

The Effects of 900 MHz Electromagnetic Radiation CD4+ T cells, IL-2, IL-10, and IL-17a in Peripheral Blood Mononuclear Cells cultures

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ABSTRACT

The immune system is able to react to discrete environmental stimuli like electromagnetic field (EMF). To investigate the effects of radiofrequency EMF (RF-EMF) on T helper cells, our study aimed to determine that exposure to 900 MHz RF-EMF affects Th1, Th2, and Th17a cells in peripheral blood mononuclear cells (PBMC) cultures. The PBMC cultures from healthy subjects were exposed to 900 MHz RF-EMF (38 V/m, SAR 1.194 W/kg) with durations of 15, 30, 45, and 60 minutes and distances of 0 and 5 cm or without irradiation, respectively. After 48 hours culture, the effects of RF-EMF exposure on the number of CD4+ T cells, IL-2, IL-10, and IL-17a were evaluated using flow cytometer. There was a decreased CD4+ T cells at 15 & 30 minutes exposure and, which then became almost the same at 60 minutes exposure, to those without. IL-2 and IL-10 increased significantly, but decreased with the duration of exposure, approximates the control. Interleukin-17a was decreased significantly compared to controls. The effect of 900 MHz RF-EMF exposure on the percentage of CD4+ T cells, IL-2, and IL-10 was temporary. The RF-EMF causes reduction of IL-17a.

Keywords

900 MHz, RF-EMF, T helpers

Introduction

The frequent use of non-ionizing radiation exposure RF-EMF in the last decades has increased the interest in the evaluation of its effects on health. The interest is mostly motivated by the broaden use of mobile phones for telecommunication worldwide. Each country in the world may implement different frequencies for mobile networks. The 900, 1800 MHz bands in the range of RF waves are used in the Global System for Mobile Communications (GSM) networks. Low-power RF, indicated by a few studies, may trigger a biological effect in target tissues or cells. Nevertheless, whether or not these biological consequences culminate to adverse health is still not clear [1].

The immune system is a crucial part of the network of the homeostatic neuro-endocrine-immune. It is devised to maintain its optimal activity by identifying its 'self' and 'non-self' components, to deliver regenerative support for injured tissue, and to defend the body from infections. The immune system is the quintessential biological system to investigate the concomitants of the EMF because the immune system can respond to distinct albeit remarkably subtle environmental stimuli [2].

The growing interest of biological impact of EMF has been observed for many years. However, the non-equivocal results of specific effects of EMF were not obtained until now. The results of non-thermal effects of EMF do not fulfil the entire characteristic of the specific effects. In the present study examined the effects of electromagnetic waves on Th cells. Our study aims to determine that exposure to 900 MHz RF EMF affects Th1, Th2, and Th17a cells in PBMC cultures.

Material and Method

Study Design

Our study was experimental research conducted in a laboratory with a randomized post-test-only controlled group for PBMC one. Peripheral blood mononuclear cells cultures were mixed in vitro with RPMI 1:10 culture media and divided into 13 treatment groups. PBMCs were exposed to 900 MHz EMF-RF with various durations and distances. PBMCs were cultured for 48 hours 37°C. Next, PBMCs were examined using flow cytometer for CD4+ T cells expressed IL-2, IL-10, and IL-17a.

Subjects and Samples

The sample was obtained consecutively on the healthy control of healthy volunteers of students and employees in the Medicine Faculty of Universitas Brawijaya. The total sample consisted of 36 participants.

Venous blood samples were collected from 4 healthy volunteers (age 20 to 35 years). The samples (12 mL) were taken from the antecubital vein, using a 21G needle, into vacuum tubes containing heparin (Becton Dickinson Biosciences) as the anticoagulant for PBMC isolation. The material was isolated under prior patient's consent and the data were analyzed anonymously. The research was approved by the local Ethics Committee of Medical Faculty, Universitas Brawijaya (letter number: No. 146/EC/KEPK/05/2018). All research was performed in accordance with the relevant guidelines and regulations.

