesity. Adult overweight prevalence has doubled from 17.1% to 33 % between 1993 and 2014. [3, 4]. Obesity is related with excessive accumulation of lipids in adipocytes. This causes an increase in cell size coupled with an increase in adipogenesis. Adipogenesis consists of two related stages: the determination of MSCs into preadipocytes and the differentiation of preadipocytes into mature fat cells. The accumulation of lipids reflects the preadipocytes differentiation into adipocytes. This process is complex regulated by the increased expression of multiple genes and transcription factors.

Adipocytes secrete a number of transcription factors which are central to the regulation of lipid and glucose metabolism, immune responses, and pathophysiological changes [5]. The transcriptional regulation of adipogenesis is relatively well-characterized, with C/EBPα and PPARγ as the two main regulators which control adipogenic genes.

AP-2 is a terminal adipocyte differentiation marker and acts as cytoplasmic lipid chaperone. AP-2 regulates the cellular uptake of long-chain fatty acids in a pathway connecting obesity and fatty acid metabolism [6]. Interestingly, a number of transcriptional factors and intracellular signaling pathways have been shown to control the differentiation of MSCs into osteoblastic or adipocyte cells [7].

Over the last decade, it has become clear that low-level of chronic and local inflammation induced by obesity [8]. The fatty acid-binding proteins (FABPs) have been identified in adipocytes and macrophage. They are a family of small cytoplasmic proteins that sized 14-15 kDa. FABPs act as chaperones of cytoplasmic lipid and play a role in the cellular flow of fatty acids and other lipid signals through their interaction with functional targets.

AP-2 is the predominant FABP present in adipocytes. The primary physiological role of these proteins has been recognized only through the development of genetic models to investigate their function in mice. A lack of AP-2 causes a decrease in triglycerides, increased insulin sensitivity when made obese in these experimental models [6].

Pulsed electromagnetic field (PEMF) has been widely researched regarding its benefits to help cure bone diseases. PEMF especially in the extremely electromagnetic field range with frequencies below 300 HZ has attracted a lot of attention. At this frequency PEMF does not have enough energy to damage DNA. It also does not cause thermal and ionizing effects on cells and tissues. PEMFs allow the current to circulate through a Helmholtz coil to generate magnetic field effects with pulsed intervals which may simulate the microenvironment of physiological activities in organisms during motion.

The technology is safe, eco-friendly, non-invasive, and simple. It also prevents the introduction of infection, has a large applicability, and has few complications. PEMFs have been used for close to 40 years as a means of non-invasive treatment of bone fracture and nonunion [9]. According to Du et al, the inhibitory effects of ELF-MF on obesity could be linked to the adipogenesis differentiation of MSCs. Exposure of 7.5 Hz, 0.4 T ELF -EMF inhibited the differentiation MSCs to adipogenic through JNK-dependent Wnt signaling pathway. It suggests that ELF-EMF has the inhibitory effect on obesity through the inhibition of differentiation of MSCs into adipocytes [7]. Nichols reported that when obese (Ob/Ob) mice increased their activity, lost weight and fat within in a 6-days when they were exposed with 0.5 T direct current electromagnetic fields [10].

Other study reported that electromagnetic fields also reduced human abdominal obesity[11]. Sari et al performed a PEMF exposure of 2 mT in MSCs culture for 10 minutes / day for 14 days. PPARγ and ADIPOQ expression are the main transcription factors in adipogenesis. The expression of PPARγ and ADIPOQ were lower in the PEMF exposure group than in the control group.

The highest expression occurred on the 7th day of PEMF exposure. PEMF exposure is reported to have the potential to inhibit adipogenesis in MSCs. [12]. In the present study, we examined the effect of PEMF exposure on adipocyte differentiation of MSCs. We hypothesized that the inhibiting effect of PEMF on obesity could be related to the differentiation of MSCs into adipocytes.

Material and Methods

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. The MSCs in this study were extracted from adipose tissue donors used by Pawitan et. al., 2013 [13].The donor was a healthy 19 years old girl. MSCs were taken from the cryopreservation tank and then warmed in a water bath at 37^{o}C. After the thawing process, cells were transferred in a tube containing 9 ml of complete medium.

