

Sinusoidal 50 Hz magnetic fields do not affect structural morphology and proliferation of human cells *in vitro*

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Summary. The effects of electromagnetic fields on several processes related to cell physiology and proliferation are currently being investigated. Although the results are still not conclusive and even conflicting, there seems to be a fairly good agreement on the early effects of electromagnetic fields on the generation of free radicals and on Ca^{++} -intracellular concentration and transport. To evaluate the long-lasting consequences of these precocious events, we examined the effects of short- and long-term magnetic field exposure on structural organization (cytokeratin or actin detection), proliferation (bromodeoxyuridine incorporation and propidium iodide staining), colony forming ability and viability (trypan blue exclusion test) of highly proliferating MCF-7 cells (from human breast carcinoma) and on slowly proliferating normal human fibroblasts (from healthy donors). Cells were exposed to either 20 or 500 μ T sinusoidally oscillating (50Hz) magnetic fields for different lengths of time (1 to 4 days). Short (1 day)- and long (4 days)-time exposure to the two intensities did not affect MCF-7 growth and viability, colony number and size, or cellular distribution along the cell cycle; neither were the cell morphology and the intracellular distribution and amount of cytokeratin modified. Similarly, no modifications in the actin distribution and proliferative potential were observed in normal human fibroblasts. These findings suggest that under our experimental conditions, continuous exposure to magnetic fields does not result in any appreciable effect in both normal and tumor cells *in vitro*.

Key words: Breast cancer cells, Fibroblasts, Cell cycle, Cytometry, Immunocytochemistry

Introduction

The exposure of human populations to electromagnetic fields (EMFs) has been dramatically increasing in recent years, mostly due to the wide use of electrical equipments in daily life. Rules have therefore been established by the International Radiation Protection Association (IRPA), in order to limit population exposure to EMFs. Many studies have been performed on the effects of magnetic fields (MFs) of low intensity and low frequency, such as those produced both in the domestic environment by electric appliances and in the open environment by power distribution lines. Many of these studies were performed for epidemiological purposes, others were aimed at evaluating the tumor promoting or mutagenic effects of MFs. In addition, the consequences of EMF exposure were evaluated on cell-free systems *in vitro* (Goodman et al., 1993; Blank, 1992; Wang and Hladky, 1994), on chick embryos (Ubeda et al., 1994; Veicsteinas et al., 1996) or adult rats (Margonato et al., 1993, 1995) *in vivo*, on human amniotic cells (Nordenson et al., 1994), and on mammalian nerve cells, for which neurite growth (Trillo et al., 1996) and neurotransmitter amino acid synthesis (Zecca et al., 1991) were studied. In intact cell systems in culture, several processes related to cell physiology, proliferation and death have been investigated, such as Ca^{++} signaling, ornithine-decarboxylase activity, change of gene (e.g., *myc*, *jun*, *SRC* family) expression, membrane channel conductance, production of reactive oxygen species or superoxide radicals, ATPase activity, and DNA synthesis (reviewed in Lacy-Hulbert et al., 1998 and in Fanelli et al., 1999). The results are still not conclusive and often even conflicting.

Recently Lacy-Hulbert et al. (1998) pointed out that the somewhat poor comparability and reproducibility of the experimental conditions used (e.g., the field exposure procedure and cell systems chosen) may result in the lack of unequivocal evidence of biological response induced by EMFs or MFs.

There seems, however, to be a fairly good agreement

that short-term exposure to EMFs may induce an increase of free radical generation (McLauchlan, 1992) and may also affect the intracellular concentration and transport of Ca^{++} (Lindstrom et al., 1993; Hojevik et al., 1995; Korzhseptsova et al., 1995). It is still to be elucidated whether these early and short-lasting changes may result in long-lasting effects on cell survival, growth and proliferation. In addition, in almost all the studies so far reported in the literature, exposure to MFs was associated to other environmental (either physical or pharmacological) stimuli, with a consequent (and often ambiguous) synergistic interactive effect.

