

915 MHz Microwaves and 50 Hz Magnetic Field Affect Chromatin Conformation and 53BP1 Foci in Human Lymphocytes From Hypersensitive and Healthy Persons

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We used exposure to microwaves from a global system for mobile communication (GSM) mobile phone (915 MHz, specific absorption rate (SAR) 37 mW/kg) and power frequency magnetic field (50 Hz, 15 μ T peak value) to investigate the response of lymphocytes from healthy subjects and from persons reporting hypersensitivity to electromagnetic field (EMF). The hypersensitive and healthy donors were matched by gender and age and the data were analyzed blind to treatment condition. The changes in chromatin conformation were measured with the method of anomalous viscosity time dependencies (AVTD). 53BP1 protein, which has been shown to colocalize in foci with DNA double strand breaks (DSBs), was analyzed by immunostaining in situ. Exposure at room temperature to either 915 MHz or 50 Hz resulted in significant condensation of chromatin, shown as AVTD changes, which was similar to the effect of heat shock at 41 °C. No significant differences in responses between normal and hypersensitive subjects were detected. Neither 915 MHz nor 50 Hz exposure induced 53BP1 foci. On the contrary, a distinct decrease in background level of 53BP1 signaling was observed upon these exposures as well as after heat shock treatments. This decrease correlated with the AVTD data and may indicate decrease in accessibility of 53BP1 to antibodies because of stress-induced chromatin condensation. Apoptosis was determined by morphological changes and by apoptotic fragmentation of DNA as analyzed by pulsed-field gel electrophoresis (PFGE). No apoptosis was induced by exposure to 50 Hz and 915 MHz microwaves. In conclusion, 50 Hz magnetic field and 915 MHz microwaves under specified conditions of exposure induced comparable responses in lymphocytes from healthy and hypersensitive donors that were similar but not identical to stress response induced by heat shock. *Bioelectromagnetics* 26:173–184, 2005. © 2005 Wiley-Liss, Inc.

Key words: DNA DSBs; hypersensitivity; stress response; apoptosis

INTRODUCTION

Several investigations have shown that weak electromagnetic field (EMF) can affect biological systems [Goodman et al., 1995; Adey, 1999]. Within the extremely low frequency (ELF) range, exposure at 50 or 60 Hz is of major concern because electrical appliances and power lines emit such fields. ELF has recently been classified as a possible carcinogen by IARC [International Agency for Research on Cancer, 2002]. Concern about occupational and residential exposure to the microwave frequency transmitted by mobile phones is growing. In recent papers, it was

Grant sponsor: The Swedish Council for Working Life and Social Research; Grant sponsor: Swedish Cancer Society; Grant sponsor: Svenska Elforsk AB; Grant sponsor: Civilingenjörs Förbundet; Grant sponsor: Trygg Hansa AB; Grant sponsor: Swedish Authority for Radiation Protection.

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Received for review 2 February 2004; Final revision received 15 November 2004

DOI 10.1002/bem.20103

Published online in Wiley InterScience (www.interscience.wiley.com).

reported that increased incidence of brain tumors correlated with exposure to mobile phones microwaves [Hardell et al., 2001, 2003]. Independent confirmation of brain tumors has recently been published [Lonn et al., 2004]. There is evidence that weak EMF can result in DNA damage and changes in permeability of brain–blood barrier [Lai and Singh, 1997; Persson et al., 1997].

There are a number of publications indicating that EMF can produce stress response [Lin et al., 1997; Junkersdorf et al., 2000; de Pomerai et al., 2000] and apoptosis [Simko et al., 1998; Mangiacasale et al., 2001; Olsson et al., 2001]. “Null” effects regarding all of the above-mentioned endpoints were also reported, however, accumulating experimental evidence suggest that EMF effects occur only in specific frequency and amplitude “windows” depending on several physical parameters [Adey, 1999; Binhi, 2002]. Therefore, both positive and “null” effects should be expected depending on exposure parameters.

Several biological variables are also of importance for effects of ELF and microwaves [Belyaev et al., 1999a, 2000]. In particular, the same exposure can result in apoptosis in some cells lines but does not induce apoptosis in others [Mangiacasale et al., 2001]. Recent evidence has indicated that mobile phone microwaves activate a variety of cellular signal transduction pathways, among them the hsp27/p38MAPK stress response pathway [Leszczynski et al., 2002].

The effects of ELF fields have been observed within relatively narrow “frequency windows” [Smith et al., 1987; Blackman et al., 1988, 1994; Belyaev et al., 1994; Prato et al., 1995] and “amplitude windows” [Blackman et al., 1982, 1994; Liboff et al., 1987; Lednev, 1991; Prato et al., 1995]. It has been found by Blackman et al. [1985] that the ambient static magnetic fields (SMF) can significantly influence the effects of ELF fields. The importance of SMF for the ELF effects has been confirmed in several studies [Lednev, 1991; Belyaev et al., 1994; Blackman et al., 1994; Fitzsimmons et al., 1994; Prato et al., 1995]. Therefore, the effects of weak ELF are usually observed only under specific combinations of frequency, amplitude, and SMF.

It was shown in our previous investigations, that weak EMF affected the conformation of chromatin in cells of different types including human lymphocytes [Belyaev et al., 1999a, 2000; Belyaev and Alipov, 2001a; Olsson et al., 2001]. In particular, temporal condensation of chromatin similar to condensation during apoptosis or stress response has been observed in human lymphocytes in response to 8 Hz magnetic field [Belyaev et al., 1999a; Belyaev and Alipov, 2001a]. Our present study was designed to test the possible effects of magnetic field at 50 Hz and microwaves on stress response and apoptosis in human lymphocytes.

Lai and Singh [1997] studied the effects of EMF in brain cells with the comet assay. The authors interpreted the data as showing induction of DNA breaks, but another interpretation based on changes in chromatin conformation indicative for stress response is also possible [Belyaev et al., 1999b]. New technology has recently become available to study double strand breaks (DSBs) based on analysis of DSB-colocalizing proteins. Several proteins involved in DNA repair and DNA damage signaling, such as the tumor suppressor p53 binding protein 1 (53BP1), have been shown to produce foci in response to DNA damage [Schultz et al., 2000]. In order to analyze whether EMF induced DNA damage, we performed immunofluorescence analysis of the 53BP1 protein *in situ*.

