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Short communication

Extremely Low Frequency-Magnetic Fields (ELF-EMF) occupational exposure and natural killer activity in peripheral blood lymphocytes

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ABSTRACT

Extremely Low Frequency-Magnetic Fields (ELF-MF) are possible carcinogens to humans and some data suggest that they can act as promoters or progressors. Since NK cells play a major role in the control of cancer development, an adverse effect on ELF-MF on NK function has been hypothesized. We examined NK activity in 52 workers exposed to different levels of ELF-MF in various activities. Individual exposure was monitored during 3 complete work-shifts using personal dosimeters. Environmental exposure was also monitored. ELF-MF levels in the workers were expressed as Time-Weighted Average (TWA) values. NK activity was measured in peripheral blood lymphocytes (PBL). In the whole group the median occupational TWA was 0.21 μT. According to the TWA levels, workers were classified as low exposed (26 subjects, TWA ≤0.2 μT) and higher exposed workers (26 subjects; TWA >0.2 μT). In higher exposed workers, we observed a trend to reduce NK activity compared to low exposed, but the difference was not significant. Then we selected a subgroup of highest exposed workers (12 subjects; TWA >1 μT); no difference was observed between low and highest exposed subjects in the main personal variables. Considering both *E:T* ratios from 12:1 to 50:1 and Lytic Units, a significant reduction in NK activity was observed in the highest exposed workers compared to the low exposed. Multivariate analysis showed a significant negative correlation between exposure and LU, while no correlation was evidenced with other personal characteristics. ELF-MF are considered possible carcinogens, and existing data suggest that they can act as promoters. Due to the role of NK activity in host defence against cancer, the results obtained in this study in workers exposed to ELF-MF levels exceeding 1 μT are in agreement with this hypothesis, and support the need for further investigation in this field.

1. Introduction

Exposure to Extremely Low Frequency-Magnetic Fields (ELF-MF) induced by power lines and electrical appliances is currently ubiquitous both in the environment and at the workplace. An epidemiological association between ELF-MF childhood leukemia was reported by Wertheimer and Leeper (1979). These results stimulated an intensive research into the

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possible carcinogenic effect of ELF, and in the following years several increasingly sophisticated epidemiological studies were performed. According to an overall evaluation of the results, ELF-MF are considered as possible carcinogens (NRPB, 2001; ICNIRP, 2001; Feychting et al., 2005; WHO, 2007), and in 2002 the International Association for Research on Cancer (IARC) has classified exposure to ELF-MF in the Group 2B (possible carcinogens) (IARC, 2002). This evaluation is mainly based on the results of studies on childhood leukemia, but the classification applies to all human exposure to ELF-MFs', including occupational exposure (Mild et al., 2005). Nevertheless, the evidence for other cancers except leukemia is considered inadequate (NRPB, 2001; ICNIRP, 2001; IARC 2002; Feychting et al., 2005; WHO, 2007). The conclusion is that, after more than 25 years of research, the results on carcinogenicity of ELF are still scarcely conclusive (Feychting et al., 2005; WHO 2007).

A major weakness in epidemiological studies on the adverse effect of ELF-MF is the assessment of exposure, a recognized cause for the inconclusiveness of the overall results of research in this field. The problem is that ELF are ubiquitous, have multiple sources and can vary greatly over time and in short distances; this can easily result in a large misclassification of subjects and/or groups, reducing the possibility of detecting an association between exposure and adverse effects (if any) (ICNIRP, 2001; Feychting et al., 2005).

Another problem is the lack of a definite mechanism for the carcinogenic effect of ELF: few data support a direct genotoxicity while some evidence exists that they can act as promoters or progressors (Feychting et al., 2005). Since the immune system plays a primary role in the control of cancer development, the possibility of an effect of ELF-MF on immune function has been hypothesized (Tuschl et al., 2000; NRPB, 2001), but never demonstrated to date (WHO, 2007).

NK cells represent an important effector mechanism for non-specific host defence, and play a major role in inhibiting tumour growth (Whiteside and Herberman, 1995). In animals ELF-MF were reported both to enhance or impair the activity or the number of circulating natural killer (NK) cells (McLean et al., 1991; Tremblay et al., 1996; House et al., 1996; Bonhomme-Faivre et al., 1998a; Bonhomme-Faivre et al., 2003; House and McCormick, 2000) while no effect was observed in other studies (Thun-Battersby et al., 1999). Selmaoui et al. (1996) and Graham et al. (2001) have not observed any definite evidence for field-related effects in humans, by using experimentally controlled MF exposures. Exposure in occupational and/or residential environments has been linked either to a reduction (Del Signore et al., 2000; Boscolo et al., 2001a,b; Ichinose et al., 2004; Di Giampaolo et al., 2006) or an increase (Bonhomme-Faivre et al., 1998b; Tuschl et al., 2000; Bonhomme-Faivre et al., 2003) in NK cell counts, while scant attention was devoted to NK activity to date.