The cells were cultured in a complete medium consisting of 1% antibiotic, 1%

antimitotic, 1% glutamine, 1% heparin, 10% serum platelet-rich plasma (PRP), and αMEM. 3x105 cells were grown in flasks 25 containing medium complete and cultured in an incubator at 37°C and 5% CO2.

Every 3 days the culture medium was changed. The cells were digested with Triple Select and planted 1x104 in six well culture disks when it reached 100% confluency. The cells had to differentiate themselves by replacing the media with an adipogenic induction medium. After reaching 60%-70% confluency, the complete medium is replaced with an adipocyte differentiation medium (Stem Pro® Adipogenesis Differentiation Kit). This induction medium contains 1% antibiotic, 1% antimitotic, 10% supplement, and basal medium. The adipogenic induction medium was replaced every 3 days for 14 days.

PEMF Exposure

The construction of PEMF generator has been described previously.[14] The PEMF generator included of a signal generator and a pair of 40-cm diameter Helmholtz coils. The coils were connected to a signal generator that produced repetitive square-wave pulses with a pulse duration 1 s and a frequency 75 Hz. (Figure 1).

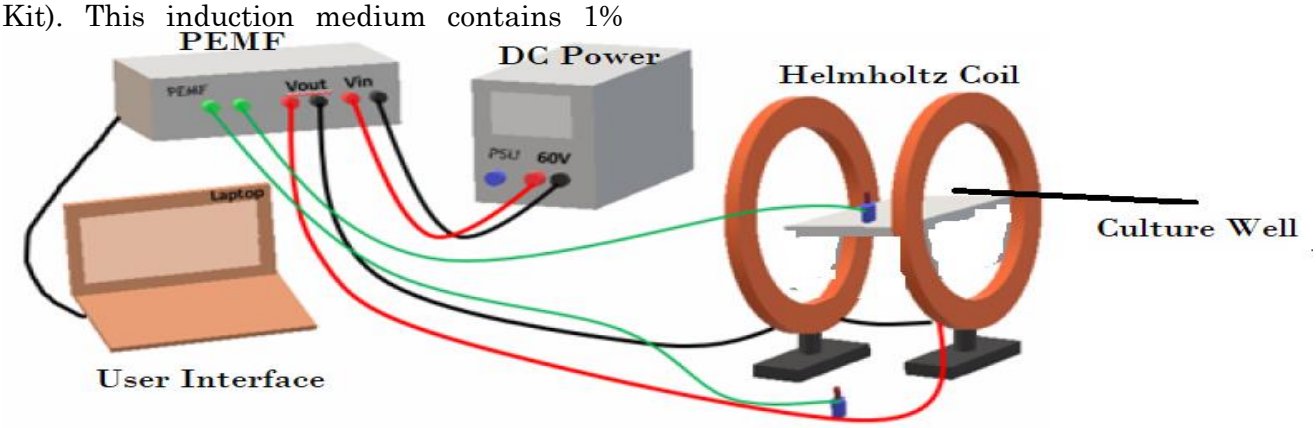

Figure 1: Schematic representation of the PEMF exposure system: (a) PEMF consisting of Helmholtz coil, pulse generator, user interface and DC power supply

The maximum magnetic field used is 2 mT with a frequency of 75 Hz. The cells were placed in flask between the coil. The cells devided into three groups namely the PEMF (P) exposure group, the external control (KL), and the incubator control (KI) group. The group exposed to PEMF received PEMF exposure with a magnetic field intensity of 2 mT, a frequency of 75 Hz. The PEMF groups were exposed to PEMF for 10 minutes a day 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.

RNA Extraction and qRT-PCR Analysis

Total RNA was isolated from MSC group day 0 (as calibrator), 2, 4, 7 and 14 by using TRIzol TM LS Reagent (Invitrogen, Carlsbad, CA, USA) following the instruction of the manufacturer. The RNA concentration and purity were analyzed using a spectrophotometer. The primers sequence used are shown in Table 1.