There is a growing concern about so-called hypersensitivity to electricity. These hypersensitive people experience several symptoms in the proximity to different sources of EMF. The symptoms are not specific and there is no known pathophysiological marker or diagnostic test for this illness [Hillert et al., 1999]. The causal relationship between EMF and symptoms reported by the afflicted individuals has not been confirmed in double-blind provocation studies [Bergqvist and Vogel, 1997; Flodin et al., 2000], however, individual responses to specific frequencies in a wide frequency range from 0.1 Hz to 5 MHz has been observed [Rea et al., 1991]. These data are in line with results of *in vitro* studies showing individual variability in response of human lymphocytes to EMF in specific frequency windows [Belyaev and Alipov, 2001a].

Here, human lymphocytes both from healthy and hypersensitive subjects were exposed to 50 Hz magnetic field under conditions which affect chromatin conformation [Belyaev et al., 2001c; Sarimov et al., *in preparation*], as well as to microwaves that previously have been shown to affect the brain–blood barrier in rats [Persson et al., 1997]. The specific research goals included (1) comparison of the effects of weak EMF on lymphocytes from healthy donors and subjects with hypersensitivity to EMF; (2) investigation of DNA breaks and apoptosis in EMF-exposed lymphocytes.

MATERIALS AND METHODS

Donors and Blood Samples

Blood samples from seven healthy donors and seven patients reporting hypersensitivity to EMF, but otherwise healthy, were obtained at the Department of Occupational and Environmental Health, Stockholm County Council, Sweden. The group of hypersensitive persons was selected based on self-reported hypersensitivity to EMF and characterized with regard to

TABLE 1. Details for Hypersensitive Subjects and Matched Control Healthy Persons

Subject	Gender	Age	Occupation	Duration of hypersensitivity (year)
1*	Female	34	Sick leave	1
2	Female	35	Secretary	—
3	Female	56	Art teacher	10
5	Female	55	Secretary	—
70*	Male	36	Unemployed	9
75	Male	31	Student	—
87	Male	46	Musician	1
88	Male	45	Physician	—
81*	Female	41	Sick leave	7
82	Female	46	Physician	—
91	Female	57	Controller	11
92	Female	58	Nurse	—
95	Female	44	Salesman	1
97	Female	43	Nurse	—

An asterisk designates cases of pronounced hypersensitivity.

symptom profile, triggering factors, time relation and avoidance behavior [Hillert et al., 1999]. The group reporting hypersensitivity to EMF consisted of two men and five women, 36–57 years old (Table 1). Control healthy subjects were matched by age (± 5 years) and gender (Table 1). All patients and controls were non-smokers and none was on any regular medication. Two persons reporting hypersensitivity were on sick leave, one was unemployed and four were working full or part time. All control persons were working. All patients reported symptoms to be triggered by electrical equipment that were not sources of light and all but two hypersensitive reported that symptoms were triggered by mobile phones. The two subjects that did not report this gave the comment that they had no experience of this exposure since they avoided mobile phones.

In all pairs of patient and matched control, the patient scored higher than the matched control in the questionnaire on symptoms (29 symptoms scored for frequency and severity, maximum scoring 232) [Hillert et al., 1999]. The mean score was 89 for patients and 12 for controls. In four patients neurovegetative symptoms (fatigue, headache, and difficulties concentrating) dominated over the skin symptoms (heat, burning sensation, tingling, and redness). In all but one hypersensitive patient the symptoms were always experienced within 24 h after exposure to a triggering factor, in most cases within 1 h. All patients reported that they tried to avoid triggering factors. Those three patients who tried as much as possible to avoid activated electrical equipment were classified as pronounced cases (Table 1). In all these three cases neurovegetative symptoms were most pronounced. Two of these patients lived in cottages in rural areas without electricity. Fresh blood samples from persons reporting hypersensitivity and

matched controls were coded and all data were analyzed in blind. Ethical permission was obtained from the Ethic Committee of the Karolinska Institutet, Stockholm, Sweden.

Chemicals

Reagent grade chemicals were obtained from Sigma (St. Louis, MO, USA) and Merck (Darmstadt, Germany). Pulsed-field gel electrophoresis (PFGE) grade agarose, λ ladder and λ digest pulse markers and low melting agarose were purchased from BioRad (Richmond, CA, USA).

Cells

Lymphocytes were isolated from peripheral blood by density gradient centrifugation in Ficoll-Paque (Pharmacia LKB, Sweden) according to the manufacturer's instructions. The cells were transferred to basal medium (BM); RPMI 1640 medium supplemented with 10% fetal calf serum, 2 mM L-glutamine, 12.5 IU/ml penicillin, 12.5 μ g/ml streptomycin (ICN Pharmaceuticals, Inc., Costa Mesa, CA, USA) at 5% CO₂ and 37 °C in a humidified incubator. Adherent monocytes were removed by overnight incubation of the cell suspension in culture flasks (Becton Dickinson & Co., Franklin Lakes, NJ, USA) at the cell density of 3×10^6 cells/ml in the volume of 10–40 ml. After this incubation, the cells in suspension were collected by centrifugation. The cell density was adjusted to approximately 2×10^6 cells/ml in fresh BM and the lymphocytes were preincubated for 2 h before exposure. The viability of cells was always above 95% as measured with trypan blue exclusion assay.

Cell Exposure

Coded samples from hypersensitive subject and matched control subject were simultaneously exposed in seven independent experiments. All exposures were performed at room temperature, 22–23 °C, in 14 ml round-bottom tubes (Falcon), 2.5 ml of cell suspension per tube, 2×10^6 cells/ml. The background ELF during exposures was not more 100 nT (rms) as measured with a three-dimensional microTeslameter (Field Dosimeter 3, Combinova, Sweden). Duration of all exposures was 2 h. In preliminary experiments, temperature was measured every 10 min during exposure in the 915 MHz/50 Hz-exposed samples. These measurements were performed at different locations across the exposed samples using a thermocouple with a precision of 0.1 °C. No changes in temperature were induced during 915 MHz/50 Hz exposures as compared to sham exposed and control samples.

Extremely low frequency. The 50 Hz exposure unit has been previously described [Alipov et al., 1994].

