ORIGINAL SCIENTIFIC PAPER

ANIMAL STUDY ON ELECTROMAGNETIC FIELD BIOLOGICAL POTENCY

IVANČICA TROŠIĆ, MIRJANA MATAUŠIĆ-PIŠL, ŽELJKO RADALJ AND IVICA PRLIĆ

Institute for Medical Research and Occupational Health, Zagreb, Croatia

Received November 1998

This recent basic research study used an animal model protocol to assess specific biomarkers of the effect of non-ionising, non-thermal radiation (2450 MHz microwave radiation at 5–15 mW/cm^2) on bone marrow, peripheral blood, and bronchoalveolar free cell populations. Of 40 male Wistar rats taken in the study, 20 animals of the experimental group were irradiated for 2 hours a day, 5 days a week, and subsequently killed on days 1, 8, 16, and 30 of the experiment. The remaining 20 rats served as control. All animals were previously intratracheally instilled with biologically inert microspheres to see the influence of irradiation on lung retention kinetics. The cell response to chosen electromagnetic irradiation was followed quantitatively and qualitatively using the standard laboratory methods. The results of peripheral blood cell response suggested a decreasing tendency in total leukocyte count and in relative lymphocyte count in the treated group. A slight increase was also observed in granulocyte count and in the absolute count of peripheral blood erythrocytes over control animals.

Key words:
bone marrow cells, bronchoalveolar free cells, non-ionising radiation, non-thermal radiation, peripheral blood cells, Wistar rats

Electromagnetic field (EMF) in the biosphere has been a natural and permanent part of human environment. The features of the field have significantly changed with development of technology, increasing the level of both the occupational and ambient human exposure. Athough wide, the entire frequency range of EMF spectrum is biologically active and affects the living matter by different underlying mechanisms (1). Natural microwave radiation in the frequency range of 100–300 GHz is low in comparison with the intensity of artificial radiation sources. Major man-made sources

Presented at the AOAC INTERNATIONAL Central Europe Subsection 5th International Symposium on Interpretation of Chemical, Microbiological and Biological Results and the Role of Proficiency Testing in Accreditation of Laboratories, Varaždin, Croatia, 21–23 October 1998.

of microwave radiation include radars, telecommunication equipment, and household appliances, particularly those using energy for heating (2). While the high-intensity microwave radiation may have harmful effects on the irradiated organism mostly through basically understood thermal mechanisms (3, 4), the low-power, non-thermal effects remain an unresolved issue. There are controversial reports about effects of exposure to non-thermal levels of microwave radiation on biological systems (5).

One of the primary concerns is to determine whether such exposure produces any adverse health effect. The aim of this pilot study was to single out specific biomarkers of the effect of non-ionising, non-thermal radiation on systemic circulation in rats. We investigated the cellular and intracellular events in bone marrow, peripheral blood, and bronchoalveolar free cell population. We also established and applied an animal model protocol to investigate the lung kinetics of harmless particles influenced by irradiation.

MATERIALS AND METHODS

Forty healthy male Wistar rats (13 week old, approximate body weight of 350 g) were used in this study. Twenty control animals were kept in normal physiological conditions and received standard laboratory food and water *ad libitum*. Light and dark cycles alternated every 12 hours. Each animal was intratracheally instilled with suspension of 0.4 ml of biologically inert particles in a concentration of 2.7×10^8/ml of physiological saline (Fluorescent carboxylated polystyrene microspheres, 2.37 μm diameter, Polyscience Inc., Warrington, PA). Details on the instillation technique have been previously reported (6). The experimental group of animals was irradiated with 2450 MHz microwaves at 5–15 mW/cm^2 2 hours/day, 5 days/week. The microwave source was a modified Micro-Chef Moulinex® generator. The applied microwave power density has been reported not to influence body temperature in rats (7). During the treatment regimen, animals were kept in Plexiglas cages. The animals did not receive food and water during exposure sessions. The microenvironment temperature for both groups of animals was 22 ± 1 °C. The experiment lasted thirty days altogether. The rectal temperature was measured before and after treatment. No significant change in body temperature was observed in treated animals when compared to the controls. The obtained results were statistically evaluated using non-parametric statistical methods (8).

All animals (N=40) received inert particles by intratracheal instillation in order to follow their kinetics inside the lung compartments under experimental conditions. The group of twenty experimental animals was divided into four subgroups which received 2x2, 7x2, 13x2 and 22x2 hours of irradiation treatment and were killed following pentobarbital anaesthesia on respective postinstillation days 1, 8, 16, and 30.

Peripheral blood was collected on the indicated times after irradiation by snipping off the end of the tail. We used standard laboratory methods (9) to examine samples for the absolute number of white and red blood cells per litre, white blood cells by type, relative number of reticulocytes, and for micronucleus occurrence in red blood cells. Two thousand red blood cells per animal were scored to distinguish erythrocytes and reticulocytes.

