EFFECT OF COMBINED IONIZING AND NON-IONIZING RADIATIONS ON THE EXPRESSION OF THE C-JUN ONCOPROTEIN

I. Lagroye, J.L. Poncy

CEA/DSV/DRR/SRCA, Laboratoire de Radiotoxicologie, BP 12, 91680 Bruyères le Châtel, France.

INTRODUCTION

The question of the effects of extremely-low frequency electromagnetic fields (ELF-EMF) on public health remains very controversial. Epidemiological studies (1, 2) indicated a correlation between ELF-EMF exposure and the incidence of childhood leukemia. Nevertheless, laboratory investigations regarding ELF-EMF genotoxic effects in *in vitro* biological systems failed to evidence any genomic damage (3-6). Recently however, attention has focused on the potential role of electromagnetic signals in cellular promotion implied in carcinogenesis process.

For instance, the transcription of oncogenes (7,8) has been reported to be responsive to ELF-EMF and the number of transformed foci in cells treated with a phorbol ester have been demonstrated to be enhanced after magnetic field exposure (9). Furthermore, cells irradiated with ^{60}Co showed increased chromosomal aberrations when they were exposed to ELF-EMF (10). Recently it was demonstrated that the number of ionizing radiation-induced micronuclei raised after ELF-EMF exposure in two of the three rat tracheal cell lines tested (11).

The aim of this study was to determine if ELF-EMF may induce the expression of the oncoprotein c-JUN, involved in cell transformation, either alone or after preexposition to gamma rays.

MATERIAL AND METHODS

Cell culture

Both cell lines used were obtained from gamma rays-irradiated rat tracheal cells in primary culture or from spontaneous immortalized clone.

Cells were maintained in HD medium (3v. Ham F12/ 1v. DMEM) with 1% of decomplemented fetal bovine serum at 37°C in a 5% carbon dioxide atmosphere.

Radiation exposures

Electromagnetic fields were generated with 2 pairs of Helmholtz coils put in a plastic box shielded with mu-metal and placed in a 5% CO_2 incubator. The electromagnetic signal consists of a sinusoidally varying field with a frequency of 50 Hz and a 0.1 mT r.m.s. amplitude, combined with a 0.05 mT static geomagnetic-like field (horizontal and vertical components).

EMF sham-exposures were performed in a similar mu-metal shielded box, without Helmholtz coils and placed in another incubator where measured background sinusoidal field was < 0.001 mT.

Confluent tracheal epithelial cells were gamma-irradiated (^{60}Co, 6 Gy, 0.8 $Gy.min^{-1}$) and control cells were mock-exposed. A uniform time of 1 hour elapsed from the irradiation time to the initiation of continuous incubation in the sham- or ELF-EMF exposure system.

Western blotting

Adapted from (12), cells were lysed 1.5, 4, 6 and 10 hours after ionizing irradiation, extracted and 10 µg protein samples were separated by SDS-PAGE on a 12% polyacrylamide gel and blotted onto a PVDF membrane. c-JUN protein was assayed using a polyclonal c-JUN/AP1 antibody (Santa Cruz Biotechnology) and the enhanced chemiluminescence (ECL) detection procedure (Amersham).

RESULTS

Experiments were done in triplicate and typical results are shown below. Two bands are usually observed and the major one was taking into account for data interpretation.

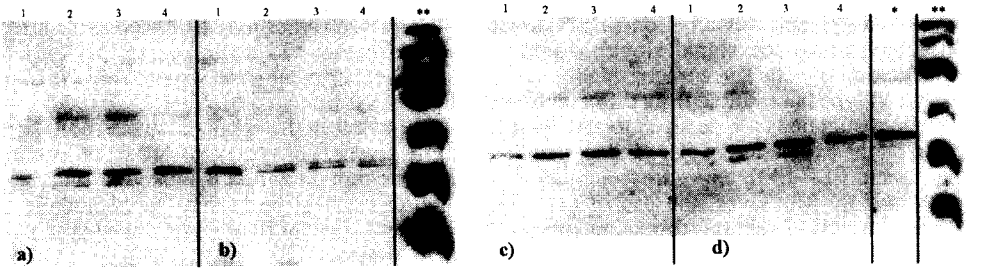

Figure: Expression of c-JUN in an epithelial tracheal cell line, after ionizing and/or non-ionizing irradiation. Cells were harvested 1.30h (**a**), 4 h (**b**), 6h (**c**) and 10h (**d**) after γ rays exposure.
(lane **1**): 0 Gy+Sham EMF; (lane **2**): 0 Gy+EMF;
(lane **3**): 6 Gy+Sham EMF; (lane **4**): 6 Gy+EMF.
*: positive control; cells were treated by Phorbol 12 myristate 13 acetate (PMA, 100 ng/ml, 1 hour).
**: molecular weight markers (97.4 - 68 - 49 - 31 - 20.1 - 14.4 kDa).

The preliminary results, obtained with a spontaneous immortalized cell line, seemed to indicate that the level of JUN protein was significantly increased in cells exposed continuously during 6 and 10 hours to ELF-EMF after a gamma rays irradiation (6 Gy) versus untreated and only gamma-irradiated cells. In this previous experiment, cells were not exposed to ELF-EMF alone, because of material constraints.
Data concerning another cell line failed to reproduce these results. Indeed, no synergistic effect of ionizing and electromagnetic fields was observed regarding the induction of JUN protein. However, under our experimental conditions, ELF-EMF alone enhanced the induction of this oncoprotein in a quite similar way as gamma rays. The figure shows that c-JUN is early induced (30 minutes after ELF-EMF exposure and 1.30 hour after ionizing irradiation) and durably expressed until 10 hours of exposure.