The aim of this study was to elucidate whether cell viability and proliferation may be affected by the simple exposure to sinusoidally oscillating MFs. Our experiments were performed in the presence of 50 Hz MFs (i.e., the frequency of the European electric power lines) of high or extremely low density flux (500 or 20 μT); the electric field intensity was negligible. These frequency and density fluxes were chosen, based on the data by IRPA for continuous exposure of either workers or the whole population. A detailed morphological and cytochemical investigation was performed using human cells, of normal and tumor origin, exposed for different times (24 hr to 72 hr) to MFs. The 24 hr exposure was chosen as the shortest suitable time to detect possible effects of MFs on cell proliferation since it corresponds approximately to the length of one cycle for our cell lines. As model cell systems, the human cell line MCF-7 (from a spontaneous breast carcinoma) was selected, due to its homogeneity, stability and high proliferation rate, whereas low-passage human fibroblasts from a healthy donor were used as an example of slowly proliferating normal diploid cells. A cytochemical and cytometric approach was chosen, as it provides the unique opportunity to investigate biological phenomena occurring in an even minor fraction of a heterogeneous cell population or affecting cells in cycle-dependent manner. Indeed, one could likely hypothesize that cells might be differently prone to the influence of MFs, depending on their proliferation potential or even on the cycle phase in which a cell is eventually positioned.

Based on these assumptions, several parameters were studied which are widely used to evaluate the effects of external (chemical or physical) agents on living cells. Namely, we evaluated cell viability and growth (as estimated by cell colony number and size), and some markers related to cell proliferation (DNA content, bromodeoxyuridine incorporation, immunopositivity for the nuclear antigen Ki-67) and to chemodifferentiation (distribution pattern and amount of cytoskeletal proteins). Both human fibroblasts and MCF-7 cells proved to be highly sensitive to exogenous agents influencing cell proliferation, differentiation and death, and have been extensively investigated for these characteristics by cytochemical and cytometric techniques (Danova et al., 1994; Pellicciari et al., 1995a,b; Mangiarotti et al., 1998a,b).

Materials and methods

Cells and experimental procedures

Human MCF-7 (estrogen-dependent mammary carcinoma) cells and normal fibroblasts derived from the derma of a 25-year-old healthy man (at the 3rd to 7th passage *in vitro*) were grown in RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum (Mascia Brunelli, Milano, Italy) and Hepes 10mM to maintain the appropriate pH. Cells in the logarithmic growth phase were seeded in flasks (25 cm^2) at a cell density of 10,000 cells/ cm^2 and 72h later were exposed to MFs, as described below. The height of the medium was always 0.13 mm in order to avoid differences in the MFs reaching the cells, among different samples. After 24hr, 48hr, 72hr and 96hr of exposure (well before the cells had reached confluence), flasks were taken out from the MFs and further incubated without exposure to the field and/or processed for the different assays, as reported below. Additional cell samples not exposed to MFs served as controls. The results were expressed as the mean (\pm standard deviation) of at least two independent experiments with three replicates for each experiment. The cell number in control samples was $1,800 \times 10^3 \pm 15\%$.

Magnetic field exposure system

A thermo-static walk-in chamber (3.5x3x2.8 m) contained the equipment for generating the MF. To assure identical temperature and growing conditions, a single chamber was used for exposed and unexposed cells.

The MF system (for details see Veicsteinas et al., 1996) consisted of 2 sets (upper and lower) of four pairs each of vertically arranged rectangular coils (spacing 320-250-320 mm). The coils (width 900 mm, height 700 mm) were wound onto a PVC frame. The external coils had 50 turns each, while the two internal coils had 25 turns each. The coils were connected so that the current flowed clockwise in the upper coils and anti-clockwise in the lower one. This scheme, similar to that developed by Battelle PNL (Miller et al., 1989) provided two (upper and lower) uniform exposure volumes (600 x 600 x 150 mm each), with the same MF strength but in opposite directions. An advantage of this arrangement is that the MF outside the two exposure volumes decreases very rapidly. Therefore it was possible to place exposed and sham-exposed cells in the same chamber with negligible interaction. The coils were connected to the mains through a continuity group, an auto-transformer (1200 VA) and a transformer (220/6-60-120 V, 1200 VA).

The direction of the field within the cell exposure volume was horizontal. The precise value of the MF in the exposure area was measured by a calibration coil, connected to an EMDEX II. The variations of the field intensities, during the experimental time, were less than

5% of the average value. At a distance of 2 m from the coils, in the sham-exposed area, the MF strength was less than 0.2% of the exposure value and mainly horizontal. The local geomagnetic field, measured by a gaussmeter (Laboratorio Elettrofisico, mod. DG6, Torino, Italy) showed a vertical component (with a downward direction) of about 34 μT in both the exposure and sham-exposure areas. The horizontal components were $B_x = 19 \mu\text{T}$ and $B_y = 7 \mu\text{T}$, where "x" is the direction of the test 50 Hz field. The 50 Hz field generated by the chamber heating system was about 0.05 μT ($B_x = 0.03 \mu\text{T}$, $B_y = 0.01 \mu\text{T}$ and $B_z = 0.04 \mu\text{T}$). Vibration, measured by an accelerometer (Mod. Egasy 25 d, Entran Devices, Fairfield, NJ, USA) attached to the coils, was $<0.05 \text{ m/s}^2$.

