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Anomalous viscosity time dependencies (AVTD); DNA double strand breaks (DSBs); electromagnetic field (EMF); microwaves (MWs); fetal bovine serum (FBS); Global System for Mobile Communication (GSM); phosphorylated histone H2AX (γ-H2AX); specific absorbed rate (SAR); transverse electromagnetic transmission line cell (TEM-cell); tumor suppressor p53 binding protein 1 (53BP1).

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Abstract

The data on biological effects of non-thermal microwaves (MWs) from mobile phones are diverse and these effects are presently ignored by the International Commission for Non-Ionizing Radiation Protection (ICNIRP) safety standards. Here, we investigated effects of MWs of Global System for Mobile Communication (GSM) at different carrier frequencies on human lymphocytes from healthy persons and from persons reporting hypersensitivity to electromagnetic fields (EMF). The changes in chromatin conformation, which are indicative of stress response and genotoxic effects, were measured by the method of anomalous viscosity time dependencies (AVTD). The 53BP1 and γ-H2AX proteins, which have been shown to co-localize in distinct foci with DNA double strand breaks (DSBs), were analyzed by immunofluorescence confocal laser microscopy. We show that MWs from GSM mobile phone affect chromatin conformation and 53BP1/γ-H2AX foci similar to heat shock. For the first time, we report here that MWs from mobile phone affect 53BP1/γ-H2AX foci are dependent on carrier frequency. In average, the same response was observed in lymphocytes from hypersensitive and healthy subjects.

Introduction

The growing public concerns about possible effects of microwave (MW) exposure from mobile phones have been discussed in many countries because of increasing usage of wireless communication systems. It has been reported by two groups that increased incidence of brain tumors is correlated with exposure to mobile phone MWs depending on duration of mobile phone use (Hardell et al. 2003; Lonn et al. 2004). The negative findings were also reported by other groups, but the results of theses epidemiological studies are not directly comparable because of methodological and other differences as has recently been reviewed (Kundi et al. 2004). The intensity levels of exposure to MWs from mobile phones are lower than the standards adopted by the International Commission for Non-Ionizing Radiation Protection (ICNIRP) (ICNIRP 1998). These standards are based on the thermal effects of microwaves resulting in heating of exposed tissues or cells. There is also evidence for non-thermal effects of MWs that suggests a possible relationship between non-thermal MW exposure and permeability of the brain blood barrier (Persson et al. 1997) and stress response (de Pomerai et al. 2000). Recent studies have described neuronal damage in the brains of exposed rats (Ilhan et al. 2004; Salford et al. 2003). In other studies, no effects of non-thermal microwaves were observed (Meltz 2003). However, experimental data suggested that MW effects occur only under specific parameters of exposure, depending on several physical parameters and biological variables (Adey 1999; Belyaev et al. 2000; Binhi 2002; Blackman et al. 1989). Dependence of the MW effects on several physical parameters, including frequency, polarization, and modulation, and also several biological variables could explain various outcomes of studies with non-thermal microwaves (Belyaev et al. 2000).

It has been described that MWs under specific conditions of exposure induce DNA strand breaks in rat brain cells as measured by single cell electrophoresis (Lai and Singh 1996; Lai and Singh 1997). The mechanisms of this effect are not understood, but could also be related to induced changes in the interaction of DNA with proteins, rather than DNA damage (Belyaev et al. 1999).

Several proteins such as the tumor suppressor p53 binding protein 1 (53BP1) and phosphorylated H2AX (γ-H2AX) have been shown to produce discrete intranuclear foci, which are believed to co-localize with DNA double strand breaks (DSBs) providing a scaffold structure for DSB repair (DiTullio et al. 2002; Schultz et al. 2000; Sedelnikova et al. 2002). According to the current model, this scaffold functions by recruiting proteins involved in the repair of DSBs (Fernandez-Capetillo et al. 2002; Iwabuchi et al. 2003; Kao et al. 2003). The scaffold is organized within a megabase-size chromatin domain around an actual DSB regardless of the type of repair that is involved (Paull et al. 2000). We have analyzed the 53BP1 foci in human lymphocytes following exposure to MWs from mobile phones using the Global System for Mobile Communication (GSM) standard at 915 MHz and have not found induction of 53BP1 foci (Belyaev et al. 2005). In contrast, we found that MWs similar to heat shock induced significant reduction in the background level of 53BP1 foci (Belyaev et al. 2005). The γ-H2AX protein was analyzed here in addition to the 53BP1 protein. The method of anomalous viscosity time dependence (AVTD) that is sensitive to various genotoxic effects (Belyaev et al. 2001; Belyaev et al. 1999) was also applied.