Accordingly, we studied the possible effect of occupational ELF-MF exposure on NK cytotoxic activity in peripheral blood lymphocytes (PBL) of workers. To reduce the risk of misclassification of exposure we measured individual ELF-MF exposure of all participants during 3 consecutive work-shifts using personal dosimeters, and also monitored environmental, non-occupational, exposure.

2. Materials and methods

2.1. Subjects

We studied a group of workers engaged in different occupational activities not involving any exposure to chemical/physical factors known to affect the immune system.

The study was designed and performed in compliance with all relevant regulations in force in our country: we presented the study to the workers, who were informed that participation was voluntary, and that they were free to withdraw from the study at any time. Then we obtained a written informed consent from all the participants. No subject refused to participate or withdraw from the study. At the end of the study, each participant received a copy of his/her results, with a brief comment, and further information was provided on demand.

Subjects were administered a questionnaire on personal history, lifestyle and occupational factors; smoking and alcohol habits, sleeping habits, job task(s), occupational or avocational exposure to chemical and physical agents; questions were asked concerning current and past diseases, and use of pharmaceutical drugs.

The following criteria were applied for eligibility to the study: no known occupational or avocational exposure to chemicals or other factors interfering with the immune system, no current acute or chronic diseases and/or pharmaceutical drugs consumption that might influence immune function.

Fifty two workers (22 men and 30 women) engaged in various occupations were included in the study: 25 in clothing production, 6 in electrical occupations, 4 in the mechanics industry, 7 in the local utilities company, 5 in schools and 5 in various activities. The main characteristics of the group are summarised in Table 1.

2.2. Exposure to magnetic fields

Individual ELF-MF exposure was measured using personal monitors (EMDEX Lite, Enertech Consultants, Campbell, CA, USA) worn in a belted pouch on the hip. To take into account within-days variability, we monitored three complete work-shifts during a normal working week. The environmental exposure was also monitored to evaluate the relative contribution of this component to overall exposure. Sampling period started at the beginning of the Monday morning work-shift (usually at 8.00–9.00 depending on the specific occupation), and the subjects wore the dosimeter until Thursday morning, to include 3 complete days. All participants were asked to note down in a one-page diary, for each of the 3 days monitored, the periods of the day spent at work, at home and elsewhere. During sleep hours, subjects were instructed to leave the instrument beside the bed, close to the body, but not near electrical devices such as an alarm-clock or radio.

ELF-MF were sampled at 10 second intervals resulting in more than 8600 records for each worker during the work-shifts (8 h for 3 days), and other 17,200 during the non-working period.

Based on the diary, each day was divided in working and non-working hours: occupational and environmental (non-

Table 1 – Main characteristics in the examined workers

Characteristics	Entire group	Low exposure (TWA ≤0.2 μT)	Higher exposure (TWA >0.2 μT)	Highest exposure subgroup (TWA >1 T)
No.	52	26	26	12
Men (%)	22 (42.3)	13 (50.0)	9 (34.6)	2 (16.7)
Women (%)	30 (57.7)	13 (50.0)	17 (65.4)	10 (83.3)
Age (yr)				
Mean ± SD	40.42 ± 7.93	44.54 ± 6.38	36.31 ± 7.24	37.33 ± 6.76
Previous exposure (yr)				
Mean ± SD	12.8 ± 8.2	15.2 ± 8.4	10.4 ± 7.4	11.77 ± 9.27
BMI (kg/m^2)				
Mean ± SD	23.38 ± 3.89	23.79 ± 3.40	22.97 ± 4.36	20.87 ± 2.38
Current smokers (%)	13 (25.0)	6 (23.1)	7 (26.9)	3 (25.0)
Non-smokers (%)	28 (53.8)	13 (50.0)	15 (57.7)	7 (58.3)
Former smokers (%)	11 (21.2)	7 (26.9)	4 (15.4)	2 (16.7)
Physical activity				
No (%)	29 (55.8)	17 (65.4)	12 (46.2)	7 (58.3)
Yes (%)	23 (44.2)	9 (34.6)	14 (53.8)	5 (41.7)

The difference among the groups is not significant.

occupational) ELF-MF exposure were estimated as Time-Weighted Average (TWA) calculated as the arithmetic mean of all measurements throughout the work-shifts and during periods not at work (Occupational and Environmental TWA respectively). For each worker the mean of TWAs for the 3 days monitored was calculated, and this value was adopted as individual TWA, to reduce possible variability of exposure. All values are expressed as microtesla (μT).