The chamber temperature was continuously monitored and controlled by thermistors (Thermelert model TH-6D, Bailey Instruments, Saddle Brook, NJ, USA) and a quartz thermocontroller (model EXR-101, Sauter, Milan, Italy), at $37.0 \pm 0.2 \text{ }^\circ\text{C}$. Two thermistors were placed near the flask containing the cell culture, one in the exposure and one in the sham-exposure volumes. Since the exposure system produced a small amount of heat, the chamber was also equipped with fans to improve temperature uniformity so that the same temperature was found in the exposure and sham-exposure volumes.

Cells were placed in the field two days after the seeding in logarithmically growing conditions and maintained for different times, as described below. Control samples were placed in the same chamber in the sham-exposure volume.

Cell counting and viability test

MCF-7 cells ($10^4/\text{cm}^2$) were seeded in tissue culture flasks. Forty-eight hours after seeding, cells were exposed to MFs for 24h, 48h, 72h and 96h. At the selected time points, flasks were taken from the field till 72h when control and treated cells were collected by mild trypsinization and stained with 0.1% trypan blue, to assess cell viability by microscope scoring. Only trypan blue-negative cells were considered as viable and counted. Human fibroblasts ($2 \times 10^4/\text{cm}^2$ at the cell seeding) were collected and counted after 4 days of exposure.

Colony formation assay

MCF-7 cells (40 cells $/\text{cm}^2$) were seeded in 25 cm^2 flasks. Forty-eight hours after seeding cells were: a) exposed to MFs for 72h and incubated for a further 24h without exposure to MFs and fixed; or b) exposed for 96h and then fixed in 70% ethanol. Then cells were stained with 2% crystal violet dissolved in 70% ethanol, and colonies were counted with an inverted microscope. Only colonies of more than 30 cells were considered. Colonies were also subjectively scored for size. Results are expressed as the percentage of colonies (\pm standard

deviation) in treated samples versus the untreated controls.

Immunofluorescence detection of BrdU labeling

At each time considered, control and MF-exposed samples of MCF-7 cells were pulse-labeled for 30 min with 10 μM BrdU (Sigma Chemical Co., St. Louis, MO, USA); the medium was then washed out, and cells were rinsed with pre-warmed fresh medium. Cells were then trypsinized and fixed with 70% ethanol for 20 min at $-20 \text{ }^\circ\text{C}$. Cell samples (about 10^6 cells each) were treated with 3N HCl for 30 min at room temperature, to denature DNA partially. After treatment with 0.1M sodium tetraborate for 15 min to neutralize HCl, cells were rinsed for 15 min at room temperature with phosphate-buffered saline (PBS) containing 0.5% Tween 20 and 0.1% bovine serum albumin (BSA) and incubated for 30 min with a 1:20 dilution of anti-BrdU mouse MoAb (Dako, Glostrup, Denmark) in PBS. Samples were then washed twice with PBS and incubated for 30 min with a 1:20 dilution of an FITC-labeled goat anti-mouse IgG (Dako) in PBS.

Immunofluorescence detection of cytokeratin

MCF-7 cells grown in slide-flasks (10^4 cells $/\text{cm}^2$) were fixed with 70% ethanol at $4 \text{ }^\circ\text{C}$, rehydrated in PBS and incubated with a 1:10 dilution of mouse anti-cytokeratin MoAb (CK-1, Dako) for 60 min at room temperature. After washing with PBS, cells were incubated for 45 min with a 1:20 dilution of an FITC-labeled goat anti-mouse IgG (Dako).

