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Genotoxic and haematotoxic damage induced by ELF magnetic fields

Danno genotossico ed ematotossico indotto da campi magnetici ELF

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Summary

Aim. **To assess genotoxic and cytotoxic properties of extremely low frequency magnetic fields.** ***Materials and methods.*** **The micronucleus test was performed on peripheral blood sampled from newborn mice exposed to a 50 Hz, 650 μT magnetic field during the whole intra-uterine life (21 days) and from adult mice exposed for the same amount of time.** ***Results.*** **Chronic exposure caused an increase in micronuclei frequency and a decrease of polychromatic erythrocyte percentage in newborn mice but not in adults.** ***Conclusions.*** **This work underlines the necessity to further investigate the effects of magnetic fields on genome integrity and haemopoiesis. Eur. J. Oncol., 13 (4), 239-244, 2008**

Key words: **magnetic fields, ELF, genotoxicity, micronucleus test**

Riassunto

Finalità. **Valutare le proprietà genotossiche e citotossiche dei campi magnetici a frequenze estremamente basse.** ***Materiali e metodi.*** **Il test dei micronuclei è stato applicato su campioni di sangue periferico prelevati da topi neonati esposti ad un campo magnetico di 50 Hz a 650 μT durante l'intera vita intra-uterina (21 giorni) e su campioni di sangue periferico prelevati da topi adulti esposti per lo stesso periodo.** ***Risultati.*** **L'esposizione cronica ha causato un aumento della frequenza di micronuclei ed una diminuzione della percentuale di eritrociti policromatici nei topi neonati, ma non negli adulti.** ***Conclusioni.*** **Questa ricerca sottolinea la necessità di studiare ulteriormente gli effetti dei campi magnetici sull'integrità genomica e sull'emopoiesi. Eur. J. Oncol., 13 (4), 239-244, 2008**

Parole chiave: **campi magnetici, ELF, genotossicità, test dei micronuclei**

Introduction

The use of electric devices is associated with the production of weak electric and magnetic fields, which oscillate with a frequency corresponding to 50 Hz (in Europe and Asia) or 60 Hz (in North America). These belong to the extremely low frequency (ELF) region of the electromagnetic spec-

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trum. Because of the very large wavelengths in the ELF range, the electric and the magnetic field propagate uncoupled. In consideration of the size of the human body and the common distance from ELF-EF and ELF-MF sources, the exposure corresponds always to "near-field" conditions (less than one wavelength)[1]. Moreover, EF are easily and efficiently shielded by many materials, whereas MF usually penetrate nonferrous material and readily enter the body.

Several research groups sought to determine whether a link existed between 50/60 Hz ELF-MF and potential adverse biological effects, in order to determine the possible mechanism of cancer risk. However, the results obtained are conflicting and comparison between them is difficult, because of the many differences in parameters (duration and periodicity of the exposure, flux intensity, endpoints investigated). In addition to studies on teratogenesis[2], tumour promotion[3] and haematology[4], several authors have examined the genotoxic properties of ELF magnetic fields. Some studies have been performed on samples taken from individuals professionally exposed. Increases in micronuclei frequencies and chromosomal aberrations have been observed in the lymphocytes of photocopying machine workers[5], powerline operators and railwaymen[6-10].

Laboratory studies (especially *in vitro*) are more abundant. Several works denied the hypothesis that ELF magnetic fields have genotoxic properties[11]. Svedenstal and Johanson[12] did not detect any increase of micronucleated erythrocytes in adult mice exposed for 90 days to a 14 mT magnetic field; the same result was obtained by Abramsson-Zetterberg and Grawé[13], using an identical field, both in adult and newborn mice; Maes *et al*[14] exposed human lymphocytes (up to 2500 mT) and found no significant effect on chromosome aberrations, sister chromatid exchanges and single-strand breaks. Moreover, McNamee *et al*[15] reported no significant effect on DNA strand breaks in cerebellar cells of immature mice exposed continuously to a 60 Hz magnetic field at 1 mT for 2 h; Testa *et al*[16] detected an absence of DNA damage (using several cytogenetic assays) in human blood cells exposed *in vitro* for 48 h to a 50 Hz, 1 mT magnetic field.

