Bratisl Lek Listy 2009; 110 (9)
526 – 529

EXPERIMENTAL STUDY

The effects of electromagnetic fields on peripheral blood mononuclear cells in vitro

Ayten Atasoy[1], Yusuf Sevim[1], Ismail Kaya[1], Mustafa Yilmaz[2], Ahmet Durmus[2], Mehmet Sonmez[2], Omay SB[2], Feyyaz Ozdemir[3], Ercüment Ovali[2]

Department of Electrical and Electronics Engineering, Faculty of Engineering, Karadeniz Technical University, Trabzon, Turkey. **feyyazozdemir@yahoo.com**

Abstract: *Objective:* A discussion about the adverse effects of electromagnetic waves on the biological life has been ongoing since the discovery of electricity in the 19th century.
Materials and methods: The primary objective of this study was to analyze the changes in the cell viability, rates of apoptosis, proliferation indices and the cell surface antigenic structures resulting from 2-, 6- and 24-hour exposure of mononuclear cells isolated from the peripheral blood to 450, 900 and 1784 MHz electromagnetic waves.
Results: Data obtained showed that electromagnetic waves didn't have any effect on the cell viability, rates of apoptosis and proliferation index. While electromagnetic waves didn't affect the HLADR and CD11b expression in the peripheral blood mononuclear cells, they decreased the CD11a expression and increased the CD49d expression.
Conclusion: These data suggest that electromagnetic signals could affect the functional capacity of the peripheral blood mononuclear cells by changing their adhesion ability. Maybe these alterations are the sign of the immune system modulation. More comprehensive studies are needed, involving higher number and more lines of cells *(Tab. 6, Fig. 3, Ref. 11)*. Full Text (Free, PDF) *www.bmj.sk.*
Key words: electromagnetic fields, peripheral blood mononuclear cells, in vitro.

A discussion about the adverse effects of electromagnetic waves on the biological life has been ongoing since the discovery of electricity in the 19th century. While the positive aspect of technologic innovation makes the life easier, it may also involve components that impair the quality of life via its certain negative effects. Electromagnetic waves generated by many natural and human-made sources can travel for long distances and play a very important role in daily life. In particular, the electromagnetic fields in the Radiofrequency (RF) zone are used in communications, radio and television broadcasting, cellular networks and indoor wireless systems. Resulting from the technological innovations, the use of electromagnetic fields gradually increases and thus people are exposed to electromagnetic waves at levels much higher than those present in the nature. Along with the widespread use of technological products in daily life, the biological effects of electromagnetic waves started to be discussed. Particularly, the dramatically increasing number of mobile phones users rise significant concerns due to its potential damage on people exposed by radiofrequency waves.

Since mobile phones are used in positions very close to the human body and require a large number of base station antennas, the public and the scientists have question marks in their mind about the impact of mobile phone networks on health. These question marks trigger a conduct of studies on this subject. Some relevant studies revealed that different dimensions of electromagnetic waves have not shown any DNA damage on different cell lines. For example, in a comprehensive review published, Brusick et al have reported no evidence regarding the direct mutagenic effect of radiofrequency signals on cells (1). The general opinion is that there is no direct evidence of hazardous effects on human health incurred by low-frequency radiofrequency waves, studies at the cellular level, which uses relatively higher frequencies, continue. In an attempt to obtain a clear picture about the effects of the electromagnetic waves on the nature, a case study has been proposed in this paper which is conducted on the human peripheral blood mononuclear cells exposed by an experimentally generated electromagnetic field. The electromagnetic field has been generated using the standard GSM mobile phone specifications and used to stimulate a mobile phone user which is in the proximity of the Fresnel zone of an antenna, as it is used in handset of the GSM radio (2).