Propidium iodide DNA staining of immunolabeled cells and dual-parameter flow cytometry analysis

MCF-7 cells grown on flasks were trypsinized, fixed and immunolabeled for either BrdU or cytokeratin, as above. Immunolabeled cells were counterstained for at least 30 min at room temperature with 5 $\mu\text{g}/\text{ml}$ propidium iodide (PI) in 0.1M phosphate buffer pH 7.2 containing 100 U/ml RNase A. Bivariate measurements of green fluorescence (identifying immunolabeled cells) versus red fluorescence (PI-DNA content) were made with a FACStar (Becton Dickinson, San José, CA, USA) flow cytometer. This was carried out under the following conditions: Argon ion laser excitation power 200mW at 488nm, 560nm beam splitter, 510-540nm band pass filter for the green fluorescence detector, and 610nm long pass filter for the red fluorescence detector. The level of background fluorescence, due to the nonspecific binding of the FITC-conjugated antibodies, was established using control cell specimens processed as previously described, but either without exposure to the primary antibodies or with exposure to a mouse serum containing no specific antigenic activity. The corresponding value of green fluorescence was used as a cut-off value above which cells were considered as

labeled. Data were collected in list-mode with a Consort 30 software program running on a Hewlett Packard computer, and presented as plots for correlated green to red fluorescence signals. This approach made it possible to study BrdU labeling or cytokeratin content in single cells, and to relate it directly to specific cycle phases. Cytometric data were evaluated with rectangular region analysis: FITC-immunolabeled cells were those with green fluorescence values exceeding the background threshold determined as reported above; the ranges for G0-G1, S and G2-M phase cells were established on the basis of the corresponding DNA content histograms.

At least 20,000 cells per sample were considered in the gated regions used for calculations.

Detection of actin with fluorescently-labeled phalloidin and immunodetection of the nuclear antigen Ki-67 in human fibroblasts

Human fibroblasts were grown on slide-flasks (17×10^4 cells/cm²); after exposure to MFs the slides were detached from the flasks, and cells were fixed with 70% ethanol at 4 °C, rehydrated in PBS and incubated with Texas-red-labeled phalloidin (Molecular Probes, Eugene, OR, USA) for 60 min. After washing with PBS, slides were finally mounted with PBS:glycerol (1:1).

For detecting Ki-67, ethanol-fixed fibroblasts were permeabilized with a solution of BSA-Tween-PBS as above, and incubated for 60 min with anti-Ki-67 monoclonal antibodies (Dako) diluted 1:10 with PBS. Cells were then incubated for 45 min with a 1:20 dilution in PBS of FITC-conjugated anti-mouse-IgG antibodies raised in goat. Slides were finally mounted in PBS: glycerol, as above.

Fluorescence microscopy

Fluorescence micrographs (Agfapan 400 film) were taken with a BX50 Olympus microscope equipped with an HBO 100 Watt mercury lamp. The filter sets used were as follows: for FITC, a BP 450-480 excitation filter, a DM500 dichroic mirror and a BA515 barrier filter, and for Texas red, BP 510-550, DM570, and BA590.

Table 1. Effect of different exposures to MFs of various intensities on MCF-7 cell viability.

FIELD INTENSITY	CELL SURVIVAL ^a ±(% of control)		
	24h ^b	48h ^b	72h ^b
20 μT	87±3	90±9	91±5
500 μT	91±3	95±14	90±19

^a: cell survival was determined at 72 h after the beginning of treatment by trypan blue dye exclusion test. Results are the mean (± standard deviation) of two independent experiments with three replicates for each experiment. ^b: time of MF exposure.

Statistical analysis

The differences between the values for the different variables of exposed and unexposed cells were compared by Student's t test for paired values or for proportions.

Results

Cell growth and proliferation

Cell growth of MCF-7 cells was not modified by the exposure to MFs of different intensity (Table 1). Cell viability after 24, 48, 72hr exposure was similar to that of untreated cells. Consistently, colony number (Table 2) and size (over 30 cells/colony) were similar for untreated and treated MCF-7 cells.

In the same experimental conditions human fibroblasts gave quite comparable results (not shown).

Cell cycle distribution and evaluation of DNA-synthesizing cells in MCF-7 cells

Flow cytometric measurements of DNA content after PI-staining indicated that, with increasing culture times a reduced number of MCF-7 cells fell in the S-phase range of DNA content. This was apparent from both the frequency distribution of the DNA histogram (not shown) and the results of the BrdU incorporation experiments (Fig. 1, Table 3). The progressively smaller percentage of BrdU-positive cells after 48 and 72 hr in culture was probably due to the cell crowding in the culture flasks, which determined the slowing of cell cycle progress.