On the other hand, other works detected positive results only in conditions of co-exposure with other mutagenic agents, such as static magnetic fields[17, 18], benzopyrene[19, 20], X-rays[21] and vinblastine[22]. These results led to the hypothesis that ELF magnetic fields are able to enhance, but not to start, a mutagenic event. Nevertheless, there has been an increase in the number of papers detecting the genotoxic properties of ELF magnetic fields alone, both with *in vivo* and *in vitro* exposure. Lai and Singh[23-25] found that rats exposed (either for 2 or 48 h) to a 60-Hz sinusoidal magnetic field at intensities of 10, 100 and 500 mT showed increases in DNA single- and double-strand breaks in their brain cells. Similar results were obtained by Svedenstal *et al*[26] with brain cells of CBA mice exposed for 14 days to 500 mT. Yokus *et al*[27] found a significant increase in 8-hydroxy-20-deoxyguanosine (indicative of oxidative DNA damage) in the plasma of rats exposed to 970 mT for 50 days. Another study, conducted on mouse m5S cells, detected a significant, dose-dependent increase of chromatid-type chromosomal aberrations at 5, 50 and 400 mT[28]. Ivancsits *et al*[29-31] reported an increase in DNA single- and double-strand breaks in human fibroblasts intermittently (50 on/100 off) exposed to a 50-Hz magnetic field at 1 mT. Pasquini *et al*[32] observed an increased frequency of micronuclei in Jurkat cells exposed for 24 h to 5 mT (50 Hz) magnetic fields. Wolf *et al*[33] found in HL-60 leukaemia cells, Rat-1 fibroblasts and WI-38 diploid fibroblasts, a peak in DNA strand breaks and in the formation of 8-hydroxy-20-deoxyguanosine after 24 and 72 h of exposure to 0.5 and 1.0 mT magnetic fields, while Winker *et al*[34] revealed a time-dependent increase of micronuclei in human diploid fibroblasts, which became significant after 10 h of intermittent exposure (50 on/100 off) at a flux density of 1 mT. In a recent study, genotoxic damage was detected using the micronucleus test on bone marrow samples from rats acutely and long-term exposed to a 50 Hz, 1 mT magnetic field[35].

The micronucleus test[36] is a simple *in vivo* assay used for detecting cytogenetic damage induced by chemical and physical mutagens. Micronuclei appear when a whole chromosome or a chromosome fragment fails to migrate with one of the two daughter nuclei formed during mitosis. The application of this assay is very popular, in particular on blood samples, as in the latter thousands of scorable

cells are present. Moreover, the presence of micronucleated erythrocytes from the peripheral circulation reflect events that occurred in a time equal to the lifespan of the circulating erythrocytes[37]. Therefore, the application of the micronucleus test on peripheral blood samples is particularly indicated for conditions of chronic exposure.

The aim of this work is to detect the possible genotoxic insult in newborn mice exposed to an ELF magnetic field during the whole intra-uterine life and in adult mice exposed for the same amount of time.

Materials and methods

Three female mice (CD-1 Swiss) were individually caged and exposed during pregnancy to 50 Hz, 650 μT magnetic field generated by a solenoid working 24 h per day, and 20 newborn mice were exposed until day three after birth (for a total of 21 days of exposure), when they were sacrificed. Seven adult mice were exposed to the same magnetic field for the same amount of time.