Briefly, it is based on three pairs of Helmholtz circular coils, which produce relatively homogenous (5% variation) magnetic field inside the working volume. The sinusoidal signal is supplied by a function generator and controlled by means of an oscilloscope and a frequency meter. The intensities of static and alternating magnetic fields were controlled in two ways: by direct measurements using a magnetometer (Sam3, Dowty Electronics Ltd., Cannock, UK) and one-dimensional microteslameter (G79, NPO Mikroprovod, Russia) and by measuring the current and subsequent calculation of the magnetic fields. Cells were exposed to a vertical 50 Hz magnetic field at the amplitude of 15 μ T. Vertical SMF was 43.5 μ T and the horizontal SMF was "nulled" by the vertical Helmholtz coils. Under these conditions of exposure, 50 Hz has been shown to induce changes in chromatin conformation as measured with AVTD [Belyaev et al., 2001c; Sarimov et al., in preparation].

GSM microwaves. Exposure of lymphocytes to microwaves was performed using a GSM900 test-mobile phone, which produced a GSM signal with controlled frequency and power level as observed under exposure from mobile phones. The output of the phone was connected by coaxial cable to a transverse electromagnetic transmission line cell (TEM cell) that has previously been described [Salford et al., 2003; Sarimov et al., 2004]. In principle, this is a spliced coaxial cable with a central electrode and an outer shield electrode with the unique characteristic of having both linear amplitude and phase response versus frequency. The fields of various waveforms including continuous waves and pulsed (or modulated) fields can be accurately generated in the TEM cell.

The construction of the TEM cell allows relatively homogeneous exposure of samples. There are 124 different channels/frequencies, which are used in GSM900 mobile communication. They differ by 0.2 MHz in the frequency range between 890 and 915 MHz. Frequency is supplied randomly to mobile phone users. The test-mobile phone was programmed to use a preset frequency. We used the channel 124 with the frequency of 915 MHz. The signal included standard GSM modulations. No voice modulation was applied. The test phone was programmed to regulate output power in pulses in the range of 0.02–2 W (13–33 dBm). This power was kept constant at 33 dBm during exposure as monitored on-line using a power meter (Bird model 43). During all exposures, two samples were exposed at one time, specific absorption rate (SAR) was 37 mW/kg and discontinuous transmission mode (DTX) was off. The SAR value was determined by measurements and calculations. Both the incident and the reflected power

at the input and the transmitted power through the TEM cell were measured for an input power of 1 W to the TEM cell. The absorbed power was then calculated and the SAR value was determined. Distribution of 915 MHz SAR in our exposure system has been recently described [Sarimov et al., 2004]. Even at peak values, the SAR was well below detectable heating.

Sham exposure, heat shock, and irradiation. Simultaneously with exposure to 915 MHz/50 Hz, the control cells were kept at room temperature, 22–23 °C, under the same conditions as the exposed cells. During all post exposure incubations, control and exposed cells were at 5% CO₂ and 37 °C in a humidified incubator. In four separate experiments, sham exposures were performed in both the ELF unit and the GSM unit with power off and compared with control cells. The sham exposed cells were kept under the same conditions as the exposed ones, including samples at room temperature. During GSM sham exposure, the cells were inside the GSM unit with power off. During ELF sham exposure, the coils were reconnected to produce anti-parallel magnetic fields of the same values as for real exposure. These magnetic fields compensated each other. No significant differences were observed between control and sham exposed cells using techniques described below. Heat shock, 41 °C for 2 h in a water bath, was used as a positive control for stress response. As a positive control for 53BP1 foci formation and apoptosis, the cells were irradiated with ¹³⁷Cs γ rays, 3 Gy, using a Gammacell 1000 (Atomic Energy of Canada Ltd., Ottawa, Canada) source. The dose rate was 10.6 Gy/min.

Apoptosis

Morphological changes associated with apoptosis, e.g., chromatin condensation, membrane blebbing and appearance of apoptotic bodies, were visualized by simultaneous staining of cells with fluorescent dyes acridine orange and propidium iodide and viewed by a fluorescence microscope (Nikon Eclipse E6000) as previously described [Belyaev et al., 2001b]. One hundred cells were scored as normal, apoptotic, or necrotic cells in each of three slides prepared for each treatment condition. The total number of counted cells was 300 per each treatment condition. All samples were analyzed 24 and 48 h after exposure by observers blind to exposure conditions.

Apoptotic fragmentation of DNA was analyzed by PFGE as described previously [Belyaev and Harms-Ringdahl, 2002]. Briefly, after two washes with PBS the cells were mixed with 1% low melting point agarose (Sigma). Agarose blocks were prepared in triplicate using 100 μ l plug molds. The cells were lysed by incubation of agarose blocks in lysis solution (0.25M

Na₂EDTA, 2% w/v sarcosyl, 10 mM Tris-base, pH 7.4) at 37 °C for 48 h. After three washes with Tris-EDTA (TE) buffer (10 mM Tris, 1 mM EDTA, pH 8.0), PFGE was run at 14 °C in 0.5× TBE buffer using a CHEF-DR II apparatus (BioRad) as follows: 1.5% agarose gel, 190 V, run time was 7 h and pulse time was ramping from 1 to 30 s. After electrophoresis, the gels were stained with 0.5 µg/ml ethidium bromide and images were acquired at appropriate saturation using a CCD camera (Gel Doc 1000, Bio-Rad, Hercules, CA). The images were analyzed using Quantiscan (Biosoft) software. Measurements of integrated optical density (IOD) were performed on the images. Calibration curves were obtained for each PFGE using three to five different concentrations of the λ digest pulse marker. The data obtained showed a linear dependence of IOD on the amount of loaded DNA (not shown), which meant that the DNA fragmentation could be quantitatively compared. The percentage of apoptotic DNA fragmentation was calculated assuming that genome of human lymphocyte contains 6 pg DNA.