In comparison to control cells, samples submitted to MFs of 20 or 500 μT did not exhibit any difference at any of the exposure times examined, as far as both DNA histograms and BrdU incorporation levels were concerned. Since the results after 20 and 500μT exposure were fully comparable, only data referring to 500μT are reported.

Table 2. Effect of different exposures to MFs of various intensities on MCF-7 colony formation^a.

FIELD INTENSITY	No. COLONIES/FLASK	
	72h ^b	96h ^b
0 μT	225±25	240±12
20 μT	225±14	253±17
500 μT	204±27	230±36

^a: cells were exposed to MFs for 72 or 96 h. After a further incubation of 3 and 4 days in the absence of MF, cells were fixed and crystal violet-stained colonies were scored and counted by an inverted microscope. Results are the mean of two independent experiments with three replicates for each experiment. ^b: time of MF exposure.

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Content and intracellular distribution of cytokeratin in MCF-7 cells

In MCF-7 cells, biparametric flow measurements of the immunopositivity for cytokeratin versus DNA content showed that the amount of cytokeratin increased during the cell cycle (as expected, due to the increasing cell size), without significant differences between control and treated cells (Fig. 2).

The immunopositivity for cytokeratin of MCF-7 cells was also examined in fluorescence microscopy. It

was apparent that both cell morphology and intracellular distribution of cytokeratin were not affected by the MF-exposure (Fig. 2).

Immunopositivity for the antigen Ki-67 expression and actin distribution in normal human fibroblasts

Normal human fibroblasts were also studied for their proliferation potential and for the distribution of actin after exposure to MFs. It is well known that normal fibroblasts are relatively slowly proliferating cells; this

Table 3. Cell cycle distribution of MCF-7 cells exposed for different times to MFs^a.

FIELD INTENSITY	TIME OF EXPOSURE								
	24 h			48 h			72 h		
	G0/1	S	G2/M	G0/1	S	G2/M	G0/1	S	G2/M
0 μ T	44 \pm 2	41 \pm 0.8	15 \pm 3.7	55 \pm 4.4	29 \pm 2.8	16 \pm 4.0	63 \pm 1.4	16 \pm 0.8	20 \pm 1.2
500 μ T	42 \pm 4.9	40 \pm 1.0	18 \pm 1.7	56 \pm 1.5	29 \pm 2.0	15 \pm 2.0	65 \pm 1.0	15 \pm 1.5	19 \pm 3.1

^a: cell cycle distribution was evaluated at the end of MF exposure as reported in Materials and Methods. Values were estimated by rectangular-region analysis, based on dual-parameter cytograms of BrdU versus DNA content, such as those in Figure 1. The differences between unexposed and exposed samples were not significant. The progressively smaller percentage of BrdU-positive cells with increasing time in culture was due to the crowding of cells in the culture flasks (see also Results).