Bone marrow samples were collected from both femurs of each animal. Both the proximal and distal ends of the femur were cut off and the bone marrow cell gently flushed out with buffered physiological saline and foetal calf serum (1:1). The procedure for preparation of the bone marrow cells was applied according the *Mazur's* technique (10).

The free lung cell population was obtained by bronchoalveolar lavage on the indicated post-instillation days. Cell response to the selected irradiation level was followed quantitatively by defining absolute and relative free cell count, and qualitatively by examination of alveolar macrophage viability and phagocytic activity and capacity (11, 12).

In addition, this study was designed to obtain information about the influence of selected electromagnetic irradiation on the retention, deposition, and kinetics of non-cytotoxic particles in the respiratory tract by analysing lung and lymph nodal tissue burdens over the course of alveolar clearance (13).

RESULTS

The results of this pilot research study have not been processed completely. Here we shall report and discuss about the cellular components of peripheral blood circulation. Figure 1 shows the total leukocyte count in peripheral blood after 2450 MHz irradiation of the exposed (G2) and the control (G1) group of animals. The decreasing tendency ($P<0.05$) in the total leukocyte count was noted during the course of experiment. The treated animals showed higher relative granulocyte count than did the control

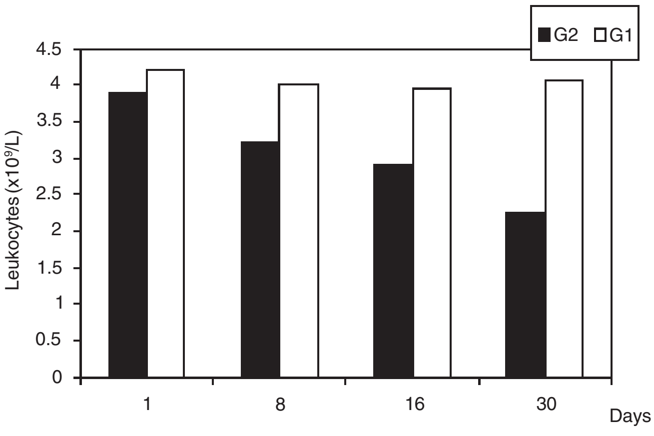

Figure 1 *The total number of leukocytes in the peripheral blood after 2450 MHz irradiation of the exposed (G2) and the control (G1) animals (N=5 per group)*

animals (Figure 2). Figure 3 shows a significantly lower lymphocyte blood percentage ($P<0.05$) in group G2 than in control animals. The absolute erythrocyte count in the peripheral circulation of irradiated animals increased over the initial eight days, only to keep falling thereafter until the end of the experiment, yet stay within the physiological range (Figure 4).

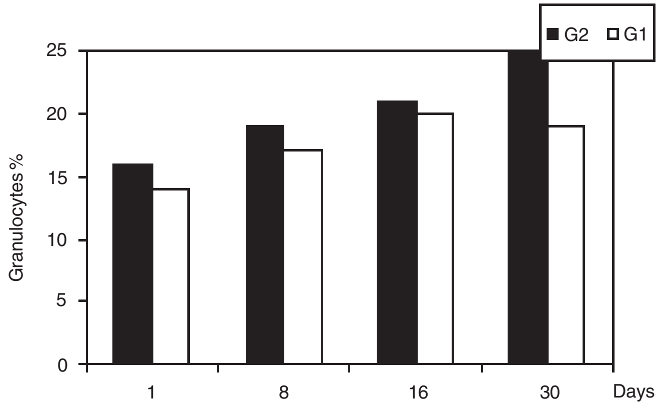

Figure 2 *Percentage of granulocytes in the peripheral blood after 2450 MHz irradiation of the exposed (G2) and the control (G1) animals (N=5 per group)*

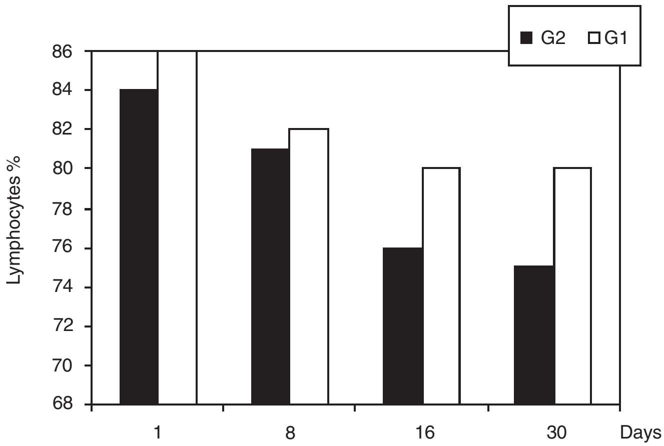

Figure 3 *Percentage of lymphocytes in the peripheral blood after 2450 MHz irradiation of the exposed (G2) and the control (G1) animals (N=5 per group)*