AVTD Measurements

The conformation of chromatin was studied by the method of anomalous viscosity time dependencies (AVTD). Cell lysis was performed as has been previously described [Belyaev et al., 1999b]. Briefly, lymphocytes were lysed in polyallomer centrifuge tubes (14 mm, Beckman) by addition of 3.1 ml lysis solution (0.25M Na₂EDTA, 2% w/v sarcosyl, 10 mM Tris-base, pH 7.4) to 0.1 ml of cell suspension. The lysates were prepared in triplicate and kept at 23 °C for 5 h in darkness before AVTD measurements. The AVTD were measured in lysates as described using an AVTD-analyzer (Archer-Aquarius, Ltd., Moscow, Russia) [Belyaev et al., 1999b]. The AVTDs were measured at the shear rate of 5.6 s⁻¹ and shear stress of 0.007 N/m². For each treatment condition, the AVTD was measured three times. Two parameters were measured: normalized maximum relative viscosity (NRV), and the relative time (Rt) when the maximum relative viscosity was observed [Belyaev et al., 1999b].

Immunostaining and Foci Analysis

Anti-53BP1 antibodies were a gift by Dr. T. Halazonetis, The Wistar Institute, University of Pennsylvania, Pennsylvania, PA. The antibodies recognize the C-terminal domain of the protein that corresponds to the breast cancer susceptibility gene-1 carboxyl terminus (BRCT) domain. The immunostaining was performed according to Schultz et al. [2000] with some modifications. Immediately after exposure the cells were placed on ice to inhibit eventual repair of DNA damage. Cytospin preparations were fixed in 4% para-

ormaldehyde at room temperature for 10 min, washed once with PBS, permeabilized with 0.2% Triton X-100 for 5 min at room temperature, washed three times for 5 min in PBS, stained with primary antibodies for 1 h, followed by three washes in PBS, incubated with secondary antibodies conjugated with FITC or Texas red, washed three times and mounted with 80% glycerol solution in PBS containing 2.5% 1,4 diazabicyclo-(2.2.2.) octane. Bisbenzimidazole (Hoechst 33258) was added at a concentration of 0.4 µg/ml to the secondary antibody for DNA staining. The images were recorded from three to five randomly selected fields of vision on a DAS microscope Leitz DM RB with a Hamamatsu dual mode cooled CCD camera C4880. One hundred cells were analyzed for each treatment condition.

Statistical Analysis

The data were statistically analyzed using Kolmogorov–Smirnov test. The data that fulfilled the normal distribution were further analyzed with Student's *t*-test. Otherwise, the data were compared by the Mann–Whitney *U*-test or by the Wilcoxon signed ranks test. A correlation analysis was performed using Spearman rank order correlation test. Results were considered as significantly different at *P* < .05.

RESULTS

Apoptosis

Measurements of apoptotic DNA fragmentation and morphological analysis of apoptosis and was performed 24 and 48 h postexposure (Fig. 1). Analysis of data pooled from 50 Hz and from 915 MHz exposures and also from heat shock (41 °C) treatment showed no significant induction of apoptosis in cells from normal or hypersensitive donors. Furthermore, analysis of data pooled from normal and hypersensitive donors showed no statistically significant effects from EMF treatments. Significant apoptotic response was seen after irradiation with 3 Gy (not shown). The data from PFGE and morphology studies were comparable (Fig. 1). The only exception was in cells from donor 3 (Table 1), where PFGE provided significant increase in apoptotic DNA fragmentation after exposure of cells to 50 Hz (*P* < .05, Mann–Whitney *U*-test). This donor was hypersensitive, but not a case of pronounced hypersensitivity. The control levels of apoptosis varied between normal and hypersensitive donors and no difference in the levels of apoptosis between groups has been found (Table 2).

Chromatin Conformation

Effects of exposure were analyzed as data pooled from all subjects, normal and hypersensitive, for each of the treatment conditions. The analysis showed