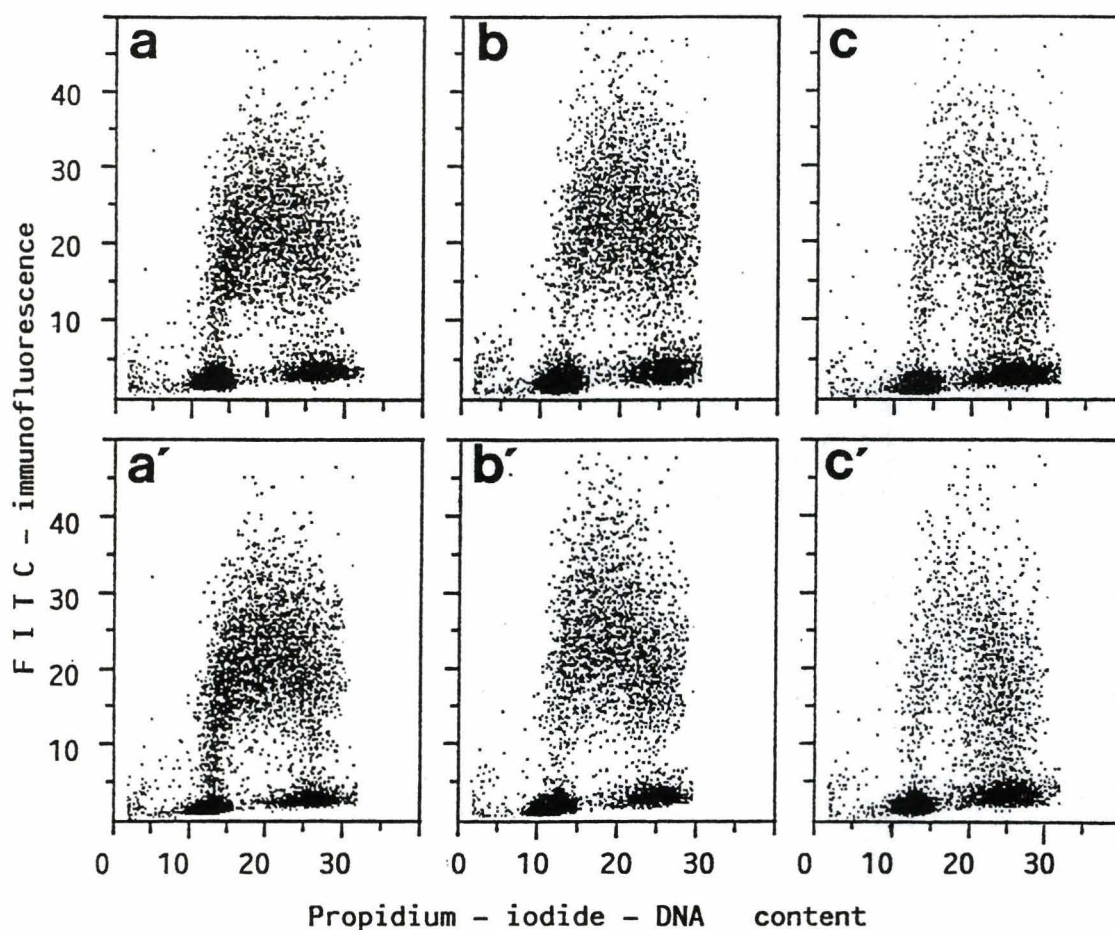
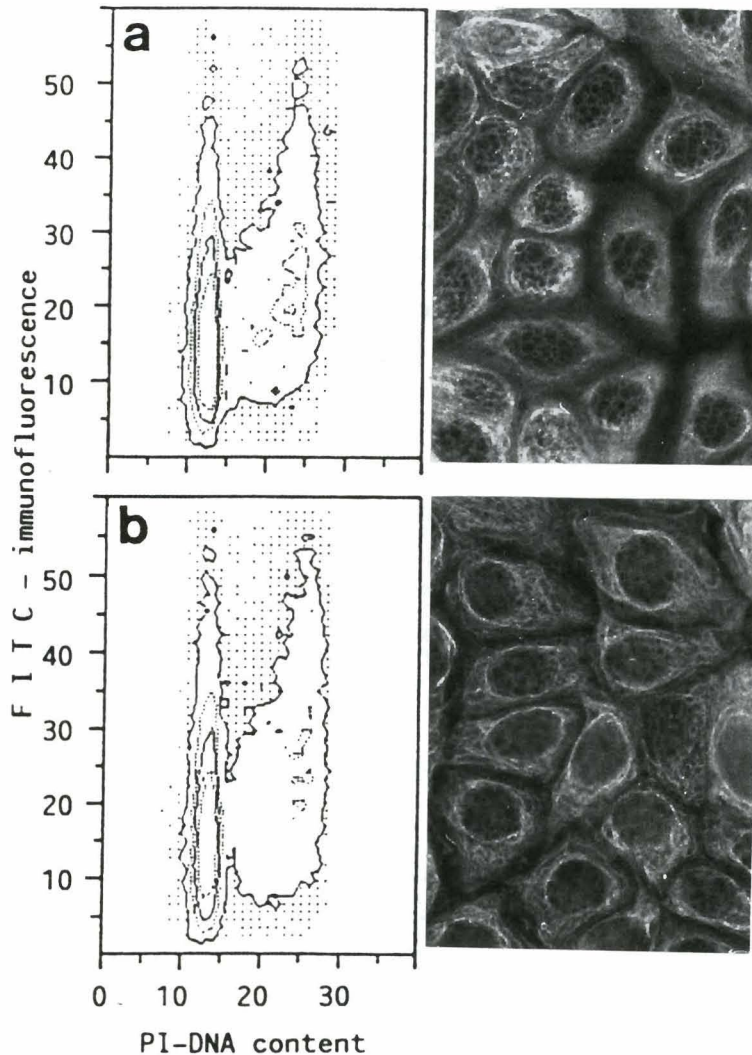