PBMCs isolation

Peripheral blood mononuclear cells were purified from heparinized peripheral venous blood samples using Ficoll-Hypaque gradients, $d=1.077$ g/mL (Sigma-Aldrich Co. LLC.). PBMCs were washed with phosphate-buffered saline (PBS) and re-suspended in 300 μ L RPMI 1640 media supplemented with 10% fetal bovine serum (FBS) and 1% glutamine/penicillin/streptomycin. PBMCs 1×10^6 cells/ml were plated in 24 well and exposed to 900 MHz EMF with four different durations of exposure (15, 30, 45, and 60 minutes) and two different distances (0 cm and 5 cm) at 37°C. After 48 hours of culture, PBMCs were harvested, in which 10 μ g/ml Brefeldin A (Golgi plug) (BD Pharmingen, San Diego, CA, USA) was given 5-6 hours prior. Golgi plug was given so that cytokines are not overly secreted into the supernatant.

Cells were taken with a micropipette and put into a 1.5 ml eppendorf tube and centrifuged 2500 rpm for 3 minutes. The cells were washed 2-3 times with 1 ml PBS. Cells were analyzed using flow cytometry.

Sample preparation of CD4, Th17, IL-2, and IL-10 for Flow cytometry

Cell surface staining was performed using mouse anti-human monoclonal antibodies (mAbs) anti-CD4 fluorescein isothiocyanate (FITC) (BD Pharmingen). The staining was performed by adding 20 μ L of each mAb to 100 μ L of separated PBMCs in the same tube, followed by 30 minutes incubation in the dark at room temperature. The tubes were washed twice with FACs buffer.

Next, fixative and permeabilizing solutions were added, followed by intracellular staining using 20 μ L of Fastimmune Anti-Human IL-2 phycoerythrin conjugate (PE) (BD Pharmingen), Mouse Anti-Human IL-10 PE (BD Pharmingen), Mouse Anti-Human IL-17a PE (BD Pharmingen). The cells were incubated for 30 minutes in the dark at room temperature and washed twice with FACs buffer.

Finally, 0.5 mL of phosphate-buffered saline was added to the washed cells prior to the measurement of CD4⁺ cells, IL-2, IL-10, and IL-17a. Sample analysis was performed with a FACS Calibur flow cytometer (Becton Dickinson, San Jose, California, USA). FACs-acquisition and analysis were performed with FACs Cell Quest Pro software (BD Biosciences). Samples were initially examined for the percentage of CD4⁺ T cells.

RF-EMF Signal generator

VSG25A Vector Signal Generator is a 100 MHz to 2.5 GHz vector signal generator, which displays a wave generator that can be set at several frequencies from 54 kHz to 180 MHz. The RF-EMF generator was obtained from The Signal Hound® company (Figure 1). The whole blood and PBMC cultures placed in the chamber were exposed to the 900 MHz EMF with specific absorption rate (1,194 w/kg) (SAR). In this study, radiofrequency radiation was used at four different duration (15, 30, 45, and 60 minutes) and two different distances (0 cm and 5 cm). Control PBMC cultures were not exposed to the EMF RF. The Generator was placed in a chamber made of aluminum layered with lead (32 cm \times 32 cm) in 37°C, with procedures according to Sulalah et al. (2019) (see Figure 1) [3].



Figure 1. VSG25A Vector Signal Generator, The Signal Hound® company

Treatment Groups

Peripheral blood mononuclear cells in this study was divided into several treatment groups: 1. PBMCs without exposure, 2. PBMCs with exposure to 900 MHz RF-EMF, a distance of 0 cm for 15 minutes, 3. PBMCs with exposure to 900 MHz RF-EMF, a distance of 0 cm for 30 minutes, 4. PBMCs with 900 MHz RF-EMF exposure, a distance of 0 cm for 45 minutes, 5. PBMCs with 900 MHz RF-EMF exposure, a distance of 0 cm for 60 minutes, 6. PBMCs with 900 MHz RF-EMF exposure, a distance of 5 cm for 15 minutes, 7. PBMCs with 900 MHz RF-EMF exposure, a distance of 5 cm for 30 minutes, 8. PBMCs with 900 MHz RF-EMF exposure, a distance of 5 cm for 45 minutes, and 9. PBMCs with 900 MHz RF-EMF exposure, a distance of 5 cm for 60 minutes.

SAR measurement

Determination of SAR received by cells based on the amount of electrical energy exposed to cells, tissue/organ conductivity, and density of tissues/organs. SAR measurements are carried out

by measuring the large electric field in the box after being given a 900 MHz frequency through the transmitter. The measurement of the electric field is carried out using the Mustool MT525 Electromagnetic Radiation Detector, which is placed just below the antenna with a distance of 5 cm by the position of the sample. The transmitter is turned on with a frequency of 900 MHz for ± 3 minutes then the detector is put into the lead-lined box and turned on. After the numbers that appear on the detector were stable, data that often appear as often as 5 times were recorded and then averaged. After obtaining the average value of the electric field, we then calculate it with the equation:

$$SAR = \frac{\sigma E^2}{\rho} \left(\frac{w}{kg} \right)$$

Where σ is the effective conductivity of a tissue/organ, ρ is the mass of the tissue/organ type (kg/m^3), E is the magnitude of the electromagnetic field (V/m). Data on blood conductivity and mass can be seen in table 1 and table 2.