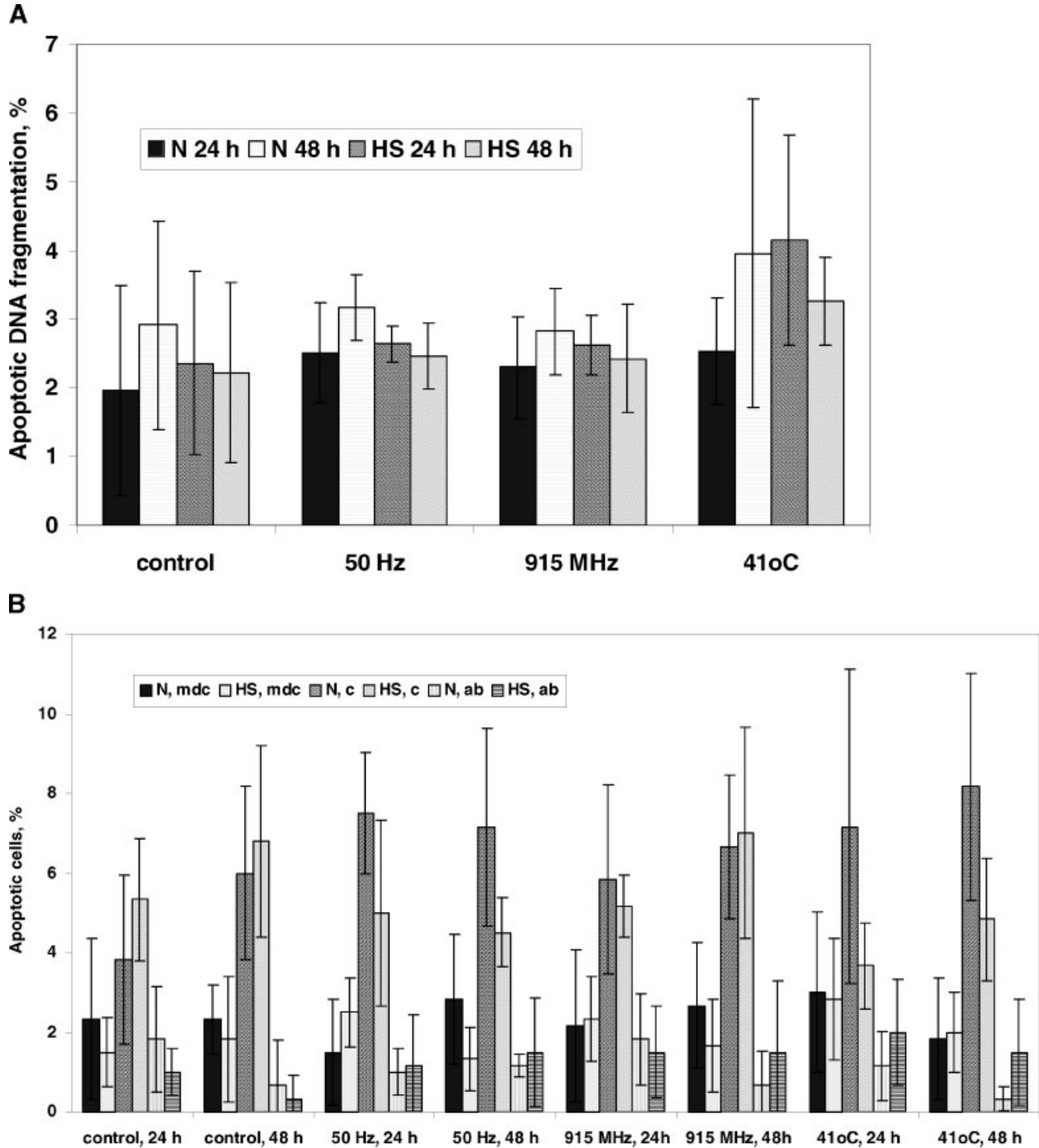


Fig. 1. The histogram shows percentages of apoptotic DNA fragmentation as measured with pulsed-field gel electrophoresis (PFGE) (A) and cells with apoptotic morphological changes (B) after exposure of cells from matched hypersensitive donors (HS) and normal controls (N). Cells were exposed to 915 MHz, 50 Hz, and heat shock (41 °C) for 2 h. Measurements were performed 24 and 48 h following exposures. Percentages for fragmented DNA, membrane-damaged cells (mdc), cells with apoptotic bodies (ab), and cells with condensed chromatin (c) are shown as mean and standard deviations of cell measurements from each of the seven matched-pairs of hypersensitive—normal donors.

TABLE 2. Percentage of Fragmented DNA (Apoptosis) as Measured by PFGE in Unexposed Lymphocytes From Hypersensitive and Control Donors 24 h Postexposure

Matched donors	Hypersensitive	Control	Wilcoxon signed ranks test, <i>P</i>
1*-2	1.8	2.6	
3-5	1.4	2.1	
70*-75	5.2	4.4	
87-88	1.6	0.2	
81*-82	1.8	1.9	.55
91-92	2.0	2.5	
95-97	2.7	0.0	
Mean	2.4	2.0	
SD	1.3	1.5	

An asterisk designates cases of pronounced hypersensitivity.

statistically significant condensation of chromatin in the pooled cells immediately after exposure to 50 Hz and 915 MHz ($P < .015$ and $P < .004$, respectively, Wilcoxon signed ranks test). Based on analysis of these pooled data, 50 Hz induced condensation almost disappeared 2 h postexposure ($P = .059$, Wilcoxon signed ranks test). At this time, the 915 MHz effect was still statistically significant ($P < .02$, Wilcoxon signed ranks test).

Exposure to 50 Hz and 915 MHz resulted in significant changes in chromatin conformation in cells from three and six donors, respectively (Table 3). This

TABLE 3. Changes in Chromatin Conformation in Response to Exposure

Subject	50 Hz		915 MHz	
	NRV	<i>P</i>	NRV	<i>P</i>
1*	0.74 ± 0.06	.02**	0.87 ± 0.04	.03**
2	1.07 ± 0.07	.34	0.98 ± 0.06	.79
3*	0.81 ± 0.09	.1	0.73 ± 0.06	.02**
5	0.81 ± 0.08	.09	0.67 ± 0.09	.03**
70*	0.91 ± 0.04	.09	0.86 ± 0.02	.003**
75	0.99 ± 0.03	.7	0.94 ± 0.05	.32
87*	0.8 ± 0.1	.18	0.81 ± 0.08	.11
88	0.97 ± 0.02	.15	0.87 ± 0.08	.19
81*	0.76 ± 0.09	.05**	0.62 ± 0.04	.001**
82	0.93 ± 0.12	.62	0.98 ± 0.19	.93
91*	0.85 ± 0.09	.21	0.93 ± 0.13	.64
92	0.72 ± 0.08	.03**	0.72 ± 0.09	.03**
95*	1.25 ± 0.43	.53	1.17 ± 0.11	.17
97	0.82 ± 0.10	.17	0.78 ± 0.07	.07

Lymphocytes from hypersensitive subjects and matched control healthy persons were exposed to 50 Hz/915 MHz and NRV was analyzed by the AVTD assay immediately after exposure. Mean and standard error of NRV measured as ratio of three EMF-exposed divided by three measurements of corresponding controls is shown along with *P*-values for effects as analyzed by the Student's *t*-test. An asterisk designates hypersensitive subjects. Double asterisks designate statistically significant effects.

effect was observed both in cells from normal and hypersensitive subjects, two and four cases, respectively (Table 3). The effect of 50 Hz and 915 MHz exposure was similar to stress response induced by heat shock at 41 °C. Two hours after the treatment ended the changes tended to disappear in cells from normal subjects, but not in cells from hypersensitive subjects (Table 3, Fig. 2).

No effects on Rt were observed (not shown). Decrease in NRV was not statistically significant when analyzed across each of the two groups. No statistically significant differences in effects on chromatin conformation were seen between the hypersensitive group and the group of matched controls as measured either immediately or 2 h after exposures/heat shock (Wilcoxon signed ranks test).

The effects reported here on chromatin condensation did not correlate significantly with the age of the persons under investigation, correlation coefficients between the age and the AVTD parameters being in the range from 0.19 to 0.45 (Spearman rank order correlation test).

Interesting to note, that both 50 Hz and 915 MHz effects were stronger in cells of pronounced hypersensitive donors (1, 70, 81) as compared with cells of matched donors (2, 75, 82) (Tables 1 and 3). However, these differences were statistically insignificant when pooled data across ages and gender for these subgroups, three controls versus three pronounced hypersensitive subjects, were compared.

