

CELLULAR RESPONSE IN EXPERIMENTAL EXPOSURE TO ELECTROMAGNETIC FIELDS

DANA DABALA¹, DIDI SURCEL², CSABA SZANTO², SIMONA MICLĂUȘ³,
MARIANA BOTOC², SEPTIMIU TOADER², OVIDIU ROTARU²

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Although the physical techniques for measuring of the electromagnetic fields (EMF) are well developed, adequate characterization of the biological effects induced by EMF is subject of discussion yet. The aim of this study was to show the effects of the chronic exposure to EMF on the immune and oxidative response. In vivo experiment was carried out on 80 Wistar rats that were divided in 4 groups as following: Two Control-groups, without exposure, sacrificed at 1 month and respectively at 3 months; Two EMF-exposed groups, sacrificed at 1 month and at 3 months. The rats were exposed to RF EMF that covers a range of the frequencies between 140-160 MHz. The following parameters were assessed: a) 3HTdR incorporation test; b) IL-1 assay; c) TNF-assay; d) Chemiluminescence's assay; e) Lipid peroxides. Our results indicate an association between electromagnetic fields and immune and oxidative response.

1. INTRODUCTION

Although the physical techniques for measuring EMF are well developed adequate characterization of the biological effects induced by EMF is subject of discussion yet. We do not know the effects that would be after a long term of exposure. Many scientific studies have been devoted to assessing what health risks are associated with EMF exposure.

In the last years there is an increasing evidence for the immunologic role and oxidative stress play a major roles in the prolonged EMF exposure. There are possibility that EMF – activated macrophages to become high secretory cells and release several factors, such as: interleukin-1(IL-1), tumor necrosis factor (TNF), prostaglandins (PGs), reactive oxygen species (ROS), lipid peroxides (LP). These metabolites are not only injurious to other cells, but their release has also been

¹ Department of Occupational Medicine, Transport Regional Public Health Center, Cluj-Napoca, Romania

² Department of Occupational Medicine, Institute of Public Health, Cluj-Napoca, Romania

³ Land Forces Academy, Sibiu, Romania, danadabala@yahoo.com

found to be chemotactic for other cells as neutrophils, damaging to connective tissue, or to lymphocytes, becoming clastogenic, mutagenic and tumor promoting (x). These reactions could contribute to the development of neoplasia following prolonged exposure to EMF.

Data from the recent experiments suggest that EMF are associated with the iron-mediated free radical generation, that can cause damage in the biologic molecules such as lipids, proteins and can profoundly affect cellular homeostasis.

The aim of this study was to show the effects of the chronic exposure to EMF on the immune and oxidative response.

2. MATERIALS AND METHODS

In vivo experiment was carried out on 80 Wistar rats that were divided in 4 groups as following (shown in Fig. 1):

1. Control 1 – group, without exposure, sacrificed at 1 month;
2. Control 2 – group, without exposure sacrificed at 3 months;
3. EMF 1 – exposed group, sacrificed at 1 month;
4. EMF 2 – exposed group, sacrificed at 3 months.

The rats were exposed four hours/day to RF EMF that covers a range of the frequencies between 140-160 MHz generated by a Motorola device. The components of EMF field were measured with an EMF 200 Monitor Water&Golterman (the measured Power density (S) was 8 ± 1 W/m²).

The animals were sacrificed at 1 month and respectively at 3 months, and the bronchoalveolar lavage was carried out. Spleen-derived lymphocytes (Ly) were prepared and were co-cultured with alveolar macrophages (AM s).

The following parameters were assessed:

- a) 3HTdR incorporation test;
- b) IL-1 assay;
- c) TNF-assay;
- d) Chemiluminescence's assay;
- e) Lipid peroxides.

a. Mitogen – Induced Lymphocyte Proliferation

Mitogen induced proliferation of spleen-derived lymphocytes (Ly) co-cultured with AMs was determined. Briefly, 1.10^6 spleen cells were cultured with autologous AMs at the Ly-MA ratio of 40/1, in presence of 1μ g phytohemagglutinin – p (PHA) (Difco) in a total volume of 1ml RPMI-1640 medium (Cibco Laboratories) supplemented with 10% FCS (Serva). After 48 h a 0.5 μ Ci of 3 H-Thimidine (3HTdR) (New England Corp, Boston Mass,

