Vol. 176, No. 1, 1991 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
April 15, 1991 Pages 194-200

# SPONTANEOUS AND MITOMYCIN-C-INDUCED MICRONUCLEI IN HUMAN LYMPHOCYTES EXPOSED TO EXTREMELY LOW FREQUENCY PULSED MAGNETIC FIELDS

M.R. Scarfi *, F. Bersani ◇, A. Cossarizza ‡, D. Monti ‡, G. Castellani ◇, R. Cadossi ‡, G. Franceschetti * and C. Franceschi ‡

* Istituto di Ricerca per l'Elettromagnetismo e i Componenti Elettronici del Consiglio Nazionale delle Ricerche, Via Claudio 21, 80125, Napoli, Italy

◇ Dipartimento di Fisica, Università di Bologna, Italy

‡ Istituto di Patologia Generale, Università di Modena, Italy

Received February 8, 1991

SUMMARY. The cytokinesis block micronucleus method, a very sensitive cytogenetic assay, was used to ascertain the possible genotoxic effects of extremely low frequency pulsed magnetic fields in phytohemagglutinin-stimulated human lymphocytes cultures from 16 healthy donors. Four conditions were studied: i) lymphocytes not exposed to the field (control cultures); ii) lymphocytes exposed to the field; iii) lymphocytes treated with mitomycin-C and not exposed to the field; iv) lymphocytes treated with mitomycin-C and exposed to the field. Mitomycin-C-treated cultures were used as control for the micronucleus method, because it is known that mitomycin-C is a potent genotoxic agent, capable of inducing micronuclei. The frequency of micronuclei in field-exposed cultures was similar to the spontaneous frequency observed in control unexposed-cultures. Moreover, the exposure to pulsed magnetic fields did not affect the frequency of micronuclei induced by mitomycin-C, suggesting that, in the experimental conditions used, this kind of field neither affected the integrity of chromosomes nor interfered with the genotoxic activity of mitomycin-C. 

In recent years a variety of cell functions have been demonstrated to be affected by extremely low frequency (ELF), low energy electromagnetic fields, including cell proliferation and DNA synthesis [1-3], ion fluxes [4], gene expression [5] and protein synthesis [6]. Cytogenetic studies in cell cultures and

Abbreviations: ELF, extremely low frequency; NMR, nuclear magnetic resonance; PMFs, pulsed magnetic fields; MN, micronuclei; FCS, fetal calf serum; Cyt-B, cytochalasim B; CB cells, binucleated cells.

0006-291X/91 $1.50

after "in vivo" exposures gave controversial results [7-11]. Moreover, epidemiological studies [12,13] suggest that the exposure to such non thermal fields may cause cytogenetic effects, but the results are still contradictory and questionable [14-16].

These findings, together with the fact that humans can be exposed more and more frequently to such physical agent (powerlines, home appliances, NMR apparatuses and therapy devices), stress the importance to ascertain whether ELF fields may represent some hazard for human health. An argued concern has been recently expressed on this topic [17,18]. In this context, the question of possible genotoxic effects is particularly relevant.

The results here presented regard the assessment of genotoxic effects induced by pulsed magnetic fields (PMFs), with characteristics similar to those employed for bone repair [19]. The cytogenetic technique used in the present work was first introduced by Fenech and Morley in 1985 [20]. It evaluates the frequency of micronuclei (MN) (small round bodies deriving from chromosome fragments or from whole chromosomes whose centromeres have lost their affinity for mitotic spindle) in cells in which cytokinesis has been previously blocked by cytochalasin B. This technique is a modification of a previous method in which MN frequency was evaluated without the block of cytokinesis [21]. The Fenech and Morley assay has some advantages when compared with other cytogenetic methods (e.g. classical karyotype assessing), such as higher sensitiveness and easier applicability to large scale screenings [22]. As far as we know, this is the first example of application of such method in bioelectromagnetic research.