Immunostaining

Our 53BP1 foci analysis included a positive control with γ rays at the dose of 3 Gy. Maximal number of foci was observed 15–30 min after irradiation (Fig. 3). Afterwards, the number of foci decreased, but even 2 h postirradiation, significant increase of foci was observed (Fig. 3). In contrast, neither cells from control subjects nor cells from hypersensitive subjects responded to 50 Hz or 915 MHz by induction of 53BP1 foci (Fig. 4). Rather, we observed a distinct EMF induced reduction in the level of 53BP1 foci both in cells from control and hypersensitive subjects (Fig. 5). Similar reductions in 53BP1 foci were observed in lymphocytes from control (Fig. 5A) and hypersensitive subjects (Fig. 5B). Under identical conditions of treatments, the amounts of foci were not significantly different between cells from matched controls and hypersensitive subjects as analyzed with the Wilcoxon signed ranks test. Therefore, the data from all experiments with cells from control and hypersensitive subjects were pooled. Statistical analysis of these pooled data has shown that both 50 Hz and 915 MHz exposures significantly reduced 53BP1 foci in human lymphocytes ($P < .04$ and $P < .006$,

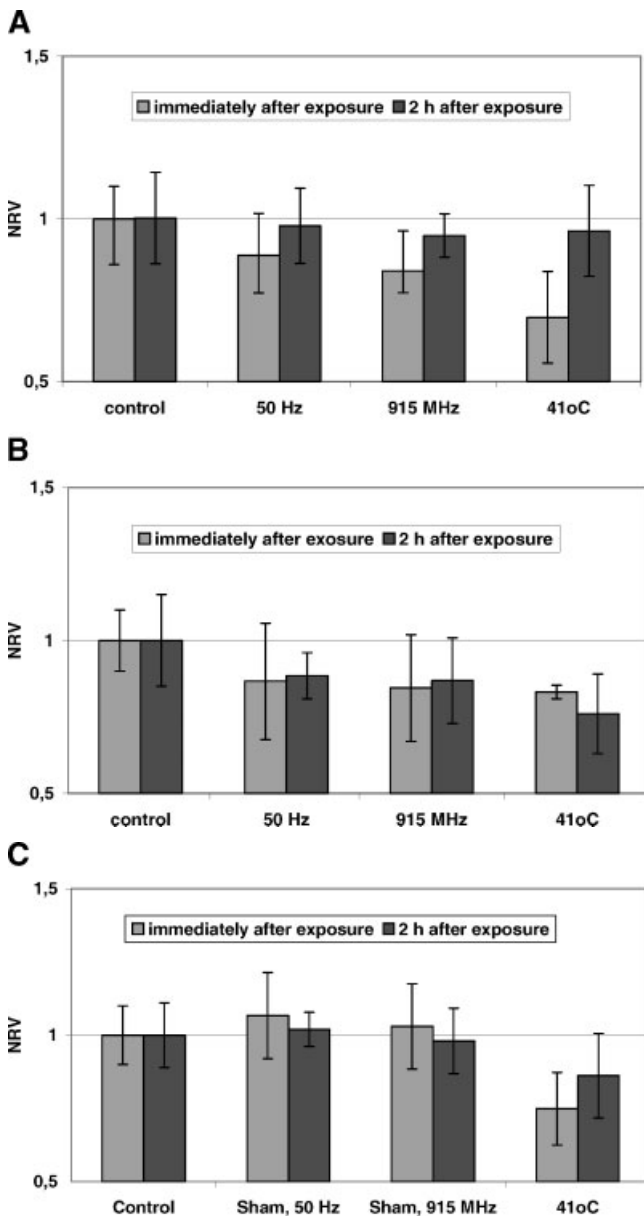


Fig. 2. Normalized maximum relative viscosity in lysates of human lymphocytes from normal controls (A) and hypersensitive subjects (B) as measured with the anomalous viscosity time dependencies (AVTD) method. The cells were exposed 2 h to 915 MHz, 50 Hz, and heat shock (41 °C) as described in the Materials and Methods section. The measurements were performed immediately after exposure (left column) and 2 h later (right column). The mean value based on experiments with cells of seven different donors and standard deviation is shown in each point. In panel C, mean values from four experiments with sham exposures of cells from healthy subjects are shown.

respectively, Mann–Whitney *U*-test). Even stronger reduction was observed after heat shock ($P < .001$, Mann–Whitney *U*-test). No statistically significant age related correlations of effects were found (Spearman rank order correlation test).

DISCUSSION

It was shown in our previous investigations that weak ELF magnetic fields and microwaves affected conformation of chromatin in *E. coli* cells, rat thymocytes, human lymphocytes, and SPD8/V79 Chinese hamster cells under specific conditions of exposure [Belyaev et al., 1994, 1999a, 2000; Belyaev and Alipov, 2001a; Olsson et al., 2001].

Chromatin conformation changes are non specific cellular responses and may be induced by diverse stimuli such as temperature, DNA intercalators, inhibitors of DNA-topoisomerases, electromagnetic fields and ionizing radiation [Belyaev et al., 1999b, 2000, 2001b; Belyaev and Alipov, 2001a]. Usually, in human lymphocytes, the NRV decreased transiently after exposure to weak ELF field in contrast to an increase in the NRV, which was observed immediately after genotoxic impacts such as irradiation [Belyaev et al., 1999b, 2001b]. Several experimental observations have suggested that the increase in the NRV is caused by relaxation of DNA domains. Single cell gel electrophoresis and halo assay confirmed this suggestion [Belyaev et al., 1999b, 2001b]. On the other hand, the decrease in the NRV can depend on chromatin condensation as well as DNA fragmentation [Belyaev et al., 2001b]. Our 53BP1 foci analysis has indicated that no DSBs were produced in response to 915 MHz/50 Hz.

Therefore, the decrease in the NRV in response to these exposures was likely caused by chromatin condensation. However, this condensation was somewhat different from the one induced by ethidium bromide (EtBr) because no decrease in *Rt* was observed here, contrary to EtBr induced condensation as described previously [Belyaev et al., 1999b]. A decrease in NRV was also induced by mild heat shock at 41 °C. These AVTD data correlate with literature data showing condensation of chromatin in mammalian cells in response to mild heat shock [Plehn-Dujowich et al., 2000].