6.7 Ci/mmol) was added and the incubation was continued for an additional 24 h. The ³HTdR incorporation was measured with a liquid scintillation counter (Beckman, Fullerton, CA). The results were presented as counts per minute (cpm) values \pm standard error of means.

b. Interleukin-1 assay

Interleukin-1 activity was assayed by measuring the capacity of dialyzed culture fluid to enhance the PHA-induced proliferation of mouse thymus cells in vitro. Thymus from parathymic lymph nodes was aseptically removed from C3H-Hei mice and homogenized. After washing the cells were suspended at $3 \cdot 10^7$ cells/ml in RPMI-1640 medium containing 10% FCS, 2 mM L-glutamine and $5 \cdot 10^{-5}$ M2-mercaptoethanol, in 50 μ l of medium. The cells were incubated for 72 h at 37°C and pulsed for 3 h with ³HTdR (20 Ci/mmol; 1 μ Ci/well). The cells were collected and the uptake of ³HTdR was determined by scintillation counting. The results are presented as mean counts per minute (cpm) values \pm standard error of means.

c. TNF assay

Tumor Necrosis Factor (TNF) activity in culture bronchoalveolar cells was determined by ³HTdR release in actinomycin D-treated L₉₂₉ target cells. Briefly, cells belonging to mouse L₉₂₉ fibroblasts were incubated for 24 h with 1 μ Ci/mol of ³HTdR in RPMI-1640 medium. The adherent cells were detached from the surface of the culture flask by gentle washing (three times) with media and were resuspended in RPMI-1640 medium containing 3 μ g/ml actinomycin D, at $3 \cdot 10^5$ cells. Five hundred microlites of target-cell suspension as added to 500 μ l of macrophage culture supernatant and incubated 24 h at 37 °C. At the end of the incubation period tubes were centrifuged at 400 g for 10 min. and ³HTdR release measured in a Beckman liquid counter. Results were expressed as counts per minute (cpm).

d. Chemiluminiscence assay

Luminol dependent chemiluminiscence (CL) assay evaluated the stimulated (Zymosan) radical oxigen species (ROS) release of AMs. Briefly ,the reaction mixture consisted of 100 μ l freshly prepared luminol (Sigma) solution (stock solution 10 mM luminol in dimethyl sulfoxid was diluted 1:50 in aqua distilated; 100 μ l RPMI-1640 containing 10 mg/ml Zymosan (Sigma), and 100 μ l cell suspension containing $2 \cdot 10^5$ AMs. The CL was measured over the next 30 min. in a

Beckman liquid scintillation counter. Results were expressed as counts per minute (cpm). The results are expressed as means \pm standard error of the means (SEM).