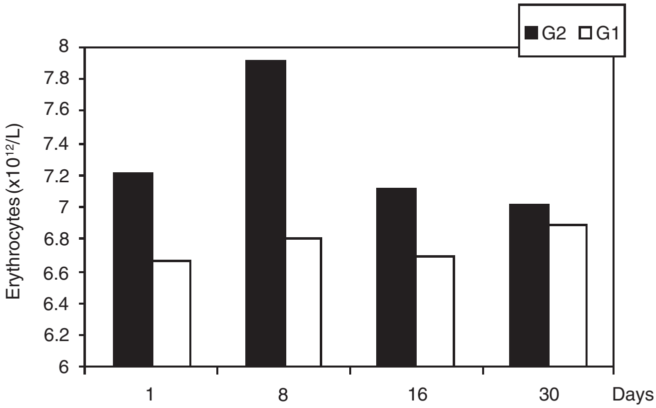

Figure 4 *The total number of erythrocytes in the peripheral blood after 2450 MHz irradiation of the exposed (G2) and the control (G1) animals (N=5 per group)*

DISCUSSION

It is essential to realise that there is no universally accepted, specific biological indicator of exposure to microwaves and that there is no reason that a single biological test would be a satisfactory indicator of exposure to microwaves. A proper investigation of biological effects of microwaves requires an understanding and appreciation of biological principles and comparative biomedicine. It should focus on biomedical parameters that take into account physiological functions such as specific and non-specific reactions and are able to distinguish adaptational and compensatory changes from pathological manifestations.

Many sources of microwave radiation are capable of causing biological damage. There is a large range of biological effects which have been reported to have positive or negative influence on the living matter. In a word, a lot of controversial results on microwave biological effects have been reported over the past decades (5, 14). Besides, there is a lack of both understanding and consensus about the underlying interaction mechanisms. *Saunders and co-workers* (15) suggest that if there are adverse health effects attributed to non-thermal microwave radiation exposure, it is important to conduct a basic research study *in vivo* to find them.

To elucidate the missing mechanisms we focused on singling out specific biomarkers of the effects of non-ionising, non-thermal radiation on quantity and quality of bone marrow, peripheral blood, and free lung cell populations. So far, the results of peripheral blood cell response to selected electromagnetic irradiation suggested a decreasing tendency in total leukocyte count as well as in the lymphocyte percentage in the treated animals. An increase in the percentage of granulocytes was observed during the experiment. The first eight days of the experiment saw an increase in total erythrocyte count in treated rats over controls, after which it kept falling until the end of experiment. *Ragan and co-workers* (16) reported significant differences in erythrocyte, leukocyte, and platelet values, and in reduced bone marrow cellularity in microwave (2880 MHz, 5–10 mW/cm^2, 3–7.5 hours/day totalling 60–360 hours) exposed groups of mice, but those findings were not consistently observed throughout the experiment. Later investigation by *Galvin and co-workers* (17) did not find differences in total and differential leukocyte counts in peripheral blood samples of pregnant mice exposed to irradiation of 2450 MHz at 30mW/cm^2 8 hours a day.

Our model is based on the widely adopted ideas about haematopoiesis in normal conditions and under irradiation. The results of this study give us an insight into the cellular dynamics, its maturation and functionality, and enable us to single out specific biomarkers of non-thermal, non-ionising radiation as a basis for further experimental research.