## MATERIALS AND METHODS

Cell preparation. 0.8 ml of defibrinated whole blood from 16 healthy donors was added to 9.2 ml of RPMI 1640 medium containing 15% fetal calf serum (FCS), 2mM L-glutamine (10 $\mu l$/ml), of phytohemagglutinin (M form, Gibco). Cells were cultured in Falcon plastic flasks (cod.3013) for 72 hours at 37°C. Cytochalasin B (Cyt-B, Sigma) was added to the cultures 44 hours after initiation, to give a final concentration of 3.0 $\mu g$/ml. Cyt-B was made up as a stock solution in dimethylsulphoxide at a concentration of 2 $mg/ml$, aliquoted and stored at -20°C. After 72 hours, each culture was washed twice in RPMI containing 2% FCS (wash medium) and cell pellets were resuspended and kept for 15 minutes at room temperature in hypotonic solution consisting of 1 part of wash medium and 4 parts of distilled water [23]. Cells were mixed and 0.3 $ml$ of cell suspension was added to each bucket of a cytocentrifuge (Cytospin,

Shandon) and spun down onto slides at a speed of 1200 rpm for 7 minutes. Slides were removed, dryed for several minutes, fixed in methanol 80% aqueous solution for 10 minutes and stained with a 10% Giemsa solution for 10 minutes. Codified slides were scored blindly by the same operator (M.R.S.) using a Leitz Dialuz microscope at 1000 × magnification. MN were identified following standard criteria [24] and their frequency was evaluated as the ratio between the cells blocked in first mitosis (binucleated cells, i.e. CB cells) presenting MN and the total number of binucleated cells.

MMC treatment. A dose-response curve of MMC was established in our laboratory, taking into account previous data from other authors, in order to obtain an adequate number of MN without affecting cell growth (data not shown). 0.033 $\mu g/ml$ of MMC (Sigma), sampled from fresh solution in sterile distilled water, was added at the start of the culture and was left in the medium throughout the whole culture period.

Field characteristics and exposure conditions. The exposure system has been previously described [3]. Briefly, it consists of a pair of horizontal Helmholtz coils connected to a pulse generator (Igea, Italy). The field characteristics are shown in fig.1. The upper diagram shows the magnetic field signal: the maximum amplitude was 2.5 mT and the pulse width (T1) 1.2 msec. The repetition frequency was 50 Hz (T=20 ms). The lower diagram shows the signal of the induced electric field as detected by a suitable coil probe. The Helmholtz coils were maintained parallel to the flasks used for cell cultures. Taking into account that the induced electric field inside the culture medium depends on the size of the flask and on the position with respect to the magnetic field direction [25], the maximum electric field can be estimated of the order of 0.5 mV/cm.

Statistical analysis. The comparison between micronuclei frequency in PMFs-exposed and control cultures with or without MMC in each subject was performed by Z test for proportions. The comparison between micronuclei frequency in the same cultures in all the subjects was performed by variance analysis and two-tailed paired Student's t test.

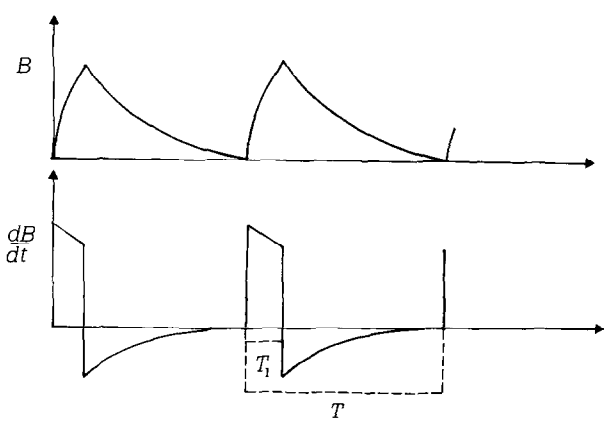


FIGURE 1. The maximum intensity of the magnetic field (upper figure) was 2.5 mT. The time derivative of the magnetic field (lower figure) was proportional to the induced electric field within the sample; $1/T = 50$ Hz; $T_1/T = 0.1$.

## RESULTS

Four conditions were studied: i) lymphocytes not exposed to PMFs (control cultures); ii) lymphocyte cultures exposed to PMFs; iii) lymphocytes treated with MMC but not exposed to the field; iv) lymphocytes treated with MMC and expose to PMFs.