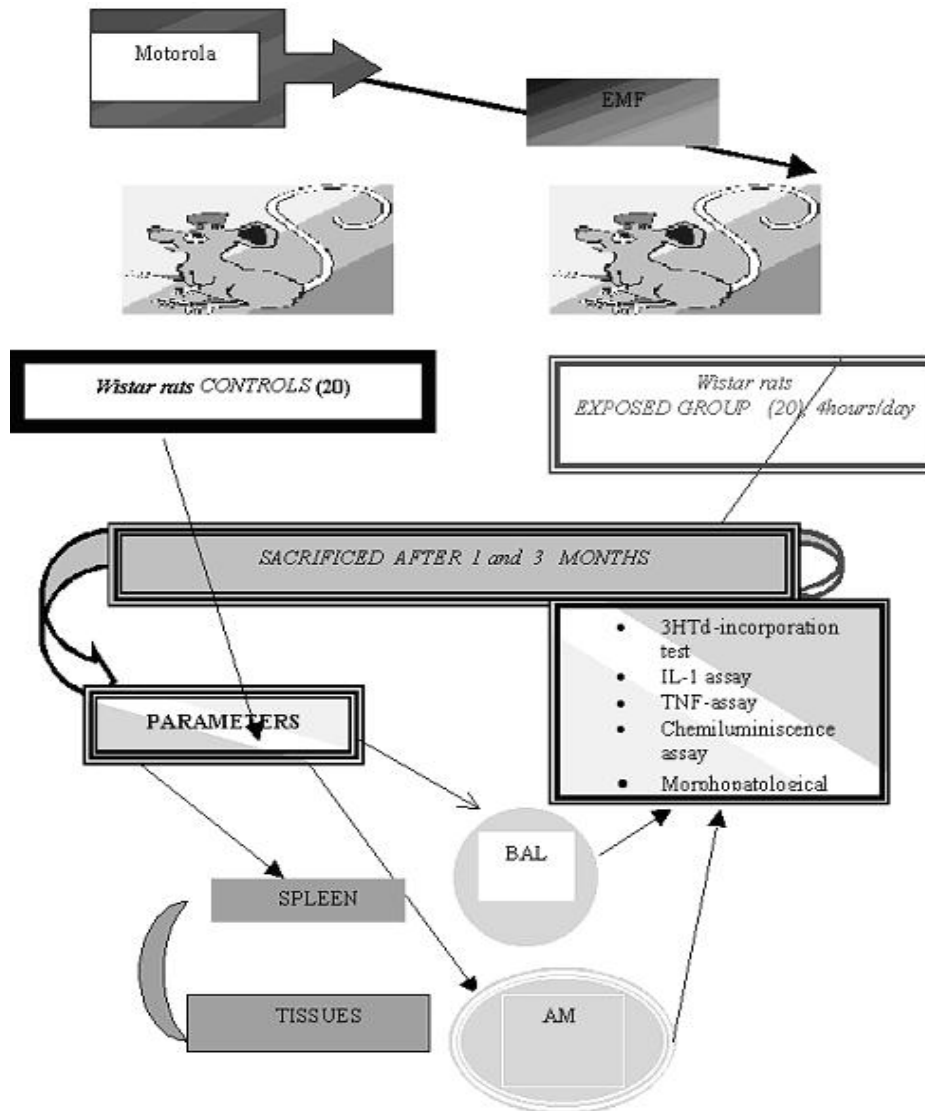


Fig. 1 – Outline of study and methods: the two groups of 20 Wistar rats exposed 4 hours /day to EMF generated from a Motorola device (Freq = 160 MHz; S ech = $8 \pm 1 \text{ Wm}^{-2}$) and the two control groups were sacrificed after 30, respectively 90 days after exposure. The following parameters from spleen, tissues and bronchoalveolar macrophages were assessed: a) 3HTdR incorporation test; b) IL-1 assay; c) TNF-assay; d) chemiluminescence's assay; e) lipid peroxides.

e. Lipid Peroxides assay

Lipid Peroxides assay is thiobarbituric acid TBA colorimetric assay of hydroperoxides and it was performed using a modification of the technique described by Asakawa and Matsushita (29). The sample (0.5 ml) was combined in a screw-capped tube with 0.5 ml of 20% trichloroacetic acid in 0.6 N hydrochloric acid 50 μ l of mM butylated hydroxytoluene, and 1.0 ml of 0.53% TBA. The tube was heated for 30 min in a steam bath, cooled in ice water, and centrifuged at 6500 g for 10 min. The absorbance of the supernatant was read at 532 nm using a Carz 219 spectrophotometer. Values for experimental samples were compared to those for standard samples containing 1,1,3,3-tetraethoxypropane.

f. Statistical Analysis

Differences between groups of the experiment were analysed for statistical significance by using Student's "t" test.

3. RESULTS

a. HTdR incorporation test (Fig. 2)

PHA-induced lymphocyte proliferation in the co-cultures containing autologous AMs showed difference in the investigated groups.

The ³HTdR incorporation was decreased in the both of the EMF – exposed groups, as compared with control groups, but with statistically significant difference (ssd) ($p > 0.01$) only in fourth group (Fig. 2).

The depressed mitogenic response at 90 days of the EMF exposure suggested a possible interference of EMF with lymphocyte response. These data suggest a possible alteration of the cellular mediated immunity with highly receptivity for diseases development in the exposed subjects.