Table 1. Blood conductivity [4].

Frequency	σ (S/m)
900 MHz	1,53
1800 MHz	2,0453
2450 MHz	2,54

Table 2. Mass density of organs

Tissue/organ	Mass density, ρ (kg/m^3)
Blood	1850
Muscle	3490
Bone	1042

Where in determining the amount of electrical energy using an electromagnetic wave detector mounted on a lead-lined box.

At 900 MHz, the measured E value is 38 V/m. SAR values for the 900 MHz frequency (figure 2).

$$SAR = \frac{\sigma E^2}{\rho} + \frac{1,53 \times (38)^2}{1850} = 1,194 W/kg$$

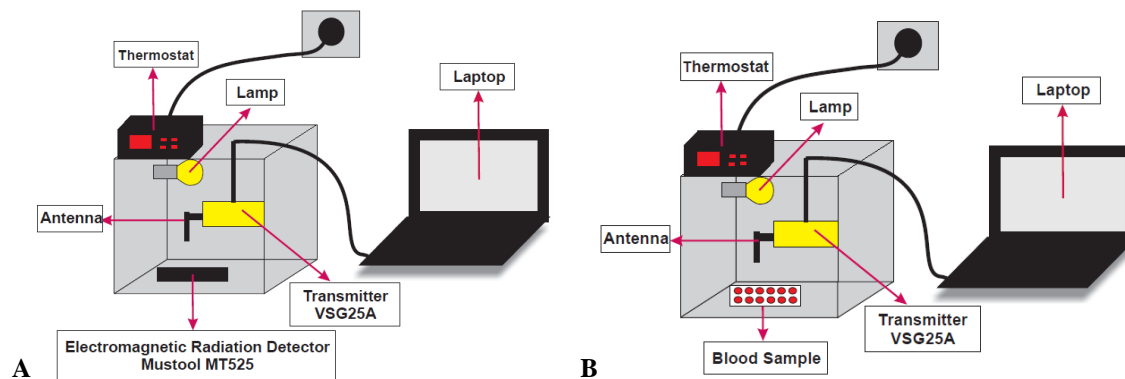


Figure 2. Schematic diagram of box/chamber for cell culture exposure to 900 MHz RF-EMF: A. SAR measurement, B. Exposure to PBMC

Statistical analysis

Results obtained from the EMF-exposed blood cells and cultures were compared with the control one. Data distribution was assessed using the Shapiro-Wilk test. Comparisons between two groups were tested for statistical significance using One Way Anova and Tukey test or the nonparametric Kruskal Wallis and Mann Whitney U-test as appropriate. All statistical analyses were performed using SPSS statistical software (version 20, SPSS Inc., Chicago, IL, USA).

Results

Table 3 showed the effect of 900 MHz EMF RF on the number of CD4+ T cells. The data showed that CD4+ T cells decreased during exposure for 15 and 30 minutes, compared to controls without exposure then increased with increasing exposure time (45 and 60 minutes) at a distance of 0 cm, approximates the control value without exposure. The effect of 900 MHz EMF RF exposure a distance of 0 cm showed an increase in IL-2 compared to controls, so did the distance of 5 cm, but IL-2 decreased with the increase of the duration of exposure, approximates the control value without exposure. The number of CD4+ T cells expressing IL-10 significantly increased compared to controls, but at an exposure at 5 cm for 60 minutes, it decreased so that the number almost the same as the control. With an exposure at a distance of 0 cm, IL-17a decreased compared to controls, but at 45 and 60 minutes of exposure, the value increased, approximates the control value. However, an exposure at 5 cm, IL-17a increased compared to controls, except for a duration of exposure of 60 minutes, IL-17a decreased.