[1]Department of Electrical and Electronics Engineering, Faculty of Engineering, Karadeniz Technical University, Trabzon, Turkey, [2]Department of Hematology, Faculty of Medicine, Karadeniz Technical University, Trabzon, Turkey, and [3]Department of Medical Oncology, Faculty of Medicine, Ankara University, Trabzon, Turkey

Address for correspondence: Feyyaz Ozdemir, MD, Dept of Medical Oncology, Faculty of Medicine, Karadeniz Technical University, 61080, Trabzon, Turkey.
Phone: +90.462.3775732, Fax: +90.462.3250518

Acknowledgement: The company, ATI, is recognized here for their contributions to this work.

Material and method

Mononuclear cell isolation from the peripheral blood

In this study, 100 ml of peripheral blood obtained from 5 healthy individuals was anti-coagulated by heparin. Heparinized peripheral blood was spread on Ficoll at a rate of 1:3. The tubes containing Ficoll and peripheral blood samples were centrifuged at room temperature for 30 minutes at 400 G. After centrifuging, plasma clustered at the top, mononuclear cells in the middle, ficoll below, and erythrocytes and granulocytes at the bottom. The remaining sections were discarded and mononuclear cells collected. The collected cells were washed by centrifuging at least two times with serum-free culture medium at a 5-fold volume.

Formation of the electromagnetic field

The electromagnetic field was formed by the placement of the biquad antenna with an aperture angle of 60 and gain of 9–11 dB inside the incubator. The reason for the use of the biquad antenna instead of an ordinary GSM antenna from a handset unit was that the biquad antenna has more uniform electromagnetic waveform around the sample caps, shown in the Figure 1, and an ordinary GSM phone antenna does not have similar electromagnetic field distribution around the experimented samples, as shown in the Figure 2. This does not mean that a user of a mobile GSM phone is perfectly stimulated here, but the experimental model was quite close to represent a biological tissue exposed by a GSM field having a 3 cm distance from the antenna. The Figure 1 shows the experimental biquad antenna where the samples were exposed. The antenna was placed in an incubator (electromag, M420B) to stabilize the temperature at the 37 °C degree. If an antenna from an ordinary mobile phone was used for the electromagnetic exposure, as in the Figure 2, then the magnetic field would not be homogeneous as it was in the Figure 1. This decision was verified by the field measurements using the device (Chauvin-Arnoux, CA.43), which is used for electrical and magnetic field measurements for GSM system. Figure 3 shows the experimental units and the whole set up.

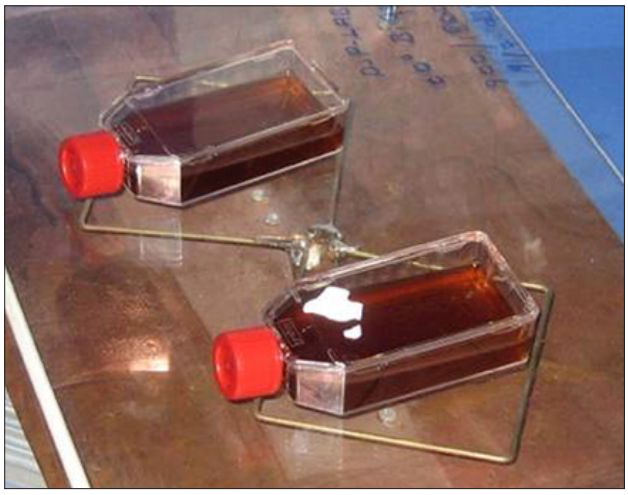

Fig. 1. The biquad antenna and the placement of samples.

Fig. 2. The size comparisons between the antenna of a mobile set and a sample cap.

Fig. 3. The experimental set.

The signal for the GSM standard was generated by a vector signal generator (Agilent, E4438C), which generates the GSM signal using its embedded software and hardware as it is described (ETSI, 1996). The transmit power was 20 dBm for all frequencies as presented in the Table 1, and measured electric fields are described in the Table 1. The field strength was increased due to frequency increase for the same level of power. The measured field strengths for the considered GSM frequency bands are given in the Table 1, where the obtained field's strengths belong to the continuous pattern generation form of GSM std. using a Pseudo

Tab. 1. The values used in experimental studies.