The solenoid was 0.8 m in length and 0.13 m in radius, with 552 turns of 2.5 mm^2 copper wire, wound in two layers in continuous forward-backward fashion around a cylinder of PVC. It was supplied by 50 Hz main power through a transformer. A voltage of 6.5 V (rms) has been applied to obtain a flux density of 650 μT (rms) at the centre of the solenoid. The field was uniform between ± 5% in the volume where the mice were exposed. The solenoid was not shielded for the electric field, as the induced electric field was negligible due to the low voltage used. The intensity of the field we adopted in the experiment was selected in agreement with the following considerations. We would test the effect of an industrial alternate magnetic field provided at the reference level assumed as international standards for the protection of the public against non-ionizing radiations. Since European Union (EU) Recommendation 1999/519 indicates the value of 2 mA/m^2 as basic limit for the induced current density in the human body for people exposed to the industrial alternate (under 1 kHz) magnetic field[38], we arranged the magnetic flux of the solenoid to obtain the same value for the induced current density in the mouse body. For a sinusoidal field the following relationship can be assumed between the induced current density J and the magnetic flux density B:

$$J = \pi \cdot f \cdot B \cdot R \cdot \sigma$$

where the frequency is f = 50 Hz and an homogeneous conductivity is taken σ = 0.2 S/m[39]. For determining the radius R, the length and the width of the mouse body has been measured. For a comparison the human thorax width and depth have been deduced from NASA-SRD-3000 ''Man-systems integration standards'' (50th percentile)[40]. The transverse sections of the mouse body and human thorax have been modelled by means of an ellipse whose axes are 88 mm and 26 mm, 392 mm and 250 mm, respectively. The ellipse areas are 1,797 mm^2 and 76,969 mm^2, respectively. The square root of the reverse ratio of these areas, equal to 6.5, is the ratio of the magnetic flux density levels which induce the same current density in the mouse body and in the human thorax transverse sections. Applying the above formula, ICNIRP Guidelines[41] estimate equal to 100 μT the magnetic flux density for the 50 Hz industrial frequency like the one able to induce a current density equal to the basic limit value (2 mA/m^2) in the human thorax. To obtain this current density, which in man is induced by a 100 μT magnetic field, we used a magnetic flux density of 650 μT for mice exposures, given the above ratio of 1:6.5.

Another four female mice were kept unexposed during pregnancy and 36 newborn mice were sacrificed at day 3 after birth. A positive control was carried out exposing five 3-day-old mice to X-rays (3 Gy), which were sacrificed 24 h later. Exposure to X-rays was performed in a Gilardoni apparatus (Gilardoni, Milano, Italy; 250 kV, 6 mA, 3 mm Al filter) at a 0.5 Gy/min dose rate for 6 min.

The temperature and the relative humidity of the animal room were 20-22°C and 40-50% respectively. Artificial lighting was from 8 am to 8 pm and commercial pellets and tap water were available *ad libitum* throughout the experimental period. The temperature inside the coils was the same as in the room.

Blood was collected from jugular veins and smears were air dried, fixed with absolute methanol and stained with May-Grünwald and Giemsa. Micronuclei were scored at 1,000x magnification using a Zeiss Axiophot. The samples were random-

ized, coded, and scored blindly by a single microscopist. For each animal, 2,000 erythrocytes were analyzed. The percentage of polychromatic erythrocytes (PCEs) was assessed on the first 1,000 erythrocytes.

The Cox transformation ($\sqrt{}$ x+0,5) was applied to stabilize the variance because the absolute values of micronuclei were not normally distributed. The Student's t-test was applied to the transformed data. The level of significance was established at $p < 0.05$. All analyses were carried out using the STATISTICA 6.0 package (StatSoft, Tulsa, OK).

Results

Table 1 shows the results of the micronucleus test performed on newborn and adult mice. In newborn mice, micronuclei frequencies of both ELF- and X-rays-irradiated mice resulted significantly higher than in the control group (both $p < 0.00001$). Concerning the PCE percentage, the observed decrease was significant both in the ELF-exposed ($p = 0.0485$) and in the X-irradiated group ($p = 0.00008$).

In adults, micronuclei frequencies were higher in the ELF- and X-rays-irradiated mice, but the difference resulted significant only in the latter ($p = 0.00001$). Similarly, the decrease of the PCE percentage was significant only in the X-irradiated group ($p = 0.00564$).