DISCUSSION

ELF-EMF have been recently suggested to act as promotor agents in carcinogenesis processes (9-11,13). As overexpression of protooncogenes and their products is involved in epigenetic mechanisms (14), we examined the ability of two epithelial tracheal cell lines to synthetize the c-JUN protein in response to ELF-EMF exposure, with or without a previous ionizing radiation acting as an initiator.

c-jun belongs to the early response genes family and is known to be induced until several hours after ionizing radiations (15) and enhancement in the transcription of oncogenes including c-jun was previously reported in T CEM-CM3 lymphoblastic cells after 1 hour exposure to a very similar electromagnetic signal, excepted the absence of the static field (7). The same group recently indicated that the same signal could increase the transcription of the ras oncogene (16). EMF were also demonstrated to alter polypeptide synthesis in salivary gland cells, with an increased expression of several non-identified proteins (17). Furthermore, messengers such as Ca^{2+} or inositol triphosphate, involved in signal transduction pathways and leading to the transcription of genes such as c-jun, have been reported to be responsive to ELF-EMF (18,19).

Rat tracheal cell lines are usefull in transformation assays using chemical agents (20) and have been demonstrated to be responsive to ELF-EMF after gamma irradiation. A synergistic effect of the combination of gamma irradiation and ELF-EMF exposure, depending however on the cell line tested (two of the three cell lines used were responsive), has been observed concerning micronuclei induction but no effect of ELF-EMF alone was detectable (11). In this study, data obtained in a spontaneous immortalized tracheal cell line suggested such a synergism on the delayed induction of c-JUN expression. In a second cell line isolated from irradiated primary epithelial cell culture, no synergistic effect was detectable but ELF-EMF exposure alone appeared having an effect on the

early induction and delayed expression of JUN protein. Being assumed that both cell lines used behaved in a different way after ionizing treatment, data reported earlier may be linked to the effect of the electromagnetic signal alone. Moreover, these effects could be linked to the level of transformation of cells since tracheal cell lines used in this experiment were issued from the same primary cell type but resulted from different treatments.
Further investigations are needeed to ascertain ELF-EMF effect in the spontaneous immortalized cell line on the expression of JUN and to elucidate the effectiveness of synergistic effect of ionizing radiation and ELF-EMF exposure in induction of oncogene protein which is related to act in cell transformation. Moreover, the use of primary epithelial tracheal cells may be of interest to determine the role of cell transformation level in the biological effect of ELF-EMF alone which may emphasize the hypothesis of ELF-EMF promotor effect.

REFERENCES

1. N. Wertheimer and E. Lepper, *Am. J. Epidemiol.* 109, 273-284 (1979).
2. D.A. Savitz, H. Wachtel, F.A. Barnes, E. John, J. Tvridk, *Am. J. Epidemiol.* 128, 21-38 (1988).
3. M.M. Cohen, A. Kunska, J.A. Astemborski, D. McCullooch, *Mutat. Res.* 172, 177-184 (1986).
4. M.R. Scarfi, F. Bersani, A. Cossarizza, D. Monti, G. Castellani, R. Cadossi, G. Franceschetti, C. Franceschi, *Biochem. Biophys. Res. Com.*, 176 (1), 194-200 (1993).
5. M. Fiorani, O. Cantoni, P. Sestili, R. Conti, P. Nicolini, F. Vetrano, M. Dachà, *Mutat. Res.*, 282, 25-29 (1992).
6. D.W. Fairbairn and K.O'Neill, *Cell. Mol. Biol.* 40, 561-567 (1994).
7. J.L. Phillips, W. Haggren, W.J. Thomas, T. Ishida-Jones, W.R. Adey, *Biochim. Biophys. Acta* 1132, 140-144 (1992).
8. H. Lin, R. Goodman, A. Shirley-Henderson, *J. Cell. Biochem.* 54, 281-288 (1994).
9. C.D. Cain, D.L. Thomas, W.R. Adey, *Carcinogenesis* 14 (5), 955-960 (1993).
10. D.E. Hintenlang, *Bioelectromagnetics* 14, 545-551 (1993).
11. I. Lagroye, J.L. Poncy. *In: proceedings of the 10th. ICRR*, Eds U. Hagen, H. Jung, C. Streffer. (Abstract) P28-38 (1995).
12. E. Schreiber et al., *Nucleic Acids Res.* 17, 6419 (1989).
13. R.W. West, W.G. Hinson, D.B. Lyle, M.L. Swicord, *Bioeletrochemistry and Bioenergetics* 34, 39-43 (1994).
14. G.L. Chan, *Int. Rev.Cytology* 70, 101-137 (1980).
15. M.L. Sherman, R. Datta, D.E. Hallahan, R.R. Weichselbaum, D.W. Kufe, *Proc. Natl. Acad. Sci. USA* 87, 5663-5666 (1990).
16. J.L. Phillips, W. Haggren, W.J. Thomas, T. Ishida-Jones and W.R. Adey, *Cancer Biochem. Biophys.* 13, 187-193 (1993).
17. R. Goodman and A.S. Henderson, *Proc. Natl. Acad. Sci. USA* 85, 3928-3932 (1988).
18. J.J.L. Carson, F.S. Prato, D.J. Drost, L.D. Diesbourg, S.J. Dixon, *Am. J. Physiol.* 259, C687-C692 (1990).
19. I.L. Korzh-Sleptsova, E. Lindström, K. Hansson Mild, A. Berglund, E. Lundgren, *FEBS Let.* 359, 151-154 (1995).
20. V.E. Steele, J.T. Arnold, J. Van Arnold, M.J. Mass..*Environ. Mol. Mutagenesis* 14, 48-54 (1989).