	450.6 MHz	900 MHz, MS, Channel 50	1784 MHz
Transmit Power	20 dBm	20 dBm Power level:Class 11, from ref. [ETSI, 1996]	20 dBm
Measured Electric Field	30 (V/m)	48.3 (V/m)	53 (V/m)

Noise Sequence as the transit data. So, if a framed packet type is chosen then the field's strength for 900 MHz would be reduced by 18.5 V/m (as it was measured), which was 48.3 V/m during the experimental trails using the continuous form of data packet. This means, the framed structure averages the field strength since each of eight frames has been used to send data from the mobile station. Peripheral blood mononuclear cells were kept in 3 cm distance from the antenna inside the incubator (37 °C) and exposed to electromagnetic field at the frequencies of 450.6 MHz, 900 MHz and 1784 MHz as shown in the Figure 1.

The analysis of the electromagnetic field's impact on the cells

Mononuclear cells isolated from the peripheral blood were inoculated in 24-well plastic flasks, with each flask containing 1 million cells, using RPMI 1640+10 % FCS. The cells inoculated on the culture flasks were exposed to 450, 900 and 1784 MHz electromagnetic waves for 2-, 6- and 24-hour periods. On the cells inside the wells exposed to electromagnetic waves at the above wavelength and period of time, the number of cells, rates of viability, proliferation index, rates of apoptosis and HLA--DR, CD11b, CD49d, CD11a antigen expressions were analyzed. Cell counts were performed via an auto-counter. For the determination of the viability, Trypan blue dye exclusion test was used. Proliferation index, rates of apoptosis and HLA-DR, CD11b, CD49d, CD11a antigen expressions were analyzed by the flow cytometry (Beckman-Coulter EPICS XL-MCL (Beckman Coulter, Nyon, Switzerland)).

Results

This study demonstrated that the exposure to electromagnetic waves at 450, 900 and 1784 MHz for 2-, 6- and 24-hour periods had no effect on the number of cells, rates of viability, proliferation index, apoptosis and HLA-DR expression and CD11b expression. The expression of CD11a, an adhesion molecule, was decreased after 6-hour incubation in the 900 and 1784 MHz signal group and after 24-hour incubation in the 1784 MHz signal group (Tabs 2, 3, 6). The expression of another adhesion molecule, CD49d, was increased after 24-hour exposure to 450, 900 and 1784 MHz electromagnetic waves (Tabs 4, 5, 6). In CD49d and CD11a expressions, no significant changes were detected compared to the controls after 6- and 12-hour incubation periods at each of the three wavelengths.

Tab. 2. The changes in the peripheral blood mononuclear cells using 900 MHz electromagnetic waves after a 6-hour incubation period.

Parameter	Control	900 MHz	p
Number of cells/µul	8.2±2.7	8.4±3.9	AD
Rates of viability %	98.6±0.5	98.6±0.5	AD
Rate of apoptosis %	8.4±2.9	14.4±6.4	AD
Proliferative index %	1.26±0.36	1.73±0.52	AD
HLA-DR expression %	58.0±65	17.4±3.7	AD
CD11a expression %	38.6±6.6	27.4±5.3	0.023
CD11b expression %	34.2±7.7	35.5±7.0	AD
CD49d expression %	68.2±3.2	66.5±1.9	AD

Tab. 3. The changes in the peripheral blood mononuclear cells using 1784 MHz electromagnetic waves after a 6-hour incubation period.

Parameter	Control	1784 MHz	p
Number of cells/µl	13.6±5.3	13.1±4.8	AD
Rate of viability %	98.6±0.5	97.6±1	AD
Rate of apoptosis %	9.3±4.5	9.7±5.2	AD
Proliferative index %	1.73±0.31	1.83±0.52	AD
HLA-DR expression %	22.7±6.4	24.6±6.4	AD
CD11a expression %	30.6±9.6	22.3±7.2	0.043
CD11b expression %	30.8±6.1	31.3±4.6	AD
CD49d expression %	76.9±5.4	75.4±5.8	AD

Tab. 4. The changes in the peripheral blood mononuclear cells using 450 MHz electromagnetic waves after a 24-hour incubation period.