Table 1: Polymerase chain reaction primer sequence

No.	Gene	Nucleotides (5'-3')
1	AP-2	F: GGAAAGTCAAGAGCACCATAAC R: GCATTCCACCACCAGTTTATC
2	GAPDH	F: CCCTTCATTGACCTCAACTACA R: ATGACAAGCTTCCCGTTCTC

AP-2 = adipocyte-binding protein-2

QRT-PCR was performed using Applied Biosystems® 7500 Fast engine and Sensi FAST ™ SYBR® Lo-ROX One-Step Kit material, which allowed detection of the PCR products by quantifying the increase in SYBR green fluorescence due to the binding of SYBR green to double -stranded DNA. Each of PCR sample had a volume of 20 µL, 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, 30s at 60°C and 30 s at 72°C. The housekeeping gene GAPDH was used as endogenous control for mRNA normalization.

All experiments were conducted in duplicate. The analysis of melting curve was performed to confirm the qRT-PCR product. The expression level was calculated based on the PCR cycle number (Ct) and it was identified using the Livak method.

Statistical Analysis

SPSS 21 for Windows was used for statistical analysis. The test of normality was carried out using the Shapiro Wilk. Homogeneity test was performed using the Lavene test. The significance level used was 0.05. Data with normally distributed are presented as Means ± SEM and non-normally distributed data as medians (range). One-way analysis of variance (ANOVA) with a post hoc Tukey test was used to compare means.

Results and Discussion

The electromagnetic field (EMF) has been used successfully in numerous physiotherapy to treat bone disorders and osteoarthritis, as well as to regenerate cartilage or reduce pain [15].

Numerous biochemical and biophysical stimuli including fluid shear stress, hydrostatic pressure, substrate strains, trophic factors, and the electromagnetic field (EMF) effect adult stem cells in their vivo micro-environment [16]. Mesenchymal stem cells (MSCs) are multi-potent cells that able to differentiate into bone, cartilage and adipose lineage. Several studies have reported an association between fat and bone mass due to MSC differentiation in the adipocytic or osteoblastic lineage. It is important to balance the osteogenic and adipogenic differentiation of MSCs in order to prevent obesity [17]. The differentiation of MSCs into adipogenic or osteoblastic cells controlled by several transcriptional factors and intracellular signaling pathways.

Aeberly reported that AP-2 concentrations in circulation are significantly high in overweight and obese. Elevated concentration of AP-2 were also significantly correlated with most of the metabolic syndrome, including insulin resistance, high blood pressure, and inflammation [18].

To examine the effect of PEMF on the adipogenic differentiation of MSCs, adipocyte specific mRNA expression AP-2 was measured by qRT-PCR method. The data obtained on day 0 (calibrator), 2, 4, 7, and 14 from each group. PEMF exposure for 14 days resulted in a decrease in AP-2 in the exposed group.

The AP-2 mRNA expression was shown in the form of relative expression that compared to the housekeeping gene, GAPDH. A comparison of AP-2 expression based on the treatment group can be seen in Figure 2. Figure 2 showed that AP2 expression in the PEMF group has a lower level compared to the outside control group and the incubator control group, both on days 2, 4, 7 and 14.