2.3. *Analytical methods*

Blood samples were collected by venipuncture on Thursday morning. Immediately after collection, blood was transferred into heparinized tubes and immediately taken to the laboratory for NK cells analysis. Individual ELF-MF exposure was blind to the co-authors involved in NK analysis.

Lymphocytes were separated using a modified Ficoll-Paque (Pharmacia Fine Chemicals, Uppsala, Sweden) density-gradient centrifugation procedure, resuspended in complete medium: i.e., RPMI 1640 supplemented with heat-inactivated fetal calf serum (100 ml/l), penicillin (950 kIU/l), and streptomycin (50 mg/l), all purchased from Gibco (BRL, Paisley, Scotland, UK), and counted using a Bürker chamber. The cell number was then adjusted to 5×10^6 cells/ml and used to prepare lower concentrations by serial two fold dilutions in complete medium. The human erythroleukemic cell line K562 was used as target (Lozzio and Lozzio, 1975).

The measurement of NK cell activity was determined using the traditional chromium-51 (^{51}Cr) release assay. The release assay has been extensively used in the evaluation of natural killer (NK) cell activity for the direct detection of target cell lysis (Brunner et al., 1968; House and McCormick, 2000).

Briefly, to label target cells, we added 100 μCi of ^{51}Cr (sodium chromate in aqueous solution, The Radiochemical Centre, Amersham, England; specific activity 250–500 mCi/mg of Cr) to 1.5 ml of medium and ~3–5×10^6 cells. After overnight incubation at 37 °C, the cells were washed, adjusted to a concentration of 5×10^4 cells/ml, and 100 μl of labelled target cells was pipetted into 96-well round bottom microplates. An equal volume (100 μl) of different concentrations of effector cells was added to give effector:target (*E:T*) ratios from 100:1 to 6:1. Assay of each concentration was performed in triplicate. After a 4-hour incubation, the plates were centrifuged and 100 μl of supernatant was immediately collected for measuring ^{51}Cr release. Spontaneous release was determined by incubating target cells with complete medium only (0.2 ml), and the maximum release by lysing cells with a detergent (Nonidet P40, BDH, final concentration 2% v/v).

The radioactivity from the ^{51}Cr release assay was measured in a CompuGamma counter (Beckman 5500). The percentage of specific marker release was calculated according to the formula:

$$\%\ \text{of specific lysis} = \frac{\text{experimental release} - \text{spontaneous release}}{\text{maximum release} - \text{spontaneous release}} \times 100.$$

Details of this method are reported in previous works (Borella et al., 1995). Values are also expressed as number of Lytic Units in 10^7 cells (LU/10^7 cells); 1 LU is defined as the number of effector cells killing 30% of the target cells.

2.4. *Statistical analyses*

The normality of the variables was evaluated with the Kolmogorov–Smirnov test; log transformation of data was applied when the distribution was not normal. Between group comparisons were assessed by Student's *t* test and/or by Chi-square test. Multiple regression analysis was performed using log LU/10^7 cell values as the dependent variable, and sex, age, BMI, smoking habits, coffee, alcohol and drug intake, physical activity, sleeping hours and duration of exposure in the current job as independent variables. We applied $p<0.05$ as significance level.

All statistical analyses were performed using the SPSS 15.0 statistical package for Windows.

3. Results

In the entire group the occupational exposure was estimated based on the mean of individual TWA of all workers included

in the study: the overall occupational mean TWA was 0.58 μT (SD 0.80), the median 0.21 μT. Similarly, we calculated environmental exposure: the mean TWA resulted 0.04 μT (SD 0.02), i.e. less than 1/10 than the occupational one. The results of individual monitoring show that, at least in the group examined, the relative contribution of the environmental component to the overall exposure is scarcely significant.

We then stratified the workers into two groups according to the occupational exposure: low exposed (TWA ≤0.2 μT; no. 26 workers) and higher exposed (TWA >0.2 μT; no. 26 workers). This cut-off (0.2 μT) was adopted based on epidemiological results suggesting an absence of any increase of the risk for chronic adverse effects below this level (Floderus et al., 1993). Low and higher exposed workers did not significantly differ in the general characteristics (Table 1).