MMC-treated cultures were used as control for the MN method, as this potent genotoxic agent increases the formation of MN with a strict dose-dependent effect. This treatment was also used as control for condition (iv). The combination of MMC-treatment and field-exposure was tested in order to verify whether the field was able to exert antagonistic or synergistic effect with the drug.

The results reported in Table 1 can be summarized as follows:

- the spontaneous MN frequency in unexposed cultures was of the same order of that observed by other authors [22];
- the frequency of MN in cultures exposed to the field was similar to the spontaneous frequency observed in unexposed cultures;
- the method was sensitive to a genotoxic agent such as MMC, as demonstrated by the significant increase of MN in MMC-treated cultures;
- cultures treated with MMC and exposed to PMFs showed a frequency of MN similar to that observed in cultures treated with MMC only.

## DISCUSSION

It is of great interest both from the biological and the epidemiological point of view to ascertain the genotoxic potential of ELF-PMFs as humans may be exposed to such non-ionizing agent in a variety of situations. In fact, the field characteristics used in the present study were similar to those used for therapy (bone repair) [19]. Moreover, the time variation of the magnetic field (dB/dt) was of the order of 1T/sec, which is similar to that present in nuclear magnetic resonance (NMR) devices used for diagnostic tests. In previous works [26], we showed that a field with same characteristics was able to enhance "in vitro" lymphocyte proliferation and to affect the IL-2 utilization and the IL-2 receptor expression, without affecting DNA repair after gamma irradiation [3,27].

TABLE 1. SPONTANEOUS AND MITOMYCIN-C (MMC) - INDUCED MICRONUCLEI FREQUENCY IN LYMPHOCYTES EXPOSED TO PULSED MAGNETIC FIELDS (PMFs)

| | Micronuclei/100 CB cells | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Subject | Control | PMFs | Z ** | p | MMC | Z | p | MMC+ PMFs | Z | p |
| 1 | 1.28 (781)* | 1.9 (1212) | 0.879 | 0.380 | 2.67 (675) | 1.734 | 0.083 | | | |
| 2 | 1.11 (1256) | 1.19 (1760) | 0.03 | 0.976 | 3.4 (1085) | 3.649 | 0.0001 | | | |
| 3 | 1.33 (1650) | 1.14 (1226) | 0.284 | 0.776 | 2.72 (1431) | 2.635 | 0.008 | | | |
| 4 | 1.29 (2009) | 1.1 (1895) | 0.398 | 0.690 | 2.84 (1618) | 3.212 | 0.001 | | | |
| 5 | 0.92 (2941) | 1.13 (2830) | 0.662 | 0.508 | 3.37 (2460) | 6.253 | 0.0001 | | | |
| 6 | 1.66 (1869) | 1.22 (2007) | 1.017 | 0.390 | 3.4 (1647) | 3.209 | 0.001 | | | |
| 7 | 1.38 (3257) | 1.02 (2652) | 1.135 | 0.256 | 2.29 (3223) | 2.638 | 0.008 | | | |
| 8 | 1.48 (4599) | 1.08 (1931) | 1.156 | 0.248 | 2.7 (2878) | 3.627 | 0.0001 | | | |
| 9 | 1.38 (3843) | 1.65 (1695) | 0.650 | 0.516 | 3.01 (962) | 3.352 | 0.0001 | | | |
| 10 | 0.86 (2561) | 1.24 (1532) | 1.019 | 0.308 | 2.37 (894) | 6.214 | 0.0001 | 2.51 (1515) | 0.078 | 0.938 |
| 11 | 1.20 (249) | 1.13 (1772) | 0.221 | 0.825 | 2.46 (5411) | 1.057 | 0.290 | 2.57 (233) | -0.11 | 0.913 |
| 12 | 0.74 (2716) | 0.66 (2630) | 0.187 | 0.852 | 2.65 (2675) | 5.338 | 0.0001 | 3.34 (2996) | 1.439 | 0.150 |
| 13 | 0.654 (1071) | 1.17 (1021) | 1.014 | 0.310 | 1.93 (1138) | 2.448 | 0.014 | 2.39 (1129) | 0.609 | 0.543 |
| 14 | 1.08 (2975) | 0.83 (2767) | 0.863 | 0.403 | 2.57 (2376) | 4.035 | 0.0001 | 2.28 (2413) | 0.558 | 0.577 |
| 15 | 1.35 (1856) | 1.05 (1710) | 0.666 | 0.505 | 2.19 (1643) | 1.765 | 0.078 | 2.09 (1388) | 0.064 | 0.949 |
| 16 | 0.99 (1513) | 1.37 (1240) | 0.747 | 0.455 | 2.18 (871) | 2.181 | 0.029 | 3.64 (1027) | 1.731 | 0.083 |
| Mean | 1.17 | 1.18 | | | 2.67 | | | 2.69 | | |
| S.E. | ±0.07 (a)*** | ±0.11 (b) | | | ±0.11 (c) | | | ±0.22 (d) | | |