So-called hypersensitivity to electromagnetic field (EMF) is a fairly new phenomenon, and etiology of the hypersensitivity to EMF is not known yet. There are

several symptoms that people experience in proximity to different sources of EMF, such as video display terminals of personal computers, electrical appliances or mobile phones. The symptoms are not specific for this illness and there is no known pathophysiological marker or diagnostic test (Hillert et al. 1999).

There is a substantial lack of knowledge in the biophysical modeling of MW-induced non-thermal biological effects. Resonance-like interactions of MWs with such targets as cellular membranes, chromosomal DNA, and ions in protein cavities have been proposed (Adey 1999; Belyaev et al. 1992a; Binhi 2002; Ismailov 1987).

Among other dependencies, dependence of non-thermal effects of MWs on frequency has been reported (Belyaev et al. 2000; Pakhomov et al. 1998). Non-thermal effects of GSM MWs at various frequencies on the conformation of chromatin in human lymphocytes have recently been studied (Sarimov et al. 2004). The data have shown that MWs from GSM mobile phone affect chromatin conformation in human normal and transformed lymphocytes at specific frequencies, 905 MHz and 915 MHz being most effective. The observed MW effects depended upon the initial state of chromatin as measured before exposure, and were similar to stress responses induced by heating (Sarimov et al. 2004). In the present study, we analyzed the effects of MWs at different frequencies on chromatin conformation and 53BP1 and γ-H2AX foci in lymphocytes from healthy and hypersensitive subjects.

Materials and Methods

Subjects and blood samples

Blood samples from 5 healthy subjects and 5 patients reporting hypersensitivity to EMF 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 regarding symptom profile, triggering factors, exposure time relationships and avoidance behavior (Hillert et al. 1999). The group reporting hypersensitivity to EMF consisted of 5 men 32-60 years old (Table 1). Control healthy subjects were matched by age (±5 years) and gender (Table 1). All hypersensitive persons and controls were working or studying. There were no smokers among the participants and no subject was on any regular medication. All hypersensitive subjects reported that symptoms were triggered by electrical devices that were not sources of light. Four of the participants reported that mobile phones also triggered symptoms. The fifth subject did not use a mobile phone and consequently did not know if this exposure would trigger symptoms. In all pairs, the hypersensitive person scored higher than the matched control in the questionnaire on symptoms (29 symptoms scored for frequency and severity, maximum score 232) (Hillert et al. 1998). In all persons reporting hypersensitivity to EMF, the neurovegetative symptoms like headache, fatigue and difficulties concentrating were more pronounced than skin symptoms. The mean scores per person for neurovegetative symptoms were 33 in the hypersensitive group and 1.2 in the control group. The corresponding scores for skin symptoms in the face and upper chest were 10 and 0.4, respectively. In all cases of reported

hypersensitivity, the symptoms were experienced within 24 hours after exposure to a reported triggering factor; in most cases within one hour. All patients reported that they tried to avoid triggering factors.

Fresh blood samples from persons reporting hypersensitivity and matched controls were coded and all data were analyzed blind. Ethical permissions were obtained from the Ethics Committee of the Karolinska Institutet, Stockholm, Sweden.

