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Electromagnetic fields low levels altered the DNA infrared region in RA-differentiated SH-SY5Y neuroblastoma cells

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ABSTRACT

Differentiated neuroblastoma cells (SH-SY5Y) were used to study the effects of low static magnetic fields (SMFs) and extremely low frequency electromagnetic fields (ELF-EMFs) on the structure of DNA.

Fourier Transform Infrared (FTIR) spectroscopy analysis showed that exposures to a SMF at 2 mT and to a 50 Hz EMF at 0.7 mT produced peak's centroid shift and significant decreases of the phosphate absorption bands in the infrared region of DNA.

The exposure also induced significant increase in reactive oxygen species (ROS) production in comparison to control confirming this scenario.

KEYWORDS

SH-SY5Y neuroblastoma cells;
FTIR spectroscopy;
Electromagnetic fields;
Reactive oxygen species;
DNA.

INTRODUCTION

The International Agency for Research on Cancer (IARC) formally evaluated the effects on human health from exposure to static and ELF-EMFs, concluding that it is possible that certain cellular processes altered by exposure to ELF-EMFs, indirectly affect the structure of DNA, causing strand breaks and other chromosomal aberrations[1].

Exposure to ELF-EMFs has been proposed as a possible explanation for the association with childhood leukaemia[2], indicating changes in DNA structure.

Previous observation[3] reported increased cell proliferation, changes in cell cycle and increased DNA damage, in HL-60 leukaemia cells exposed to 50 Hz magnetic fields at 0.5–1 mT up to 72 hours. A possible explanation is that the exposure of organic systems to ELF-EMF can interfere with some metabolic processes, modifying intracellular enzymatic pathways and causing an increase in the production of free radicals which may alter or interfere with DNA reparation or replication mechanisms, protein and lipid-containing structures[1].

Increasing free radical concentration creates oxidative stress and some biological reactions including DNA damage occur under this condition[4].

In particular, it has been shown that extremely low-frequency EMF enhanced the effect of oxidative stress on DNA damage[5].

Normal human neuronal cell cultures (FNC-B4) were exposed to a SMF of 0.2 T for a short period of 15 min[6]. It was shown a change in morphology and

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thymidine incorporation, indicating decreased DNA synthesis and consequent inhibition of proliferation.

These results led us to use neuronal-like cells as an experimental model of living cells to highlight further evidence of EMFs effects on the DNA structure.

MATERIALS AND METHODS

The human neuroblastoma cell line SH-SY5Y (CRL-2266) (ATCC, Rockville, Maryland, US) were used. Foetal bovine serum (FBS), antibiotics, Minimum Essential Medium (MEM) Eagle (M5650), Nutrient Mixture F-12 Ham (M4888), all-trans retinoic acid (RA), sodium pyruvate, phosphate buffered saline (PBS) solution and other chemicals of analytical grade were from Sigma, Milan, Italy.

Cell culture and treatment

The SH-SY5Y cells were cultured in a 1:1 mixture of MEM and Ham's F12 medium containing 10% (v/v) heat inactivated FBS, L-glutamine (2 mM), sodium pyruvate (1 mM) and maintained at 37 °C in a humidified incubator with 5% CO_2 and 95% air. Neuron-like cells were obtained by retinoic-acid-induced differentiation as previously reported[7].

The exposure system consisted of two Helmholtz coils, with pole pieces of round parallel polar faces, to produce a uniform magnetic field at the centre of the coils distance.

The Helmholtz coils were driven by a power amplifier in current mode (7570 AE, Techron, Elkhart, IN) and a function generator (Model 75, Wavetek, Plainsview, NY) to provide a direct current and it was used to generate time-varying EMFs at 50 Hz by means of a AC voltage.

Cell cultures were placed at the centre of the uniform field area, whose magnetic field intensity was monitored by a GM07 Gaussmeter probe (HIRST-Magnetic Instruments Ltd, Falmouth, Cornwall, UK).

The coils and samples were located into a incubator (series 5400-115V models, Thermo Electron Corporation, Winchester, VA, USA) in a 5% CO_2 / 95% air humidified at the temperature of 37.1 °C.

Not-exposed samples were placed into another incubator of the same model at the same physical conditions.