* Numbers in parentheses refer to the total number of CB cells scored.

** Z test for proportions (comparisons were performed with rispect to Control values, except in the MMC+PMFs case, in which Z was evaluated with respect to Z for MMC treatment only).

*** Statistical analysis performed by two-tailed paired Student's t test: a vs. b : $p = 0.929$; a vs. c : $p < 0.001$; c vs. d : $p = 0.163$ (data obtained from subjects n. 10-16). Similar results were obtained by variance analysis.

In the present work we show that in human peripheral blood lymphocytes, a kind of cells extremely sensitive to both ionizing and non ionizing radiations, the spontaneous frequency of MN was not affected by the exposure to PMFs with the above described characteristics, nor did such field interfere with the effect of a potent genotoxic agent such as MMC.

The possibility that ELF fields might interfere with other genotoxic agents have important implications, as far as the risk assessment is concerned, as in real situations humans are likely exposed to a variety of genotoxic agents concomitantly with field exposure [28].

Our findings are in agreement with the results obtained by other authors who also used ELF electromagnetic fields [29]. On the other hand, some epidemiological studies devoted to the assesment of risk involved in the exposure to powerlines and domestic electromagnetic pollution suggested a possible correlation between very week ELF electromagnetic fields and leukemia [30-32]. Moreover, in a previous paper [7], we showed that ELF an electric field produces chromosomal aberrations in bovine lymphocytes, and recently Soheir et al. found an increased MN frequency in polychromatic erythrocytes from mice exposed to strong 50 Hz electric fields by using the old MN tecnique [33]. Different results obtained by different authors likely depend on the heterogeneity of the cells tested (type of cell, species of origin etc.) and differences in culture methods, field characteristics and exposure conditions used to asses the biological effect of ELF fields.

Owing to the importance of the topic, further investigations are necessary, in order to answer the general question of the potential genotoxicity of ELF electromagnetic fields with different physical characteristics and their possible synergistic effects with other genotoxic agents.

## ACKNOWLEDGMENTS

This work, done within the framework of EURAGE, has been partially supported by MURST 40% and 60% grants to C.F. and F.B., by C.N.R. Progetto Strategico "Campi elettromagnetici e bioinformazione" to C.F., F.B. and M.R.S.. We are most grateful to Mrs Ornella Raimondi for her assistance in the preparation of the manuscript.

## REFERENCES

[1] Rodan, G.A., Bourret, L.A., and Norton, L.A. 1978) Science, 199, 690-692.
[2] Cantini, M., Cossarizza, A., Bersani, F., Cadossi, R., Ceccherelli, M., Tenconi, R., Gatti, C., and Franceschi, C. (1986) J. Bioelectricity, 5(1), 91-104.
[3] Cossarizza, A., Monti, D., Bersani, F., Cantini, M., Cadossi, R., Sacchi, A., and Franceschi, C. (1989) Biochem. Biophys. Res. Commun., 160 (2), 692-698.
[4] Walleczek, J., and Liburdy, R.P. (1990) FEBS Lett., 271(1,2), 157-160.
[5] Goodman, R., Bassett, C.A.L., and Henderson, A.S. (1983) Science, 220, 1283-1285.
[6] Murray, J.C., and Farndale, R.W. (1985) Biochim. Biophys. Acta, 838, 98-105.