Comparing newborn with adult mice, there were no significant differences between micronuclei frequencies of the control groups. On the contrary the differences between newborn and adult ELF-exposed and newborn and adult X-irradiated resulted statistically significant ($p < 0.00001$ and $p = 0.04$, respectively). The differences between the PCE percentages of the newborn and adult mice were significant in the control ($p = 0.00009$) and the ELF-exposed groups ($p < 0.00001$), but not between the X-irradiated groups.

Discussion

The purpose of this study was to investigate the possible genotoxic and haematotoxic insult in newborn and adult mice chronically exposed to a 50-Hz, 650 mT ELF magnetic field. Chronic exposure during the whole intra-uterine life (21 days), caused in newborn mice a significant increase in micronuclei frequency. This result disagrees with that of Abramsson-Zetterberg and Grawé[13], who used a weaker field (14 μT), exposing the animals for 18 days during pregnancy and sacrificing the offspring 35 days after birth. However, it confirms our previous work, with the same experimental set-up, but using the CREST-staining micronucleus assay[42].

Chronic exposure did not provoke a significant increase in micronuclei mean frequencies in adult mice. This result is in agreement with those obtained by Svedenstal and Johanson[12] and by Abramsson-Zetterberg and Grawé[13], who both exposed mice to a weaker magnetic field (14 μT), but disagrees with Erdal *et al* [35], who found a significant increase in

Table 1 - Frequencies of micronucleated erythrocytes in peripheral blood

	N. of animals	Mean ME/1000 E ± SD[a]	Mean % PCE[b] ± SD
Newborns			
Controls	36	1.39 ± 0.70	17.16 ± 5.53
ELF	20	3.50 ± 1.00***	14.44 ± 3.16*
X-rays	5	12.30 ± 4.28***	6.04 ± 1.90***
Adults			
Controls	7	1.21 ± 0.49	7.90 ± 2.25
ELF	7	1.50 ± 0.58	6.69 ± 0.80
X-rays	5	7.30 ± 2.51***	4.16 ± 0.82**

[a] ME = Micronucleated Erythrocytes; E = Erythrocytes; SD = Standard Deviation

[b] PCE = polychromatic erythrocytes;

Significance respect to sham: *$p < 0.05$; **$p < 0.01$; ***$p < 0.0001$

micronucleated erythrocytes sampled from bone marrow of adult rats exposed four hours per day for 45 days to a 1 mT, 50 Hz magnetic field.

Exposure to this intense magnetic field during the whole intra-uterine life caused a decrease of the percentage of polychromatic erythrocytes. Erdal *et al*[35] found a similar decrease in the bone marrow of ELF-exposed adult rats. This data may indicate a cytotoxic insult exerted by magnetic fields on erythropoietic organs.

The different results obtained in blood samples taken from newborn and adult mice may be due to a greater sensitiveness of the first[43, 44].

Conclusion

The results obtained in this work underline the necessity to further investigate, possibly *in vivo*, the effects of magnetic fields on genome integrity and haemopoiesis in conditions of chronic exposure.