Parameter	Control	450 MHz	p
Number of cells/µl	4.83±3.8	3.83±1.9	AD
Rate of viability %	97.67±1.0	96.3±1.0	AD
Rate of apoptosis %	21.73±6.2	27.5±7.5	AD
Proliferative index %	0.96±0.28	0.96±0.28	AD
HLA-DR expression %	12.1±2.4	14.1±2.9	AD
CD11a expression %	50.2±9.4	46.7±10.6	AD
CD11b expression %	34.2±8.8	35.5±10.6	AD
CD49d expression %	63.3±2.2	67.7±2.4	0.023

Tab. 5. The changes in the peripheral blood mononuclear cells using 900 MHz electromagnetic waves after a 24-hour incubation period.

Parameter	Control	900 MHz	p
Number of cells/µl	10.8±2.3	9.2±4.7	AD
Rate of viability %	94.0±6.1	98.3±0.5	AD
Rate of apoptosis %	26.6+ 5.9	24.2±7.0	AD
Proliferative index %	1.3±0.1	1.1±0.3	AD
HLA-DR expression %	17.0±4.9	19.2±5.6	AD
CD11a expression %	52.1±6.5	52.1±8.2	AD
CD11b expression %	33.5±7.8	36.8±5.5	AD
CD49d expression %	64.3±5.1	70.9±4.8	0.038

Discussion

The primary objective of this study was to analyze the changes in the cell viability, rates of apoptosis, proliferation indices and cell surface antigenic structures resulting from 2-, 6- and 24-hour exposure of mononuclear cells isolated from the peripheral blood to 450, 900 and 1784 MHz electromagnetic waves. Data obtained showed that electromagnetic waves didn't have any effect on the cell viability, rates of apoptosis and the proliferation index. While electromagnetic waves didn't affect HLADR and CD11b expression in the peripheral blood mononuclear cells, they decreased the CD11a expression and increased the CD49d expression.

Speculations that electromagnetic waves can be carcinogenic increased the number of relevant epidemiological and in vitro

Tab. 6. The changes in the peripheral blood mononuclear cells using 1784 MHz electromagnetic waves after a 24-hour incubation period.

Parameter	Control	1784 MHz	p
Number of cells/μl	10.7±3.1	11.2±3.6	AD
Rate of viability %	97.3±0.5	98.0±0.8	AD
Rate of apoptosis %	21.6±6.6	19.6±9.0	AD
Proliferative index %	2.2±0.4	2.1±0.3	AD
HLA-DR expression %	25.1±7.8	29.7±10.9	AD
CD11a expression %	74.4±10.2	49.2±1.8	0.004
CD11b expression %	32.5±8.2	32.6±5.2	AD
CD49d expression %	80.1±3.0	83.7±1.3	0.023

studies. Some epidemiological trials have published data stating that the exposure to high-frequency electromagnetic fields may be associated with lymphatic and hematopoetic cancer. A survey conducted in people living around the Vatican radio station reported more childhood leukemia cases than expected (3). Similar data were also obtained from another study performed by Hocking et al in Australia (4). Hocking et al reported a higher leukemia incidence among adults and children living 2 km around Television transmitter stations. However, in these studies, it's stated that a definite correlation can not be established due to the scarcity of leukemia cases and due to the fact that no measurements were performed in leukemia patients on exposure to radiofrequency waves. A study by Morgan et al conducted on 195 775 subjects working in units related to wireless device manufacturing, design and tests detected that mortality associated with brain cancer, leukemia and lymphoma is not higher in this population compared to the normal population (5). In a study performed in Denmark, the analysis of 450 085 mobile phone users revealed no increase in the brain cancer incidence (6).