Infrared spectroscopy

For FTIR analysis cells were disaggregated using a trypsin solution to form single cell suspensions for FTIR measurements[8,9].

Indeed, the absorption in the spectral region assigned to nucleic acids can change following apoptosis[10].

For each spectrum 128 interferograms were collected and co-added by Fourier transformed employing a Happ-Genzel apodization function to generate a spectrum with a spectral resolution of 4 cm^{-1}.

Infrared (IR) spectra of water solution were subtracted from acquired spectra at the corresponding temperature. Each measure was performed under vacuum to eliminate minor spectral contributions due to residual water vapour.

The IR spectra were baseline corrected by means of automatic baseline scattering correction function, to subtract baselines from spectra, which allows to get spectra with band edges of up to the theoretical baseline.

The spectra were successively area normalized for exposed and not-exposed samples and vector normalization was used. In addition, interactive baseline rubberband correction was used to minimize water band contributions.

Measurement of intracellular ROS

Intracellular reactive oxygen species (ROS) production was quantified by 22 ,72 -dichlorofluorescein diacetate (H_2DCF-DA). At the end of each treatment, RA differentiated SH-SY5Y cells, grown in 25 cm^2 culture flasks at a density of $5x10^5$ cell/ml, were incubated with 5μM H_2DCF-DA (dissolved in DMSO) for 30 min at 37 °C, and the fluorescence intensity was analyzed as previously described[7].

RESULTS AND DISCUSSION

Previous FTIR spectroscopic analyses of neuronal-like cells exposed to EMFs showed a shift down of the amide I and II and the appearance of a peak around 1740 cm^{-1}[11], indicating relevant changes in the overall conformational states within the cell[8].

Alterations in the amide modes were also observed after exposure of proteins to low intensities EMFs[12-15] giving a conclusive proof that EMFs alter proteins

secondary structure.

FTIR spectroscopy was used here in the infrared region from 1250 to 950 cm^{-1}, assigned to nucleic acids, to study the influence of EMFs on the DNA structure.

Exposure to SMF

Typical spectra obtained after exposure to SMF at 2 mT for 3 and 9 h are shown in Figure1(A-B).

The vibration bands around 1230, 1080 and 984 cm^{-1} can be assigned to the phosphate bands corresponding to asymmetric stretching mode $^{as}PO_2^-$, symmetric streching $^{s}PO_2^-$ and $^{s}PO_3^{--}$ symmetric stretching, respectively[16-18].

The ratio exposed/not-exposed of the integrated area of $^{as}PO_2^-$ (from 1260 to 1200 cm^{-1}) and of $^{s}PO_2^-$ (from 1120 to 1020 cm^{-1}) decreased significantly ($P<0.01$) after exposure, suggesting alterations in DNA/RNA.

After 9 h of exposure we observed a significant ROS increase in comparison to control (about 12 ± 2 %, $p<0.05$, see (TABLE 1), confirming previous results on increasing levels of oxidative DNA damage due to EMFs, which led to the conclusion that EMF can be a stimulus to induce activated states of the cell enhancing free radicals release[3,19,20].