The comparison of NK activity in PBL of low *vs* higher exposed workers is presented in Fig. 1a: the % of lysis at the different *E:T* ratios is always reduced in higher exposed workers, even if the difference is not statistically significant. Also by expressing NK activity as mean Lytic Units (LU/10^7 cells) the comparison shows a clear decreasing trend (47.41 *vs* 74.57 in higher and low exposed respectively; $p=0.075$) (Fig. 2a).

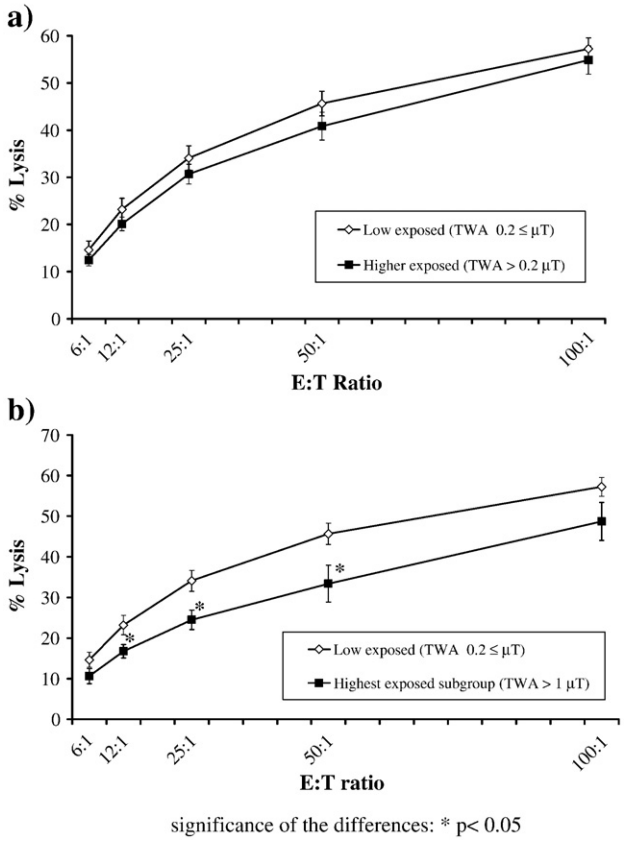

Fig. 1 – a) NK activity at different *E:T* ratios (mean ± SE) in 26 low exposed workers (TWA ≤0.2 μT) *vs* 26 higher exposed workers (TWA >0.2 μT). b) NK activity at different *E:T* ratios (mean ± SE) in 26 low exposed workers (TWA ≤0.2 μT) *vs* 12 workers exposed at ELF-MF levels >1.0 μT.

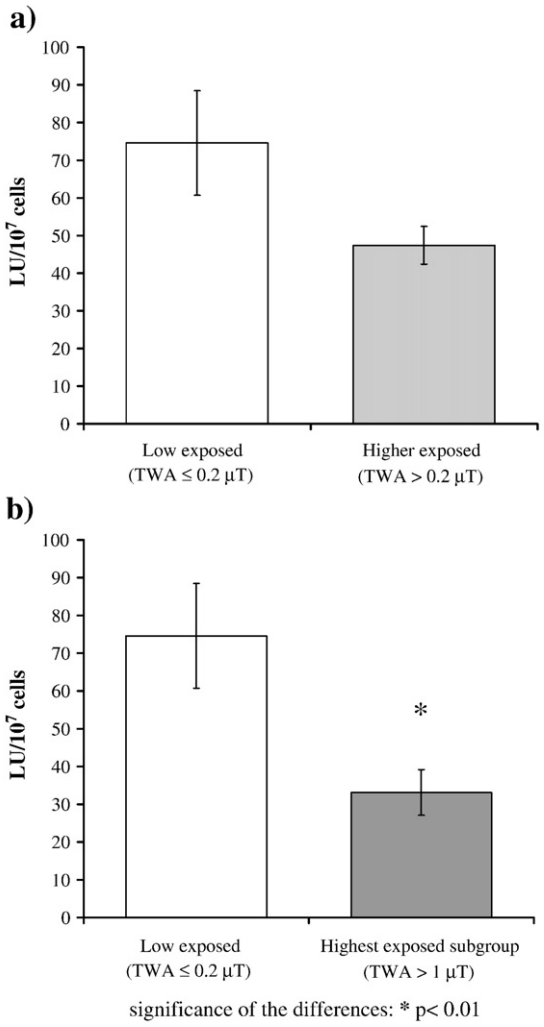

Fig. 2 – Comparison of number of Lytic Units (LU), mean ± SE, in low *vs* higher (a), and in low *vs* the subgroup of workers exposed to TWA levels exceeding 1 μT (b).