REFERENCES

1. *Roberts NJ, Michaelson SM, Lu S-T*. The biological effects of radiofrequency radiation: a critical review and recommendations. Int J Radiat Biol 1986;50:379–420.
2. *Franekić J, Koren Z*. Biological effects of non-ionising radiation. In: Aničić D, ed. 1997 Yearbook of the Croatian Academy of Technical Sciences. Zagreb: Hermes izdavaštvo, 1997:159–69 (in Croatian).
3. *Nekić K, Latin V, Garaj-Vrhovac V, Koren Z, Franekić J*. Genotoxic effects of Doppler ultrasound and microwave radiation. Period Biol 1994;96:389–90.
4. *World Health Organization (WHO)*. Radiofrequency and microwaves. Environmental Health Criteria No. 16. Geneva: WHO, 1981.
5. *McManus T*. Biological effects of non-thermal pulsed and amplitude modulated RF electromagnetic fields and related health hazards. In: Bernhardt JH, Matthes R, Repacholi MH, eds. International Seminar on Biological Effects of Non-Thermal Pulsed and Amplitude Modulated RF Electromagnetic Fields and Related Health Risk. Proceedings. Munich: International Commission on Non-Ionizing Radiation Protection and World Health Organization, 1996:15–24.
6. *Trošić I, Mataušić-Pišl M, Brumen V, Horš N*. The role of intratracheal instillation and bronchoalveolar lavage in the investigation of mechanisms underlying lung disorders – An animal model. Arh hig rada toksikol 1996;47:405–11.
7. *Djordjević Z, Kolak A, Dioković H, Ristić P, Kelečević Z*. Results of our 15-year study into the biological effects of microwave exposure. Aviat Space Environ Med 1983;54:539–42.
8. *Willemsen EW*. Understanding statistical reasoning. In: Atkinson RC, Freeman J, Thompson GF, eds. Library of Congress Cataloging in Publication Data. San Francisco, CA: 1974;168–96.
9. *Softić N*. Haematological laboratory tests. Zagreb: Sveučilišna naklada Liber, 1984 (in Croatian).
10. *Mazur L*. Induction of micronucleated erythrocytes by MEA, AET, WR-2721 and X-rays. Mutat Res Environ 1995;334:317–22.
11. *Lehnert BE*. Alveolar macrophages in a particle »overload« condition. J Aerosol Med 1990;3 (suppl 1):S-9–S-29.
12. *Seemayer NH, Happel A, Behrendt H, Hadnagy W, Tomingas R*. Comparison of cytotoxicity of airborne particulates to rat and human macrophages. J Aerosol Sci 1990;21:387–91.

13. *Snipes MB, Olson TR, Yeh HC*. Deposition and retention of patterns for 3-, 9-, and 15-μm latex microspheres inhaled by rats and guinea pigs. Exp Lung Res 1988;14:37–50.
14. *Elder JA*. A Reassessment of the biological effects of radiofrequency radiation: Non-cancer effects. A report to the Office of Radiation Programs. Durham, NC: US EPA, 1987.
15. *Saunders RD, Cridland NA, Kowalcyuk CI, Sienkiewicz ZJ*. *In vivo* biological studies relevant to low level RF health effecs. In: Bernhardt JH, Matthes R, Repacholi MH, eds. International Seminar on Biological Effects of Non-Thermal Pulsed and Amplitude Modulated RF Electromagnetic Fields and Related Health Risk. Proceedings. Munich: International Commission on Non-Ionizing Radiation Protection and World Health Organization, 1996:145–61.
16. *Ragan HA, Phillips RD, Buschbom RL, Busch RH, Morris JE*. Hematologic and immunologic effects of pulsed microwaves in mice. Bioelectromagnetics, 1983;4:383–96.
17. *Galvin MJ, MacNicols GL, McRee DI*. Effect of 2450 microwave radiation on hematopoiesis of pregnant mice. Radiat Res, 1984;100:412–17.

Sažetak

PROUČAVANJE BIOLOŠKIH UČINAKA ELEKTROMAGNETSKOG POLJA U POKUSNIH ŽIVOTINJA

Ovo pilotsko istraživanje provedeno je na animalnom modelu s namjerom da se uoče specifični biomarkeri učinka neionizirajućeg, netermalnog zračenja (2450 MHz, 5–15 mW/cm^2) na stanice koštane srži, periferne krvi i slobodnu staničnu populaciju pluća. U pokus smo uzeli 40 štakora mužjaka soja Wistar, od kojih je kontrolna skupina imala 20 životinja. Pokusnu skupinu životinja zračili smo 2 sata na dan, 5 dana u tjednu. Zatim su životinje žrtvovane 1, 8, 16. i 30. dana pokusa. Svaka je životinja prije početka pokusa intratrahealno instilirana biološki inaktivnim česticama kako bismo dobili uvid u utjecaj zračenja na njihovu retenciju u plućima. Kvantitativni i kvalitativni stanični odgovor na izabrano zračenje pratili smo standardnim laboratorijskim metodama. U usporedbi s kontrolnim životinjama, rezultati staničnog odgovora u perifernoj krvi pokazuju tendenciju snižavanja ukupnog broja leukocita, ali i relativnog broja limfocita u tretiranih životinja te lagano povišenje broja granulocita i apsolutnog broja eritrocita.

Ključne riječi:
bronhoalveolarne slobodne stanice, neionizirajuće zračenje, netermalno zračenje, stanice koštane srži, stanice periferne krvi, Wistar štakori

Requests for reprints:

Ivančica Trošić, Ph.D.
Institute for Medical Research and Occupational Health,
Ksaverska cesta 2, P.O. Box 291, HR–10001 Zagreb, Croatia
E-mail: *itrosic@imi.hr*