Fig. 1. Dual-parameter cytograms of FITC-immunopositivity for BrdU (identifying S-phase cells, in ordinate) versus DNA content after PI staining (in abscissa) of MCF-7 cells 24, 48 and 72 hr after exposure to MFs (500 μ T, lower row); the upper row refers to the corresponding unexposed controls. a, b, c: 24, 48 and 72 h respectively.



makes it difficult to obtain large cell samples to be measured in flow cytometry. Therefore the percentage of proliferating fibroblasts was estimated on cells grown on glass coverslips by evaluating the immunopositivity for the nuclear antigen Ki-67, which is a widely used marker for cycling cells (reviewed in Mangiarotti et al., 1998a). It was found that the exposure to MFs does not induce significant changes in the percentage of Ki-67-positive cells (the percentage of Ki-67 positive cells was 90 ± 5.3 and 93 ± 5.2 , in control and treated samples, respectively).

The distribution of actin (as shown by the staining with Texas-Red-labeled falloidin: Fig. 3) was similar in exposed and unexposed fibroblasts.

Discussion

The reports on the long lasting effects of MFs (or EMFs) on animal cells and tissues appear largely inconsistent (Lacy-Hulbert et al., 1998). In spite of the evidence that MFs and EMFs have nor genotoxic nor cytotoxic potential (McCann et al., 1998), a pool of positive results have been published. For instance, a rise in the proliferation of HL-60 cells exposed to 2mT (Schimmelpfeng and Dertinger, 1997) and of chick embryo fibroblasts exposed to increasing field

Fig. 2. Dual-parameter cytograms of FITC-immunopositivity for cytochrome c versus PI-DNA content (left column) and cytochrome c distribution pattern in the cytoplasm of MCF-7 cells (right column; final magnification: $\times 670$). **a.** unexposed controls; **b.** 72 hr exposure to MFs ($500 \mu\text{T}$).

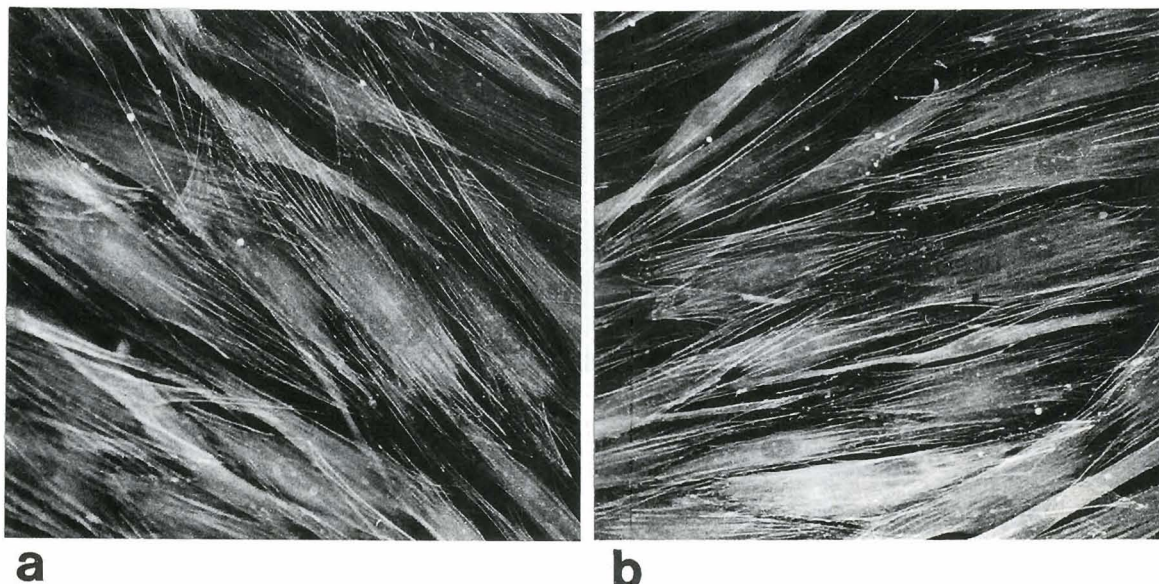


Fig. 3. Texas-red immunopositivity for actin microfilaments in normal human fibroblasts before (**a**) and after (**b**) exposure to MFs ($500 \mu\text{T}$) for 72 hr. $\times 650$

frequencies (50-100 Hz) and intensities (from 0.05 to 0.7 mT) has been reported, although no significant effects were observed in a field of 60 Hz, even at 0.7 mT (Katsir et al., 1998). The poor reproducibility of the experimental conditions, and the wide variability of the cell systems used (either *in vivo* or *in vitro*) could account for such an overall inconsistency. In particular, the exposure to MFs was often performed in association with other perturbing chemical or physical agents, such as tamoxifen, melatonin or ionizing radiation (Harland and Liburdy, 1997; Lagroye and Poncy, 1997), with a synergistic effect.