Chemicals and reagents

Reagent grade chemicals were obtained from Sigma (St. Louis, MI, USA) and Merck KgaA (Darmstadt Germany). Double cytoslides coated with polylysine and cytoslide chambers were purchased from ThermoShandon (Pittsburg PA, USA) . Anti-53BP1 antibody (anti mouse) was kindly provided by Dr. T. Halazonetis, The Wistar Institute, University of Pennsylvania, USA. The antibody recognizes the C-terminal domain of the protein that corresponds to the BRCT domains. Anti-Phosphorylated histone H2AX (γ-H2AX) (anti rabbit) was purchased from Trevigen-BioSite (Täby, Sweden).

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 bovine serum (FBS), 2 m*M* L-glutamine, 50 IU/ml penicillin, 50 µg/ml streptomycin (ICN Pharmaceuticals, Inc., Costa Mesa, CA, USA) and incubated at 5% CO2 and 37°C in a humidified incubator. Adherent monocytes were removed by overnight incubation of the cell suspension in culture flasks

Falcon (Becton Dickinson & Co., Franklin Lakes, NJ, USA) at a cell density of 3×10^6 cells/ml in a volume of 10-40 ml. After this incubation, the cells remaining 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 pre-incubated for 2 h at 37°C before exposure. The viability of cells was always above 98% as measured with a trypan blue exclusion assay.

Cell exposure

In five independent experiments, coded samples from hypersensitive subjects and matched control subjects were exposed simultaneously. All exposures were performed at 37^oC in an humidified CO_2-incubator, in 14 ml round-bottom tubes (Falcon), 2.5 ml of cell suspension per tube, 2×10^6 cells/ml. Duration of all exposures was 1 h. Lymphocytes were exposed to MWs using a GSM900 test-mobile phone (model GF337, Ericsson, Sweden) as previously described (Belyaev et al. 2005; Sarimov et al. 2004). Briefly, the output of the phone was connected by the coaxial cable to a transverse electromagnetic transmission line cell (TEM-cell). 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 MHz and 915 MHz. We used channels 74 and 124 with frequencies of 905 and 915 MHz, respectively. The signal included standard GSM modulations. No voice modulation was applied. Discontinuous transmission mode was off during all exposures. GSM signal is produced as 577 μs pulses (time slots), with an interpulse waiting time of 4039 μs (7 time slots). The power was kept constant during exposures, at 2 W (33 dBm) in pulse, as monitored on-line using a power meter (Bird 43, USA). The specific absorption rate (SAR) was determined

by measurement and calculation. Transmitted and reflected power was measured using a power meter (Hewlett-Packard 435A, USA) and a coaxial directional coupler (Narda 3001-20, USA). A signal generator (Agilent 7648C, USA) connected to a power amplifier (Mini-circuit ZHL-2-8-N, USA) was used. The SAR was calculated from the absorbed power and the mass of the sample to be 37 mW/kg. Good correlation between these measurements and calculations using the finite different time domain (FDTD) method has been confirmed (Sarimov et al. 2004). Because of non-equal distribution of SAR through the exposed volume, the minimal and maximal FDTD-derived SARs were 2.5-fold lower and 3.3-fold higher, respectively, as compare to the mean values. All these SAR values were well below thermal effects. Temperature was measured in the MW-exposed samples before and after exposure with a precision of 0.1^{o}C. No changes in temperature were induced during exposures.

At the place of exposure, static magnetic field was 18±2 μT as measured by means of a magnetometer (Sam3, Dowty Electronics Ltd., England) and background extremely low frequency magnetic field was not more than 200 nT, rms, as measured with a three-dimensional microteslameter (Field dosimeter 3, Combinova, Sweden).

In each experiment, the cells from the same blood samples were exposed in the same TEM-cell to MWs at 915 MHz, 905 MHz and sham-exposed with MWs off. The cells were exposed in sequence and the order of exposure was randomized. Heat treatment in a water bath, at 41^{o}C and 43^{o}C, was used as a positive control for stress responses. As a positive control for genotoxic effect, the cells were irradiated with ^{137}Cs γ-rays, at 3 Gy, using a Gammacell 1000 (Atomic Energy of Canada Limited, Ottawa, Canada) source. The dose rate was 10.6 Gy/min.