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References

1. Polk C, Postow E. Handbook of biological effects of electromagnetic fields, 2nd ed. New York: Academic Press, 1995.
2. Levin M. Bioelectromagnetics in morphogenesis. Bioelectromagnetics 2003; 24: 295-315.
3. Lacy-Hulbert A, Metcalfe JC, Hesketh R. Biological responses to electromagnetic fields. FASEB J 1998; 12: 395-420.
4. Ali FM, Mohamed WS, Mohamed MR. Effect of 50 Hz, 0.2 mT magnetic fields on RBC properties and heart functions of albino rats. Bioelectromagnetics 2003; 24: 535-45.
5. Iravathy Goud K, Hasan Q, Balakrishna N, *et al*. Genotoxicity evaluation of individuals working with photocopying machines. Mutat Res 2004; 563: 151-8.
6. Nordenson I, Mild KH, Nordstrom S. Clastogenic effects in human lymphocytes of power frequency electric fields. Radiat Environ Biophys 1984; 23: 191-201.
7. Nordenson I, Mild KH, Ostman U. Chromosome effects in lymphocytes of 400 kV-substation workers. Radiat Environ Biophys 1988; 27: 39-47.
8. Nordenson I, Mild KH, Jarventaus H, *et al*. Chromosomal aberrations in peripheral lymphocytes of train engine drivers. Bioelectromagnetics 2001; 22: 306-15.
9. Skyberg K, Hansteen IL, Vistnes AI. Chromosome aberrations in lymphocytes of high-voltage laboratory cable splicers exposed to electromagnetic fields. Scand J Work Environ Health 1993; 19: 29-34.
10. Valjus J, Norppa H, Jarventaus H. Analysis of chromosomal aberrations, sister chromatid exchanges and micronuclei among power linesmen with long-term exposure to 50-Hz electromagnetic fields. Radiat Environ Biophys 1993; 32: 325-36.
11. Vijayalaxmi G, Obe G. Controversial cytogenetic observations in mammalian somatic cells exposed to extremely low frequency electromagnetic radiation: a review and future research recommendations. Bioelectromagnetics 2005; 26: 412-30.
12. Svedenstal BM, Johanson KJ. Leukocytes and micronucleated erythrocytes in peripheral blood from mice exposed to 50-Hz or 20-kHz magnetic fields. Electro Magnetobiol 1998; 17: 127-43.
13. Abramsson-Zetterberg L, Grawé J. Extended exposure of adult and fetal mice to 50 Hz magnetic field does not increase the incidence of micronuclei in erythrocytes. Bioelectromagnetics 2001; 22: 351-7.
14. Maes A, Collier M, Vandoninck S, *et al*. Cytogenetic effects of 50 Hz magnetic fields of different magnetic flux densities. Bioelectromagnetics 2000; 21: 589-96.
15. McNamee JP, Beller PV, McLean JRN, *et al*. DNA damage and apoptosis in the immature mouse cerebellum after acute exposure to a 1 mT, 60 Hz magnetic field. Mutat Res 2002; 513: 121-33.
16. Testa A, Cordelli E, Stronati L, *et al*. Evaluation of genotoxic effect of low level 50 Hz magnetic fields on human blood cells using different cytogenetic assays. Bioelectromagnetics 2004; 25: 613-9.
17. Tofani S, Ferrara A, Anglesio L, *et al*. Evidence for genotoxic effects of resonant ELF magnetic fields. Bioelectrochem Bioenerg 1995; 36: 9-13.
18. Miyakoshi J, Yoshida M, Shibuya K, *et al*. Exposure to strong magnetic fields at power frequency potentiates X-ray induced DNA strand breaks. J Radiat Res 2000; 41: 293-302.
19. Cho YH, Chung HW. The effect of extremely low frequency magnetic fields (ELF-EMF) on the frequency of micronuclei and sister chromatid exchange in human lymphocytes induced by benzo(a)pyrene. Toxicol Lett 2003; 143: 37-44.
20. Moretti M, Villarini M, Simonucci S, *et al*. Effects of co-exposure to extremely low frequency (ELF) magnetic fields and benzene or benzene metabolites determined *in vitro* by the alkaline comet assay. Toxicol Lett 2005; 157: 119-28.
21. Ding GR, Nakahara T, Miyakoshi J. Induction of kinetochore-positive and kinetochore-negative micronuclei