The fact that no significant evidences were detected in the above epidemiological trials supporting the suspicions that exposure to electromagnetic waves could result in cancer is in line with the in vitro studies. The effects of electromagnetic fields on different cell lines were studied in the last 30 years and no evidence on their direct or indirect DNA damage were detected. Maes (7) and Vijayalaxmi (8) exposed peripheral blood cells to 935 and 2450 MHz electromagnetic field and reported no DNA damage in cells after 2-hour periods. Malyapa (9, 10) studied the effects of 2450 MHz electromagnetic signals on human gliablastoma cells and Mouse fibroblast cell lines and detected no DNA damage in cells, including the 24-hour period. In a study similar to our, Tice et al demonstrated that 837 and 1909.8 MHz radiofrequency waves didn't result in a significant DNA damage in leukocytes as a result of 3- and 24-hour exposures (11). In line with the literature data, our study revealed that 450, 900 MHz and 1784 MHz electromagnetic signals didn't have any effect on the rates of viability, apoptosis and proliferation (markers of DNA damage); however they changed the expression of certain adhesion molecules such as CD49d and CD11a in these cells. These data suggest that electromagnetic signals could affect the functional capacity of the peripheral blood mononuclear cells by changing their adhesion ability. Maybe these alterations are a sign of the immune system modulation. More comprehensive studies are needed, involving higher number and more lines of cells.

The important feature of this study is that all samples were exposed to the electromagnetic field generated by the GSM standards and the handset of the GSM phones was comprehensively experimented as in daily life.

References

1. Brusick D, Albertini R, McRee D et al. Genotoxicity of radiofrequency radiation. DNA/Genetox Expert Panel. Environ Mol Mutagen 1998; 32 (1): 1—16.

2. Nielsen JQ, Pedersen GF. In-network performance of handheld mobile terminal. IEEE Transactions on Vehicular Technology 2006; 55 (3): 903—916.

3. Michelozzi P, Capon A, Kirchmayer U et al. Adult and childhood leukemia near a high-power radio station in Rome, Italy. Am J Epidemiol 2002; 155 (12): 1096—1103.

4. Hocking B, Gordon IR, Grain HL, Hatfield GE. Cancer incidence and mortality and proximity to TV towers. Med J Aust 1996; 165 (11—12): 601—605.

5. Morgan RW, Kelsh MA, Zhao K, Exuzides KA, Heringer S, Negrete W. Radiofrequency exposure and mortality from cancer of the brain and lymphatic/hematopoietic systems. Epidemiology 2000; 11 (2): 118—127.

6. Johansen C, Boice J Jr, McLaughlin J, Olsen J. Cellular telephones and cancer — a nationwide cohort study in Denmark. J Natl Cancer Inst 2001; 93 (3): 203—207.

7. Maes A, Collier M, Van Gorp U, Vandoninck S, Verschaeve L. Cytogenetic effects of 935.2-MHz (GSM) microwaves alone and in combination with mitomycin C. Mutat Res 1997; 393 (1—2): 151—156.

8. Vijayalaxmi, Leal BZ, Szilagyi M, Prihoda TJ, Meltz ML. Primary DNA damage in human blood lymphocytes exposed in vitro to 2450 MHz radiofrequency radiation. Radiat Res 2000; 153 (4): 479—486.

9. Malyapa RS, Ahern EW, Straube WL, Moros EG, Pickard WF, Roti Roti JL. Measurement of DNA damage after exposure to electromagnetic radiation in the cellular phone communication frequency band (835.62 and 847.74 MHz). Radiat Res 1997; 148 (6): 618—627.

10. Malyapa RS, Ahern EW, Straube WL, Moros EG, Pickard WF, Roti Roti JL. Measurement of DNA damage after exposure to 2450 MHz electromagnetic radiation. Radiat Res 1997; 148 (6): 608—617.

11. Tice RR, Hook GG, Donner M, McRee DI, Guy AW. Genotoxicity of radiofrequency signals. I. Investigation of DNA damage and micronuclei induction in cultured human blood cells. Bioelectromagnetics 2002; 23 (2): 113—126.

Received February 9, 2009.
Accepted May 28, 2009.