The accepted markers of stress response such as induction of heat shock proteins should be tested to verify molecular mechanisms for the observed effects of 915 MHz/50 Hz. No heating was observed in samples exposed to 915 MHz/50 Hz. Therefore, the effects could not be attributed to heating induced by the exposure systems used. The cells were treated at room temperature, which is more than 10 °C below their normal growth temperature, so it is unlikely that a heat-induced, normal stress-response would occur.

Several proteins involved in DNA repair form distinct nuclear foci after DNA breakage. These include the Rad51 protein [Haaf et al., 1995], the BRCA1 protein [Scully et al., 1997], Mre11-Rad50-Nbs1 complex

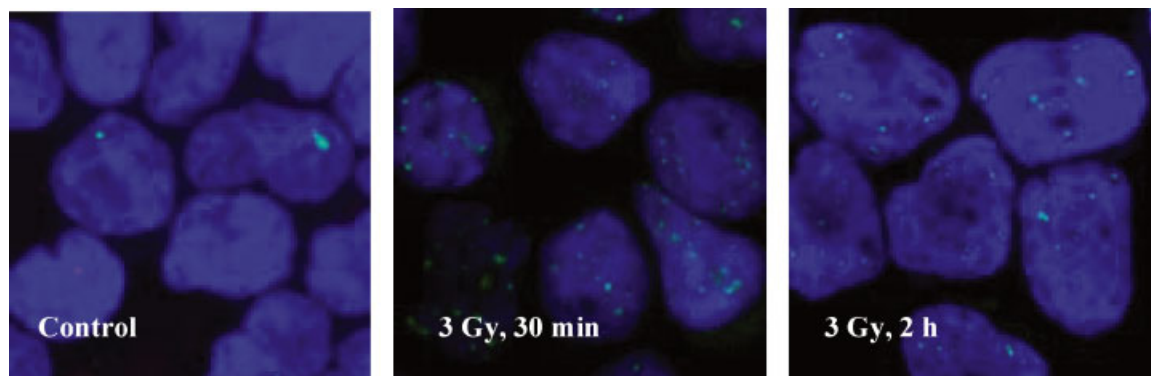


Fig. 3. Panels show 53BP1 foci (stained in green with FITC) 30 min after 3 Gy γ -irradiation of human lymphocytes (counterstained in blue with Hoechst 33258) and 53BP1 foci remaining 2 h after irradiation, as measured by immunostaining with antibody to 53BP1 protein. The images were recorded using a DAS microscope Leitz DMRB at the magnification of 700. [The color figure for this article is available online at www.interscience.wiley.com.]

[Maser et al., 1997; Nelms et al., 1998], CDKN1A [Jakob et al., 2000], γ -H2AX [Rogakou et al., 1999], and 53BP1 [Schultz et al., 2000]. An increasing body of data provides evidence that 53BP1 protein, along with γ -H2AX and mre11-Rad50-nbs1 complex colocalize with DSB in so-called foci in response to genotoxic insults [Maser et al., 1997; Schultz et al., 2000; Sedelnikova et al., 2002]. Analysis of foci formation is a more sensitive assay as compared to other available techniques to measure DSBs such as PFGE or neutral comet assay. Using this sensitive technique we did not find any genotoxic effects of 915 MHz and 50 Hz under the specific conditions of exposure applied here. No apoptotic responses were detected in cells exposed either to 915 MHz or 50 Hz, even though relatively sensitive tools were used [Belyaev et al., 2001b; Olsson et al., 2001; Belyaev and Harms-Ringdahl, 2002]. In particular, statistically significant induction of 50 kb apoptotic DNA fragmentation was consistently detected in lymphocytes following γ -irradiation at low doses

of 5, 10, and 20 cGy [Belyaev and Harms-Ringdahl, 2002]. We assumed that the exposures to 915 MHz and 50 Hz under conditions employed here did not affect significantly apoptosis in human lymphocytes.

Effects of microwaves have been shown to depend on several biological and physical parameters such as frequency, flux density, cell density, and presence of divalent ions and radical scavengers during exposure [Blackman et al., 1989; Adey, 1999; Belyaev et al., 2000]. The ELF effects are also dependent on variety of physical and biological parameters [Smith et al., 1987; Blackman et al., 1988, 1994; Belyaev et al., 1994, 1999a; Prato et al., 1995]. Moreover, exposure of human lymphocytes or V79 SPD8 cells to magnetic field at 8 Hz (21 μ T rms) resulted in DNA fragmentation [Belyaev et al., 2001c; Olsson et al., 2001]. Therefore, the absence of genotoxic effects under the specific parameters of exposure used in this study does not support a conclusion on absence of genotoxic effects of ELF and microwaves in general.

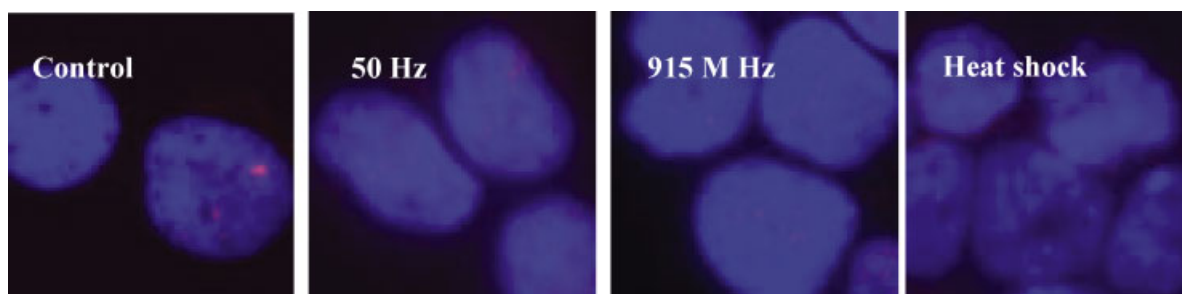


Fig. 4. Panels show typical images of human lymphocytes (counterstained in blue with Hoechst 33258) with 53BP1 foci (stained in red with Texas red) as revealed by immunostaining of 53BP1 protein. Foci were seen in control cells. Fewer foci were observed in cells immediately after 2 h exposure to 50 Hz, 915 MHz, and heat shock, 41 $^{\circ}$ C during 2 h. [The color figure for this article is available online at www.interscience.wiley.com.]