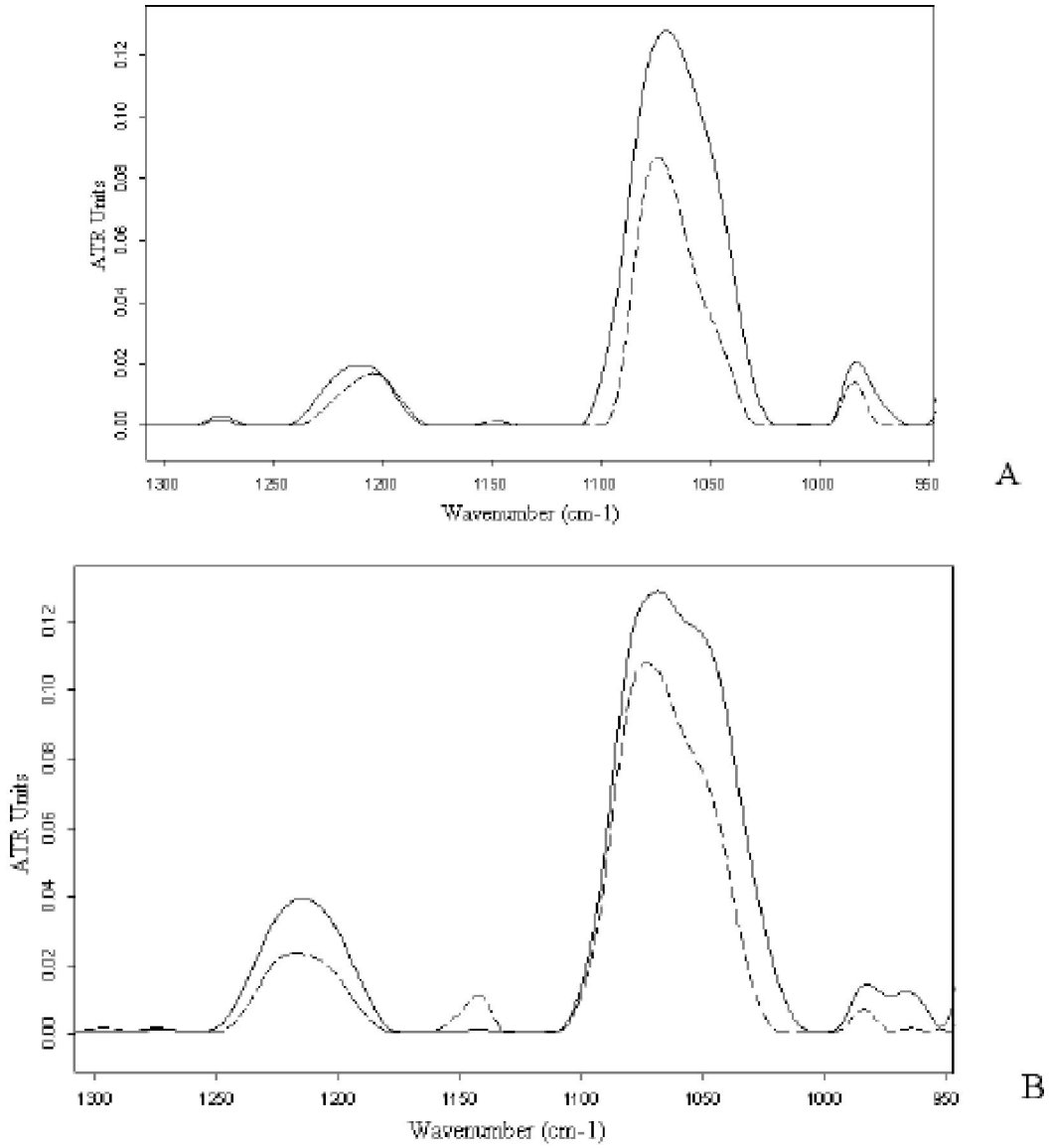

Figure 1 : Representative spectra from 1250 to 950 cm^{-1} of SH-SY5Y neuroblastoma cells sample after 3 h (A) and 9 h (B) of exposure to 2 mT SMF (exposed sample spectra are represented by the dotted lines). The decrease in phosphate vibration bands can be observed.

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TABLE 1 : Reactive oxygen species (ROS) levels in the n RA-differentiated SH-SY5Y neuroblastoma cells after exposure to SMF (* p<0.05 in comparison to controls). Values (three-four observations) are reported as relative DCF fluorescence (f/mg proteins). *p<0.05 (Student's test) in comparison to controls. DCF = dichlorofluorescein.

Time of exposure (h)	DCF fluorescence (% of control)	
3h	1.7 ± 0.2	2.2 ± 0.3
9h	5.5 ± 0.4	12.0 ± 2.1*

Exposure to ELF-EMF

Exposure of differentiated neuroblastoma cells for 3 h to a 50 Hz EMF at 0.1 mT did not produce significant alteration in the DNA region (Figure 2-A)

In contrast, exposure to 50 Hz EMF at 0.7 mT induced significant decrease in intensity ($p<0.01$) of the phosphate bands $^{as}PO_2^-$ and $^{s}PO_2^-$; in addition, their peak's centroid shifted of 9 cm^{-1} and 3 cm^{-1}, respectively, after exposure, as can be observed in Figure 2-B.

The significant decrease in the intensity of the phosphate absorption bands following high EMFs intensity suggests that EMFs can alter the DNA structure in neuronal-like cells.