AVTD measurements

The conformation of chromatin was studied by the method of anomalous viscosity time dependencies (AVTD). Cell lysis was performed immediately after exposure as has been previously described (Belyaev et al. 1999). Briefly, lymphocytes were lysed in polyallomer centrifuge tubes (14 mm, Beckman, USA) by addition of 3.1 ml lysis solution (0.25 *M* Na_2EDTA, 2% w/v sarcosyl, 10 m*M* Tris-base, pH 7.4) to 0.1 ml of cell suspension. The lysates were prepared in triplicate and kept at 23°C for 4 h in darkness before AVTD measurements. The AVTDs were measured at a shear rate of 5.6 s^{-1} and shear stress of 0.007 N/m^2. Normalized relative viscosity was used to characterize condensation of chromatin (Belyaev et al. 1999).

Immunostaining and foci analysis

Immediately after exposure, the cells were placed on ice for 1 hour to prevent the repair of eventual DSBs. Cytoslide samples were prepared by using cytospin centrifugation according to the manufacturer's instructions (ThermoShandon). The immunostaining was performed according to Schultz *et al*. (Schultz et al. 2000) with some modifications. Cells were fixed in cold 3% paraformaldehyde in PBS, pH 7.4, permeabilized with cold 0.2% Triton X-100 in PBS (for 15 and 10 min, respectively), stained with primary antibody 53BP1 (1:20) and γ-H2AX (1:100) prepared in 2% FBS in PBS for 1h, followed by 3 washes in cold PBS and incubated for 1 h with secondary Hexo goat anti-mouse IgG (H+L) antibody conjugated with Alexa fluor 488 (Molecular Probes, Inc., Eugene, OR, USA) together with Zymax goat anti-rabbit IgG Cy3 conjugate (Zymed, San Francisco, CA, USA), both in 2% FBS and in 1:200 dilution, followed by 3 washes in cold PBS. After 20 min DNA staining in ToPro-3-iodide (Molecular Probes,

10 μM in PBS, prepared from 1 mM stock solution in DMSO) and 5 min washing in PBS, cytoslides were mounted with equilibration solution and antifade reagent (Slow fade Light Antifade Kit, Molecular Probes) and sealed with cover slides. The images were recorded using a confocal laser scanning microscope Zeiss Axiovert 100M (Germany) from 5-10 fields of vision that were randomly selected from two slides per treatment condition. Through focus maximum projection images were acquired from optical sections 1.00 μm apart and with a section thickness of 2.00 μm in the Z-axis. Resolutions in X-,Y-axis were 0.20 μm. Seven optical sections were usually acquired for each field of vision and a final image was obtained by projection of all sections onto one plane. The foci were counted in the cells from these final images using LSM 510 software. For each experimental condition, 300-600 cells were analyzed. All images were analyzed blind as to exposure parameters.

Statistical analysis

A statistical power was set to 0.80 as based on previously obtained data on effects of GSM MWs on human lymphocytes (Belyaev et al. 2005; Sarimov et al. 2004). The data were analyzed with the Mann-Whitney U-test, Kruskal-Wallis test or by the Wilcoxon matched pairs signed rank test. A correlation analysis was performed using Spearman rank order correlation test. Results were considered as significantly different at $p<0.05$.

Results

Viability

The viability of unexposed cells as measured by the trypan blue exclusion assay varied between normal and hypersensitive subjects in the range of 0.01-2%. No statistical difference in the levels of viability between these groups was found.

Chromatin conformation

Statistically significant decrease in AVTD corresponding to chromatin condensation was observed in cells of 5 subjects (301, 302, 406, 606, and 607, Table 2) out of 10 at the frequency of 915 MHz ($p<0.05$, Mann-Whitney U-test) (Table 2). In contrast, significant increase in AVTD that corresponds to decondensation of chromatin was observed after 915 MHz only in cells from subject 403. MWs at 905 MHz resulted in either significant condensation, subject 607, or decondensation, subject 403, or no effects (Table 2). These data suggested that effects of MWs might be frequency dependent, and that differing responses might be observed in cells from different individuals. Similar interindividual variability was observed in response to the heat shock, especially at 43^{o}C, where two subjects responded by condensation (subjects 406, 707) and two by decondensation (subjects 302, 403). No statistically significant differences between the effects on chromatin conformation were seen in cells from controls and hypersensitive groups as measured after either MW exposures or heat shock ($p>0.05$, Wilcoxon matched pairs signed rank test). The data pooled from all subjects, normal and hypersensitive, were analyzed for each treatment condition. The analysis of these pooled data showed a statistically significant effect of MW exposure at 915 MHz ($p<0.0223$, the Mann-Whitney U-test).