Table 3. The comparison of the number of CD4+ T cells, IL-2, IL-10, and IL-17a-expressing CD4+ T cells between the two groups based on variable exposure durations

900 MHz	Duration (in minute)	CD4+ (mean±SD)	IL-2 (mean±SD)	IL-10 (mean±SD)	IL-17a (mean±SD)
0 cm	60	27.69±0.94 ^a	29.38±0.32 ^a	33.88±0.47 ^a	30.40±1.01 ^a
	45	29.25±0.73 ^b	34.05±0.40 ^b	30.05±0.55 ^b	32.51±1.74 ^b
	30	16.94±0.56 ^c	20.56±1.31 ^c	18.74±0.99 ^c	18.93±0.29 ^c
	15	18.54±0.81 ^d	28.02±1.11 ^{ad}	27.68±0.61 ^d	29.45±0.29 ^a
	Control	26.86±3.14 ^b	17.73±8.87 ^{ad}	19.64±12.59 ^e	31.58±12.48 ^{bd}
	p-value	<0.001*	0.002*	0.001*	0.002*
5 cm	60	35.16±4.19 ^a	18.29±0.30 ^a	19.30±0.76 ^a	15.85±0.27 ^a
	45	31.28±4.07 ^{ab}	37.55±0.70 ^b	32.05±0.44 ^b	31.78±0.85 ^b
	30	19.76±3.47 ^{cd}	22.18±1.04 ^c	30.69±0.60 ^c	32.11±0.57 ^{bc}
	15	23.75±5.69 ^{de}	24.47±1.23 ^d	45.33±0.61 ^d	47.13±0.71 ^d
	Control	26.86±3.14 ^c	17.73±8.87 ^e	19.64±12.59 ^e	31.58±12.48 ^e
	p-value	<0.001*	<0.001‡	0.001*	0.002*

*Kruskal-Wallis test followed by Mann-Whitney test, ‡ANOVA test followed by Tukey test

^{a-c}Mean values followed by different superscript letters within each column indicate that they differ significantly (p<0.05)

Discussion

The normal immune system which functions normally is important for its defensive, pro-regenerative and tolerogenic roles. Several experiments showed EMF has varying biological effects. Thus, it is suspected that EMF may contribute indirectly to the surfacing and progressing of various malignancies [2].

The data showed that CD4+ T cells decreased during RF-EMF exposure for 15 and 30 minutes, compared to controls without exposure then increased with increasing exposure time (45 and 60 minutes). At a distance of 0 cm, approximates the control value without exposure. The mechanism of the decrease might be through apoptosis. RF-EMF more often causes a decrease in cell number rather than stimulates cell proliferation. At a distance of 0 cm IL-2 increased compared to controls, so did the distance of 5 cm, but decreased with the duration of exposure, approximates the control value without exposure. This temporary decrease and then back to the normal was likely due to the normal homeostasis to the external stimuli.

Tuschl et al. reported GSM signal has no statistically significant effects on PBMCs. The study evaluated some immune parameters including IL-2 intracellular production. They found that the stimulation is not associated with EMF's adverse effects on the human immune system [5]. What is more, Waldmann et al. proved that RF EMF had no genotoxic effects on human peripheral lymphocytes [6].

Experimental study by Szymanski et al. utilized mononuclear cells (PBMC) isolated from the blood of healthy donors as samples. The samples were exposed twice, 15 min each, to 900 MHz pulse-modulated radiofrequency radiation (20 V/m, SAR 0.024 W/kg). Their data showed that EMF RF potentially have immunotropic effects. After the second day of exposure, the saturation of IL-2 receptor rose significantly. It suggested that PBMC, specifically human lymphocytes, are sensitive to immune-modulatory effect's of 900 MHz EMF [2].

From our study on IL-10 CD4+, the number of CD4+ T cells expressing IL-10 significantly increased compared to controls, but at a distance of 5 cm exposure with a time of 60 minutes, it decreased almost the same as the control. The increase in IL-10 in our study was transient, because in the sample group with longer exposure the value was almost the same as in the control. In our study, the expression of IL-17a as a representation of Th17 cells showed a significant decrease in PBMCs exposed to 900 MHz RF-EMF, with a distance of 0 cm, but at 45 and 60 minutes of exposure, the value increased, approximates the controls one, contrarily with 5 cm: IL-17a increased compared to controls, except for those 60 minutes, decreased. Whether this is beneficial or harmful still needs further research, and how about the effects of chronic exposure, still needs further study.