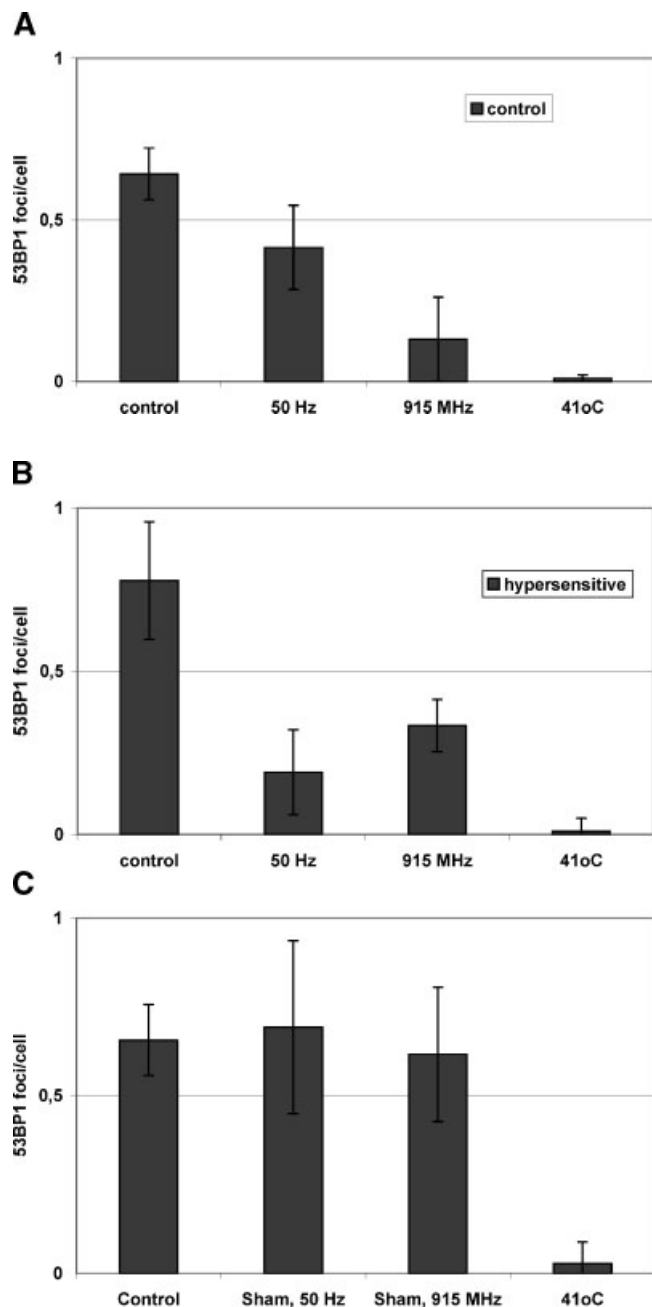


Fig. 5. 53BP1 foci in human lymphocytes of matched controls (A) and hypersensitive (B) subjects before and after exposure to 50 Hz, 915 MHz and heat shock, 41 °C, as measured by immunostaining with antibody to 53BP1 protein following 2 h treatment. Mean values for amounts of foci per cell from three independent experiments and standard errors of mean are shown. No 53BP1 foci were induced by exposures and heat shock. Instead, reduced background level was seen after all treatments. The effects of 915 MHz, 50 Hz and heat shock were statistically significant (Mann–Whitney *U*-test) as analyzed the pooled data. In panel C, mean values from four experiments with sham exposures of cells from healthy subjects are shown.

A novel result of this study is the reduction in the number of 53BP1 foci in response to heat shock, ELF and microwaves. These immunostaining data correlated with the AVTD results providing evidence that 915 MHz and 50 Hz under specific conditions of exposure induced a stress-like response. We hypothesize that chromatin condensation, which was detected by the AVTD technique, reduced the availability of DNA breaks to 53BP1 antibody. In addition, chromatin condensation might also block accessibility of DNA for nucleases and DNA repair enzymes. Our data suggest that 53BP1 foci along with AVTD measurements may provide a new tool to analyze stress response.

Comparison of pooled data obtained with 50 Hz and 915 MHz did not show significant differences in effects between groups of controls and hypersensitive subjects. This result might be explained by the heterogeneity in groups of hypersensitive and control persons. Even if there was such a difference, it would be masked by the large individual variation between donors, which was observed in both control and hypersensitive groups. Whether this individual response has a genetic component remains to be elucidated. An additional problem may be the lack of objective criteria for selection of a study group consisting of persons that are truly hypersensitive to EMF, although this has yet to be proven. Any observation associated with reported hypersensitivity may also be a result of other factors associated with long suffering of ill health regardless of the background. In the hypersensitive group, the subjects who were classified as “pronounced hypersensitive,” showed the stronger response to both 915 MHz and 50 Hz as compared with matched control subjects. However, these data are based only on three cases and may be considered only as a possible trend before new investigations with extended groups will be performed. Another trend was a prolonged change in chromatin conformation in cells of hypersensitive subjects (Fig. 2B). The observed trends for effects of longer duration in lymphocytes from hypersensitive subjects and for generally stronger effects in lymphocytes from pronounced hypersensitive subjects deserve further investigations.

CONCLUSION

Weak 50 Hz magnetic field and nonthermal microwaves from mobile phone induced stress-like responses in human lymphocytes similar to heat shock. These effects included changes in chromatin conformation and reduced level of 53BP1 foci. No significant difference in response was observed between lymphocytes from hypersensitive and healthy subjects.

ACKNOWLEDGMENTS

We are thankful to Dr. T. Halazonetis (The Wistar Institute, University of Pennsylvania, Philadelphia, PA) for kind donation of antibodies to 53BP1, Dr. L.-E. Paulsson and Dr. G. Anger (the Swedish Authority for Radiation Protection, Stockholm, Sweden) for verification of the experimental units for exposure to ELF and microwaves, Dr. G. Nindl (Indiana University School of Medicine) for generous presentation of the results reported at the 24th Annual BEMS Meeting (Quebec, Canada, 2002), and Ms. Eva Thunberg (Department of Public Health Sciences, Division of Occupational Medicine, Karolinska Institutet, Stockholm, Sweden) for obtaining and coding of blood samples. Special thanks to Dr. C. Blackman (EPA, Research Triangle Park, NC) for reading the article and valuable comments.

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