Immunostaining

Our 53BP1/γ-H2AX foci analysis included a positive control with γ-rays at the dose of 3 Gy. Significant increase in the number of foci was observed 1 h after irradiation (not shown). In contrast, neither cells from control subjects nor cells from hypersensitive subjects responded to 915 MHz by induction of foci (Table 3). We observed a distinct MW-induced reduction in the level of 53BP1/γ-H2AX foci both in cells from control and hypersensitive subjects in response to 915 MHz (Fig. 1, Table 3, 4). Very similar reductions in 53BP1/γ-H2AX foci were observed in lymphocytes from control and hypersensitive subjects in response to heat shock at 41^{o}C and 43^{o}C (Table 3, 4, Fig. 2A, B). The response to 905 MHz was not consistent among subjects, and either increase or decrease or no effect was observed in number of foci, dependent on subject (Table 3, 4).

For each subject, we verified the hypothesis that MW exposure affects formation of 53BP1 and γ-H2AX foci. For this purpose, we compared effects of microwave exposures with sham (multiple comparisons of sham, 905 MHz and 915 MHz) using the Kruskal-Wallis ANOVA by ranks. This comparison showed that MWs affected both 53BP1 and γ-H2AX foci in cells from each tested person (Table 5).

We next verified the hypothesis that the effect of MW exposure was frequency-dependent. This was done by comparison of MW effects at 905 MHz and 915 MHz for cells from each subject by the Mann-Whitney U-test. This comparison showed that MW effects on 53BP1 foci depended on frequency in cells from nine subjects (except for subject 606) and effects on γ-H2AX foci depended on frequency in cells from six subjects (except for subjects 302, 406, 607, and 708) (Table 6).

Under identical conditions of treatment, the numbers of 53BP1 and γ-H2AX foci were not significantly different between cells from matched controls and hypersensitive subjects as compared using the Wilcoxon matched pairs signed rank test. Therefore, the data from all experiments with cells from control and hypersensitive subjects were pooled. Statistical analysis of these pooled data showed that 915 MHz exposure significantly reduced the number of 53BP1 and γ-H2AX foci in human lymphocytes (Table 3, 4). Despite the fact that no heating was induced by microwave exposure, the reduction in the number of 53BP1 and γ-H2AX foci was larger than after heat shock at 41^{o}C (Table 3, 4). In the case of γ-H2AX foci, this reduction was even larger than after heat shock at 43^{o}C (Table 4). Importantly, the pooled effects of MWs were statistically significantly different at 915 MHz and 905 MHz for both 53BP1 and γ-H2AX foci, $p<0.0125$ and $p<0.0357$, respectively (Wilcoxon matched pairs signed rank test).

For all treatment conditions, a correlation between 53BP1 and γ-H2AX foci was observed ($R=0.64$, $p<0.00001$, Spearman rank order correlations test). However, the majority of 53BP1 and γ-H2AX foci did not co-localize and the co-localization did not exceed 7 %.