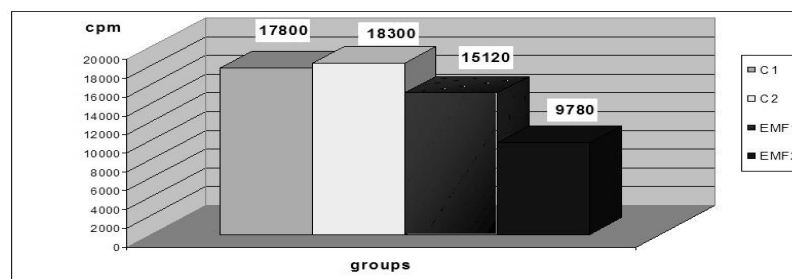


Fig. 2 – ³HTdR INCORPORATION TEST. Effect of the chronic exposure to EMF on the ³HTdR incorporation by PHA splenic Lys in the presence of the autologous AMs, at 30 and 90 days after EMF-exposure of the rats.

b. IL-1 assay (Fig. 3)

IL-1 production in supernatants from the AMs obtained from investigated groups was assayed by measuring the capacity of the culture supernatants to co-stimulate the mitogen – induced tymphocyte proliferation. The differences in the generation of IL-1 production from investigated groups was observed. The EMF1-group presented slightly decreased values of the IL-1 activity as compared to the Controls (without ssd). The EMF2-group pointed out an important decrease of the level of the IL-1. The differences were with statistically significance reported to the Controls. The EMF-2 group presented decreased values of the IL-1 activity as compared to the EMF-1-group (with out ssd), but with statistically semnificativ differences as compared to the Control 2-group .

Our data suggest that in the AMs from EMF-exposed rats can be important modifications of the IL-1 level, that can interfere with Ty lymphocyte proliferation, and with other Lys activities in the presence of the PHA.

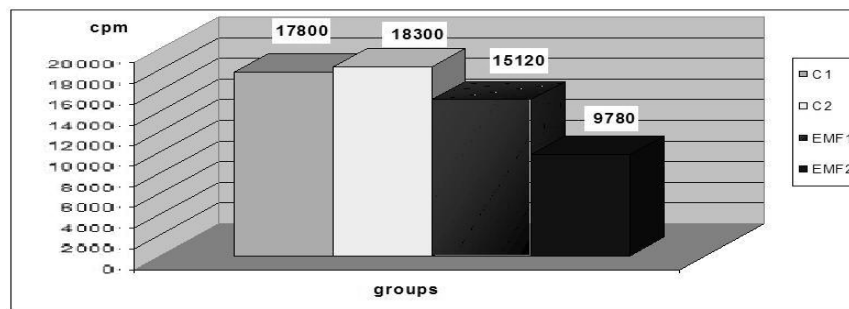


Fig. 3 – IL-1 assay. Effect of on the chronic exposure to EMF on the IL-1 release by AMs obtained by BAL, 30 and 90 days after EMF-exposure of the rats.

c. TNF – assay (Fig. 4)

Effect of the chronic exposure to EMF on the TNF release by rats AMs, obtained by BAL, 30 and 90 days after EMF-exposure of the rats. Increased values of the cytokines (TNF) were found in the 3 and 4-groups, with ssd ($p > 0.01$) for both of the groups, but the TNF values of the EMF 2-group are very important increased. These data suggest that chronic exposure to EMF can be associated with proliferative, degenerative or inflammatory processes, possible induced by active biologic molecules, between them, highly level of the TNF can be an important factor.

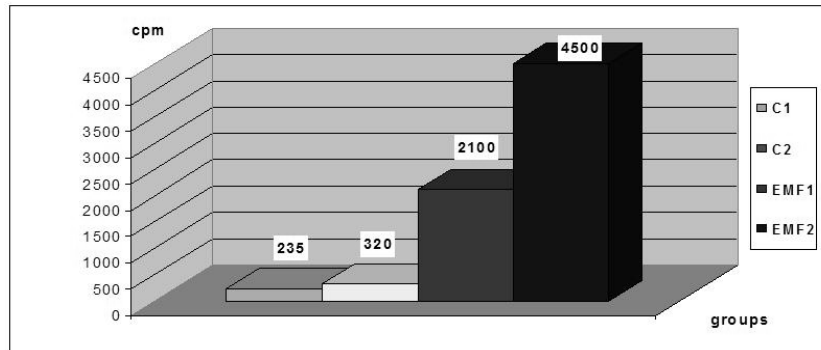


Fig. 4 – TNF – assay. Effect of the chronic exposure to EMF on the TNF release by rats AMs, obtained by BAL, 30 and 90 days after EMF – exposure of the rats.

d. Chemiluminescence (CL) assay (Fig. 5)

The oxidative response of the AMs belonging to the investigated groups measured by luminol dependent CL, evidence significant differences between EMF-exposed groups and control groups.