In order to assess whether MF exposure may influence *per se* some structural and kinetic characteristics of human cell populations, the present experiments were performed using MFs only, in the absence of the electric field and of any additional pharmacological treatment. Two human cell lines (i.e., tumor MCF-7 cells and normal diploid fibroblasts) in culture were used as relatively simple biological models, to investigate whether MFs might interfere with cell viability, organization and proliferation. These two cell lines have largely been used to test the effects of environmental (either physical or chemical) stresses. They grow in culture with different cell cycle kinetics and they represent reproducible and relatively homogeneous systems by which statistically significant results may be obtained.

The 4-day exposure time used in the present investigation may be considered as relatively long, since it corresponds to approximately 4-5 cell cycles for both fibroblasts and MCF-7 cells, under unperturbed conditions. In particular, for the MCF-7 cell strain used, 4-day treatment proved to deeply affect cell growth and the proliferation potential, and even to induce apoptosis after treatment with tamoxifen (Danova et al., 1994) or *all-trans* retinoic acid (ATRA, Mangiarotti et al., 1998b).

In the present study, the cell counting and colony forming assay showed that cell growth of both normal and tumor cells was not affected by MFs at magnitudes of 20 and 500 μ T.

The cytochemical and cytometric data demonstrated that in MCF-7 cells not only the fraction of S-phase cells, but also the fractions of cells in the other cycle phases did not change upon exposure to MFs. Since not even a delay in cell cycle progression was observed up to 4 days of MF exposure, a reduction of cell proliferation at longer times cannot be hypothesized. Using antibodies against the nuclear protein Ki-67, a reliable proliferation marker molecule (Mangiarotti et al. 1998a), we also showed that the proliferation potential of normal fibroblasts was fully comparable before and after exposure to MFs.

This is consistent with other reports in the literature which demonstrate that several epithelial (both normal and tumor-derived) cell lines (Liburdy et al., 1993; Pacini et al., 1999; Loberg et al., 2000) and fetal lung fibroblasts (Wiskirchen et al., 2000) do not undergo

significant changes in their viability and growth upon long-lasting (up to some days) exposure to either static or oscillating MFs only. No difference in cell survival and turnover was ever found in different organs and tissues (liver, heart, lymph nodes, blood, testis) of adult rats after prolonged exposure to MFs, irrespective of the high or low proliferation potential of the cell populations considered (Margonato et al., 1993, 1995).

Our cell models, which are either rapidly (MCF-7) or slowly (fibroblasts) proliferating, proved to be highly sensitive to a wide spectrum of exogenous agents influencing cell proliferation, differentiation and death (Danova et al., 1994; Pellicciari et al., 1995ab; Mangiarotti et al., 1998a,b). However, these cells did not show apparent changes in their respective kinetic parameters, before and after treatment with MFs. This feature was paralleled by the evidence that, in MCF-7 cells, there was no change in the amount and intracellular distribution of cytokeratin (which is a unique marker of epithelial differentiation, and whose changes in distribution and quantity may be considered as signs of altered chemodifferentiation). In fibroblasts too, actin cytoskeleton was not apparently modified after MF exposure.

We cannot exclude the occurrence of transient alterations in some physiological parameters, at short time after exposure to MFs. In fact, this was already found to take place for Ca^{++} and ornithine decarboxylase on some cell lines (Byus et al., 1987, Korzhseptsova et al., 1995). However, data in the literature also indicate that other cells, such as human lymphocytes and rat pituitary cells or human amniotic cells, do not exhibit changes in the intracellular concentration of Ca^{++} or in the activity of ornithine decarboxylase, upon MF exposure (reviewed in Lacy-Hulbert et al., 1998). Based on the present evidence, these early events (if any) should be reversible and/or not influential for both fibroblasts and MCF-7 cells, suggesting that long-term exposure to oscillating sinusoidal MFs does not determine steady, long-lasting effects on the morphology, cytoskeletal organization, viability and proliferation of these human cultured cells of normal and tumor origin.

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