[7] D'Ambrosio, G., Di Berardino, D., Iannuzzi, L., Lioi, M.B., Mostacciuolo, E., Scaglione, A., Scarfì, M.R. (1985) J. Bioelectricity, 4, 279-284.
[8] Cohen, M.M., Kunska, A., Astemborski, J.A., and McCulloch, D. (1986) Mutat. Res., 172, 177-184.
[9] Cohen, M.M., Kunska, A., Astemborski, J.A., McCulloch, D., and Paskewitz, D.A. (1986) Bioelectromagnetics, 7, 415-423.
[10] D'Ambrosio, G., Massa, R., Di Berardino, D., Lioi, M.B., Scaglione, A., and Scarfì, M.R. (1988) J. Bioelectricity, 7 (2), 239-245.
[11] Nordenson, I., Hansson Mild. K., Nordstrom, S., Sweins, A. and Birke, E. (1984) Radiat. Environ. Biophys., 23, 191-201.
[12] Bauchinger, M., Hanf, R., Schmid, E., and Dresp, J. (1981) Radiat. Environ. Biophys., 19, 235-238.
[13] Nordenson, I., Mild, K.H., Ostam, U., and Ljungbeg, H. (1988) Radiat. Environ. Biophys., 27 (1), 39-47.
[14] Scarfì, M. R., Franceschetti, G., Lioi, M.B. (1989) Alta Frequenza, 58, 337-339.
[15] Carstensen, E. L. (1987) in "Biological Effects of Transmission Line Fields", Elsevier, New York.
[16] Franceschetti, G., Ghandi, O. P. and Grandolfo, M. eds. (1989) in "Electromagnetic Biointeraction: Mechanisms, Safety Guides, Protection Guides" Plenum Press New York.
[17] Abelson, P.H. (1989) Science, 245 (4915), 241.
[18] Shulman, S. (1990), Nature, 345, 463.
[19] Borsalino, G., Bagnacani, M., Bettati, E., Fornaciari, F., Rocchi, R., Uluhogian, S., Ceccherelli, G., Cadossi, R., Traina, G.C. (1988) Clin. Orthop. Rel. Res., 237, 256-263.
[20] Fenech, M., and Morley, A.A. (1985) Mutat. Res., 147, 29-36.
[21] Evans, H.J., Neary, G.J. and Williamson, F.W. (1959) Int. J. Radiat. Biol., 1, 216-229.
[22] Fenech, M. and Morley, A.A. (1986) Mutat. Res., 161, 193-198.
[23] Perry, P.E., and Thomson, E.J. (1986) Cytogenet. Cell Genet., 41, 121-125.
[24] Heddle, J.A., Constance B.L., Saunders E.F., and Benz D. (1978) Cancer Res., 38, 2983-2988.
[25] Mc Leod, B.R., Pilla, A.A. and Sampsel, M.W. (1983) Bioelectromagnetics, 4, 357-370.
[26] Cossarizza, A., Monti, D., Bersani, F., Paganelli, R., Montagnani, G., Cadossi, R., Cantini, M., and Franceschi, C. (1989) FEBS Lett., 248 (1-2), 141-144.
[27] Cossarizza, A., Monti, D., Sola, P., Moschini, G., Cadossi, R., Bersani, F., and Franceschi, C. (1989) Radiat. Res., 118, 161-168.
[28] Milham, S. (1982) N. Engl. J. Med., 307, 249.
[29] Takahashi, K., Kaneko, I., Date, M., Fukada, E. (1987), Experientia, 43, 331-332.
[30] Wright, W., Peters, J.M., Mook, E.M. (1982) Lancet ii, 8308, 1160-1161.
[31] Severson, R.K. et al. (1988) Am. J. Epidemiol., 128 (1), 10-20.
[32] Wachtel, H. et al. (1988) Am. J. Epidemiol., 128 (1), 21-38.
[33] Soheir, M., El Nahas and Hanaa A.O. (1989) Environ. Mol. Mutagen, 13, 107-111.