The most highly values of the CL were detected in EMF-2-group. These data suggest that chronic exposure to the EMFs is associated with elevated levels of the ROS.

In the chronic exposure to the EMFs, increased levels of the TNF and ROS, could play a key role in development of the immune, inflammatory and fibrotic processes and these could be first steps in cancerous process development.

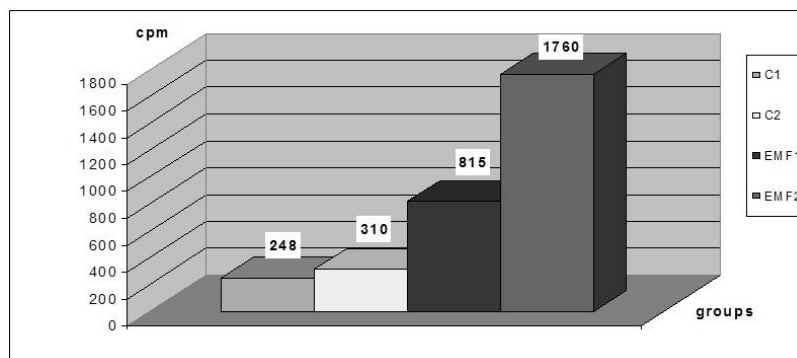


Fig. 5 – LUMINOL-DEPENDENT CHEMILUMINESCENCE ASSAY. Effect of the chronic exposure to EMF on ROS release from the rats AMs, obtained by BAL, 30 and 90 days after EMF – exposed rats.

e. Lipid peroxides (Fig. 6)

In our experiment, TBA assay evaluated the effect of the EMFs on the release of the LP in the AMs treated with thibarbituric acid (TBA), at 1 and 3 months after rats exposure. The most highlly values of the LP were detected in EMF-2-group, but the differences were with statistically significant (ssd) versus to Controls in the both of the exposed groups.

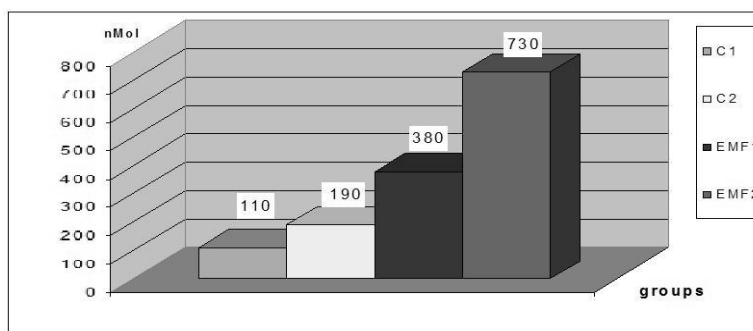


Fig. 6 – LIPID PEROXIDES ASSAY. Effect of the chronic exposure to EMF on the release of the LP in the TBA-treated AMs, at 30 and 90 days after EMF-exposed rats.

These data suggest association of the EMF-chronic exposure with an important suppression of the immune response and with the generation of the highly levels of the LP, ROS, TNF. These active biologic molecules could be the first step in the induction of the tissue injury. Although these factors can play a key role in development of the immune, inflamatory and fibrotic reactions, the processes developed are still unelucidated.

4. CONCLUSIONS

Although this important issue are not yet resolved in this study, our results showed that EMFs caused changes in the immune system, possible mediated by the supression of the T cells activity and by increase of the cytokines' release, in special TNF. Our results clearly show that the exposure to EMF is connected to an increased release of free radicals. The increased levels of the ROS and LP pointed out alterations in the oxidative stress parameters in the rat's macrophages that were exposed to EMF.

The changes in oxidative activity of cells (maybe via Fenton reaction, but not only) may be a marker of genotoxic action (by provoking DNA damage, related with cancer risk of EMFs).

Our results indicate an increased risk regarding development of the biological effects in EMF exposure, correlated with intensity and period of the exposure. The chosen condition of exposure to EMF, with equivalent power density (S) around 8 W/m^2 (below ICNIRP limits), correlated and integrated in the perspective of our results, shows that exposure to EMF has effects on immune and oxidative activity of the cells, even below ICNIRP safety limits. This may constitute an argument for further more complex studies in this domain, to attest the necessity to change the actual safety international standards.

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