in CHO cells by ELF magnetic fields and/or X-rays. Mutagenesis 2003; 18: 439-43.
22. Verheyen GR, Pauwels G, Verschaeve L, *et al*. Effect of coexposure to 50 Hz magnetic fields and an aneugen on human lymphocytes, determined by the cytokinesis block micronucleus assay. Bioelectromagnetics 2003; 24: 160-4.
23. Lai H, Singh NP. Acute exposure to a 60 Hz magnetic field increases DNA strand breaks in rat brain cells. Bioelectromagnetics 1997; 18: 156-65.
24. Lai H, Singh NP. Melatonin and N-tert-butyl-a-phenyl-nitrone block 60-Hz magnetic field-induced DNA single and double strand breaks in rat brain cells. J Pin Res 1997; 22: 152-62.
25. Lai H, Singh NP. Magnetic-field – induced DNA strand breaks in brain cells of the rat. Environ Health Perspect 2004; 112: 687-94.
26. Svedenstal BM, Johanson KJ, Hansson MK. DNA damage induced in brain cells of CBA mice exposed to magnetic fields. In Vivo 1999; 13: 551-2.
27. Yokus B, Cakir D, Akdag MZ, *et al*. Oxidative DNA damage in rats exposed to extremely low frequency electromagnetic fields. Free Radiat Res 2005; 39: 317-23.
28. Yaguchi H, Yoshida M, Ding GR, *et al*. Increased chromatid-type chromosomal aberrations in mouse m5S cells exposed to power-line frequency magnetic fields. Int J Radiat Biol 2000; 76: 1677-84.
29. Ivancsits S, Diem E, Pilger A, *et al*. Induction of DNA strand breaks by intermittent exposure to extremely-low-frequency electromagnetic fields in human diploid fibroblasts. Mutat Res 2002; 519: 1-13.
30. Ivancsits S, Diem E, Jahn O, *et al*. Intermittent extremely low frequency electromagnetic fields cause DNA damage in a dose-dependent way. Int Arch Occup Environ Health 2003; 76: 431-6.
31. Ivancsits S, Diem E, Jahn O, *et al*. Age-related effects on induction of DNA strand breaks by intermittent exposure to electromagnetic fields. Mech Age Dev 2003; 124: 847-50.
32. Pasquini R, Villarini M, Scassellati-Sforzolini G, *et al*. Micronucleus induction in cells co-exposed *in vitro* to 50 Hz magnetic field and benzene, 1,4-benzenediol (hydroquinone) or 1,2,4-benzenetriol. Toxicol in vitro 2003; 17: 581-6.
33. Wolf FI, Torsello A, Tedesco B, *et al*. 50-Hz extremely low frequency electromagnetic fields enhance cell proliferation and DNA damage: possible involvement of a redox mechanism. Biochem Biophys Acta 2005; 1743: 120-9.
34. Winker R, Ivancsits S, Pilger A, *et al*. Chromosomal damage in human diploid fibroblasts by intermittent exposure to extremely low-frequency electromagnetic fields. Mutat Res 2005; 585: 43-9.
35. Erdal N, Gürgül S, Celik A. Cytogenetic effects of extremely low frequency magnetic field on Wistar rat bone marrow. Mutat Res 2007; 630: 69-77.
36. Schmid W. The micronucleus test. Mutat Res 1975; 31: 9-15.
37. Schlegel R, MacGregor JT. The persistence of micronuclei in peripheral blood erythrocytes: detection of chronic chromosome breakage in mice. Mutat Res 1982; 104: 367-9.
38. EU Council Recommendation July 19, 1999, 1999/519/CE, Annex II, EU L199/59.
39. CENELEC, 1995. ENV 50166-1 European Standard, Annex B.3.
40. NASA. 1995. NASA-STD-3000 Man-Systems Integration Standards, Revision B, Vol. I, 3.2.
41. ICNIRP Guidelines. Health Phys 1998; 75 (4): 494.
42. Udroiu I, Cristaldi M, Ieradi LA, *et al*. Clastogenicity and aneuploidy in newborn and adult mice exposed to 50 Hz magnetic fields. Int J Radiat Biol 2006; 82: 561-7.
43. Bishop JB, Witt KL, Tice RR, *et al*. Genetic damage detected in CD-1 mouse pups exposed perinatally to 3'-azido-3'-deoxythymidine and dideoxyinosine via maternal dosing, nursing, and direct gavage. Environ Mol Mutagen 2004; 43: 3-9.
44. Udroiu I, Ieradi LA, Cristaldi C, *et al*. Detection of clastogenic and aneugenic damage in newborn rats. Environ Mol Mutagen 2006; 47: 320-4.