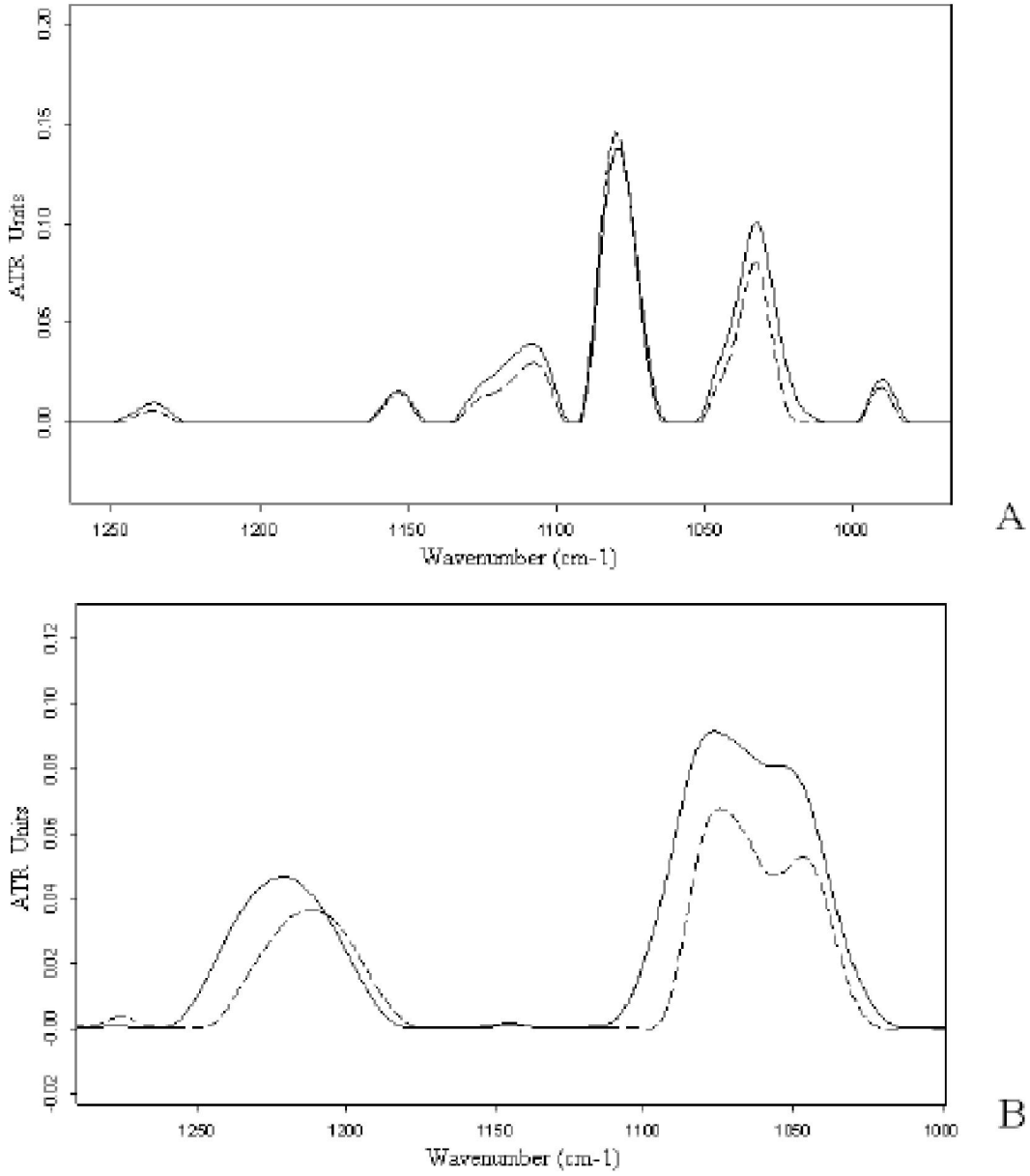

Figure 2 : Representative spectra from 1250 to 950 cm^{-1} of SH-SY5Y neuroblastoma cells sample after exposure to 50 Hz EMF at 0.1 mT (A) and 0.7 mT (B) (exposed sample spectra are represented by the dotted lines). The decrease in phosphate vibration bands can be clearly observed at the intensity of 0.7 mT.

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