Dabrowski et al. used 1300 MHz pulse-modulated microwaves at 330 pps as an exposure to samples of microculture system of mononuclear cells isolated from healthy donors' peripheral blood. The samples were stimulated in an anechoic chamber at mean power density of S 10 W/m² (1 mW/cm²) and the SAR 0.18 W/kg. Albeit the irradiation decreased the spontaneous incorporation of 3H-thymidine, there were no change in the proliferative response of lymphocytes to PHA and to Con A. Moreover, there were no changes happen in T-cell suppressive activity (SAT index) and the saturation of IL- 2 receptors. Nevertheless, the lymphocyte IL-10 production increased significantly in the culture supernatants [7].

Tests conducted in laboratory produce different results from the epidemiological studies. Because, humans are subjected to a varying intensity of a variety of different frequencies and signals. But, only one RF-EMF field frequency is used in laboratory. Moreover, RF-EMF influences people permanently but not in laboratory. Therefore, it is suspected that exposure time carries a significant impact [2]. Frequency, amplitude, SAR value, exposure conditions, and duration of exposure influence the effects of EMF exposure, together with the characteristics of the exposure-subjected cell types.

It is suspected that exposure to non-thermal RF-EMF does not cause any effect at very low levels, below the tissue heating threshold. In 2013, the International Agency for Research on Cancer, because there is “limited evidence” for the carcinogenicity, classified RF-EMF as a possible carcinogen for humans. More recently, the relationship between RF-EMF exposure and disease development have not confirmed by any epidemiological study. In addition, no experimental findings was found to provide a mechanistic explanation for such an outcome. Therefore, there has not been any established biological or biophysical mechanism of action existed so far [8].

Federal Communications Commission (FCC) and other regulatory agencies, claimed that only factors with thermal mechanism affecting health. Therefore, exposure limits are set based only on thermal effects. Nevertheless, many studies, in vivo and in vitro, proved that significant harmful biological effects occur due to the effects of non-thermal RF exposure [9-10]. In our study, the temperature was maintained within the physiological range, 36.5-37°C, to eliminate thermal effects disrupting the cellular immune system. Thus, changes occurred in our study must be due to non-thermal mechanisms.

A considerable proportion of studies have investigated the “non-thermal” effects of RF in the cells and tissues, showing that this effect is mediated by generation of Reactive Oxygen Species (ROS) like hydroxyl radical °OH, hydrogen peroxide H₂O₂ [1]. But, the results of study by Kazemi et al., 2015 did not show any difference in ROS production after exposure to the 900 MHz RF EMF with power 2W (a wavelength of about 33.4 cm) in the lymphocyte rich population samples used.

Conclusion

According to the results obtained in this study, it is concluded that 900 MHz electromagnetic field exposure on the percentage of CD4+ T cells, IL-2, and IL-10 was temporary. Sixty minutes of PBMC exposure to RF-EMF with a distance of 5 cm causes a significant reduction of IL-17a. Exposure to electromagnetic fields at low levels (non-thermal) can impair immune function if it occurs continuously for a certain period of time. The question of whether non-thermal RF exposure can alter cellular function in vitro still remains unresolved. Scientific evidence shows that the current safety standards for low-frequency EMF are inadequate because most of the standards were made by only considering thermal effects. Therefore, the authorities need to re-evaluate safety standards. Should be more research done to understand the precise effects of these waves in real terms. However, further studies are needed to define the molecular mechanism of this effect.

Author Contributions:

1. Maimun Zulhaidah Arthamin: conceptualization, methodology, formal analysis, investigation, data curation, original draft preparation, review, and editing.
2. Anis Sulalah: methodology, investigation, and review
3. Resvina: methodology, investigation, and review
4. ChomsinSulistya Widodo: methodology, validation, and review
5. Agustina Tri Endharti: methodology, validation, review and editing
6. Edi Widjajanto: conceptualization, validation, and review

Acknowledgement

The authors thank Mrs HeniEndrawati, SSi for her excellent technical work in PBMC culture, Mr WahyudhaNgatiril Lady, SSi for his excellent technical work in flow cytometry, Mr. Rudy Yuwono, ST., M.Sc for additional knowledge about SAR and the properties of electromagnetic waves, OsaFatiana Rea and Farisa Nur Afifa for help with chamber fittings and SAR measurements.

Limitation and future direction

Limitation of this study:we did not examine the mechanisms of EMF exposure in CD4 + T cells, and did not study the effects of chronic exposure. Future direction: Further research is needed regarding the role of intracellular calcium ion and free radicals as a non-thermal EMF mechanism.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper, and that this study was conducted in the absence of any commercial or financial relationship that could be construed as a potential conflict of interest.

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