We then decided to evaluate NK activity in the group of the highest exposed workers, i.e. excluding the intermediate ELF-MF levels: we selected 12 workers (10 in clothing production and 2 substation workers from a local utility company) exposed to TWA levels exceeding 1 μT (highest exposed workers), and compared NK activity *vs* the low exposed. The main characteristics of the subgroups are shown in Table 1: again, the difference between the two groups in personal variables was not significant.

The comparisons of NK activity in the highest exposed workers *vs* the low exposed are shown in Figs. 1b and 2b: the cytolysis curve shows a significant reduction of the activity at 12:1, 25:1 and 50:1 *E:T* ratios (Fig. 1), and the number of LU/10^7 is significantly lower (Fig. 2).

Multivariate regression analysis showed a significant negative correlation between NK activity, evaluated as LU, and exposure ($r=-0.291$, $p<0.05$), while no correlation was observed with demographic and professional characteristics (data not presented).

4. Discussion and conclusions

In the evaluation of the results we must consider that, at least in industrialized countries, exposure to ELF-MF is currently ubiquitous, so virtually all workers are exposed to some level; so the selection of groups of "not exposed controls" is almost impossible. Nevertheless, available epidemiological data support the hypothesis that the occurrence of long term adverse effects in humans is not expected at ELF-MF levels lower than 0.2 μT (Floderus et al., 1993). Accordingly, it is reasonable to consider subjects exposed to levels lower than 0.2 μT proxy as "not exposed subjects".

We measured individual ELF-MF exposure level using personal dosimeters, and monitored 3 complete days to account for possible within-days variability of exposure, so that the TWA levels calculated can be considered representative of the real individual exposure of the examined workers. Furthermore, the environmental exposure was also monitored. In the group of 26 low exposed workers both occupational and environmental exposure was lower than 0.2 μT, so we decided to consider this group as the control group.

We compared the results obtained in this group *vs* the higher exposed workers (TWA above 0.2 μT): a clear trend to a reduction in NK cell activity in PBL can be observed (Figs. 1a and 2a). The difference becomes significant when low exposed are compared to the subgroup of the highest exposed workers (TWA >1 μT): both the cytolysis curve (*E:T* ratios: 12:1, 25:1 and 50:1) and number of Lytic Units (LU) are significantly lower ($p<0.01$) (Figs. 1b and 2b).

We tried to identify factors other than ELF-MF exposure to explain the results obtained.

The main factors known to affect NK activity, including recent infections, vaccinations, radiological examinations, pharmaceutical drugs or other chemical or physical agents interfering with NK activity were excluded in the examined group. No significant difference was observed among the groups for all the personal variables. Mean age was relatively higher in the low exposed, (Table 1) but this could lead to a reduction, rather than to an increase in NK activity, as observed. Both men and women were included in this study: the proportion of women was lower among higher and highest exposed subjects, nevertheless no significant gender-related differences in NK function were observed in our workers at multivariate analysis, and the correction of results for gender (data not reported) did not modify the results.

Workers included in the study were engaged in various occupations in different sectors, excluding, or at least minimizing, the possible occurrence of unknown occupational factors inducing the observed results.

Accordingly, we could not identify reasons other than ELF-MF exposure to explain the reduction in NK activity observed in our workers.

To our knowledge this is the first study reporting this effect in humans, even if suppression of NK activity was observed in experimental exposure in animals (House et al., 1996; House and McCormick, 2000; Canseven et al., 2006).

As regards epidemiological research in humans, the large majority of authors evaluated NK cell count rather than NK activity. In the only similar study found, Ichinose et al. (2004) failed to evidence any exposure related effect on NK activity in 60 electric utility workers, even if a reduction in NK cell counts was observed.

In 15 workers exposed to levels of ELF-MF exceeding 1 μT, a reduction in blood NK cells was reported by Boscolo et al. (2001a), while a rise was reported by Bonhomme-Faivre et al. (1998a, 2003) in 13 and 6 workers exposed to 0.2–6.6 μT respectively, and by Tuschl et al. (2000) in 13 induction heating workers.

In conclusion, our data suggest that occupational exposure to ELF-MF, at least at TWA levels exceeding 1 μT, may induce a reduction of NK activity in PBL.

The biological significance of these changes is still to be elucidated, but we have to consider that ELF-MF are possible carcinogens (Group 2B), and available data suggest that they could act as promoters or progressors (IARC, 2002): due to the role played by NK activity in host defence against cancer, the effect of ELF-MF observed in this study is possibly in agreement with this hypothesis. This result supports the need for further investigation.

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