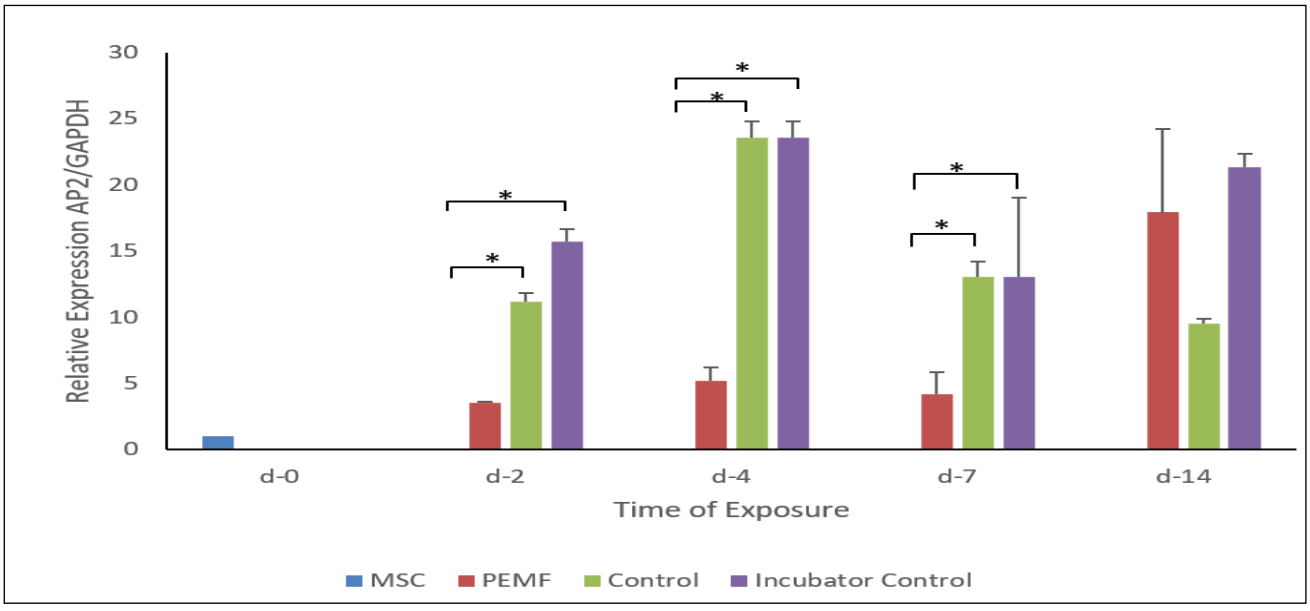

Figure 2: Expression level of AP-2 indices stimulated by pulsed electromagnetic filed (PEMF). There were significant differences in the relative expression of AP-2 to GAPDH between the PEMF exposure group and the external control group and the control incubator on days 2, 4 and 7. On day 14 there was no significant difference in AP-2 expression between groups. The data are expressed as the mean ± SE. *P < 0.05

The results showed that exposure to PEMF had a potency to inhibit adipogenesis. The inhibition of adipogenesis showed by decreased expression level of AP-2. These finding are generally consistent with finding of previous studies. The inhibition of adipogenesis with PEMF exposure is supported by research conducted by Du et al., 2015. In this study, the results showed that PEMF exposure with a frequency of 7.5 Hz, magnetic field magnitude 0.4 T, and exposure time 2 hours/day for 15 days of adipogenesis can inhibit the expression of adipogenic genes.

In this study, the gene expression was only carried out on the last day of exposure, so it could not be seen how the pattern of gene expression occurred during the 15 days of the exposure time. Lu et al also reported that the 20-Hz/2 mT PEMFs also suppress the adipokine expression, AP-2, and other transcription factors of adipogenesis in bone marrow mesenchymal stem cells (BMSCs). Adipogenesis is highly regulated by numerous gene expression. The peroxisome proliferator-activated receptor (PPAR) and the CCAAT/enhancer binding proteins (C/EBPs) are considered to be the primary early regulators of adipogenesis in mammalian cells. While the formation of mature adipocytes controlled by adiponectin, fatty acid synthase (FAS) and fatty acid binding protein 4 (FABP4) [19].

PPARγ is a main regulator of the adipogenesis differentiation and plays a key role in regulating the storage of lipid. PPAR-γ increases uptake of fatty acid through the transcriptional regulation of fatty acid-binding protein 4 (Fabp4)/adipocyte protein 2 (AP-2). [20]. Sari et al has reported that PEMF exposure decreased the relative expression of PPAR-γ. The decrease in PPAR-γ expression is consistent with the decrease in AP-2 obtained in this study.

There are some limitations to our study. Many studies show that in the MSCs differentiation of adipogenic and osteogenic, there exists a reciprocal relationship. Our study revealed that PEMF acted on MSCs to inhibit differentiation to adipocytes but we didn't evaluate the transcription factor of osteoblastic differentiation. In the previous publication, we have reported that PEMF exposure in improve bone fracture healing process.

The higher expression level of RANK, RANKL and OPG gene have indicate that PEMF induce the osteoblastic differentiation.

Conclusion

PEMF exposure has an inhibitory effect on adipogenesis of MSCs. However, it is not clear if this effect is direct or indirect PEMF exposure decreased the relative expression of AP2 mRNA.

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