Discussion

It has been previously shown that non-thermal microwaves affected conformation of chromatin in *E. coli* cells, rat thymocytes and human lymphocytes under specific conditions of exposure (Belyaev et al. 1992b; Belyaev et al. 2002; Belyaev et al. 2000; Sarimov et al. 2004). Usually, in human lymphocytes, the AVTD decreased transiently after exposure to non-thermal microwaves as opposed to the increase in

AVTD observed immediately after genotoxic impacts, such as ionizing radiation or chemicals (Belyaev et al. 2001; Belyaev et al. 1999; Sarimov et al. 2004). Several experimental observations have suggested that the increase in the AVTD is caused by the relaxation of DNA domains (Belyaev and Harms-Ringdahl 1996). Single cell gel electrophoresis and halo assay have confirmed this suggestion (Belyaev et al. 2001; Belyaev et al. 1999). On the other hand, the decrease in AVTD can be caused by either chromatin condensation or DNA fragmentation (Belyaev et al. 2001; Belyaev et al. 1999). Since no 53BP1/γ-H2AX foci were produced in response to 915 MHz, the decrease in the normalized maximum relative viscosity induced by the 915 MHz exposures was likely caused by chromatin condensation. Both decrease and increase in AVTD were induced by heat shock at 41^{o}C and 43^{o}C, depending on subject (Table 2). In contrast to a previous study (Sarimov et al. 2004) where cells were exposed to MWs at room temperature, MW exposure was performed at 37^{o}C in the present study. Bearing in mind the previously observed dependence of MW effects on temperature (de Pomerai et al. 2000), the data from these two studies should be compared with care. The AVTD data from both studies show that MWs and heat shock result in either condensation or decondensation of chromatin in human lymphocytes dependent on the subject and the duration and temperature of treatment. No heating was observed in samples exposed to microwaves. Therefore, the MW effects were not caused by heating.

The analysis of the 53BP1/γ-H2AX foci is a more sensitive assay as compared to other available techniques to measure DSBs, such as pulsed field gel electrophoresis or neutral comet assay. Using this sensitive technique we did not find any genotoxic effects of 915 MHz under the specific conditions of exposure employed here. In contrast, this

frequency persistently decreased the level of foci. Therefore, we confirm here our previous finding that exposure at 915 MHz reduces 53BP1 foci in a manner similar to heat shock, suggesting that this frequency affects cells in a manner similar to a stress factor (Belyaev et al. 2005; Belyaev et al. 2002). The duration of exposure was 2 h in the previous study (Belyaev et al. 2005). Here, we show that even shorter exposure, 1 h, produces significant reduction in the 53BP1 level.

In contrast to 915 MHz exposures, MWs at 905 MHz could either decrease or increase the number of foci depending on the subject. Does it mean that 905 MHz exposures induce DSBs in those cases where foci increased? The data obtained here neither exclude nor directly support such a possibility. We should also state that we do not really know the details of the subjects' physiological status, and therefore that may be the determining factor.

Frequency-dependent inhibition of DNA repair by non-thermal microwaves has previously been found (Belyaev et al. 1992a, 1992b). The novel result of this paper is that both 53BP1 and γ-H2AX foci can be decreased similarly by heat shock and MWs from mobile phones. We hypothesize that stress-induced chromatin condensation reduces either availability of DNA breaks to enzymes and antibodies or disrupts DNA repair machinery that involves binding of 53BP1/γ-H2AX proteins to DSBs. If repair is affected according to the second of these hypotheses, the obtained results may have a connection to genotoxicity and cancer.

We show here for the first time that the vast majority of 53BP1 and γ-H2AX foci do not co-localize in either sham-control or MW/heat shock treated lymphocytes. It should be noted that the formation of these foci deal with phosphorylation of 53BP1/γ-

H2AX proteins (DiTullio et al. 2002; Fernandez-Capetillo et al. 2002). It is thus possible that the observed effects of MW and heat shock at the level of 53BP1/γ-H2AX foci formation was due to a change in phosphorylation. Recent evidence has indicated activation of stress-induced pathways in cultivated cells in response to MWs (Leszczynski et al. 2002). Their publication 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). Whether activation of stress response pathways relates to apoptosis, brain blood barrier permeability, or increased cancer in humans remains to be investigated.

The comparison of pooled data obtained with all treatments did not show significant differences between the groups of controls and hypersensitive subjects. This result might be explained by the heterogeneity in groups of hypersensitive and control persons. Even if there is such a difference, it would be masked by the large individual variation between subjects, which was observed in both control and hypersensitive groups. An additional problem may be the lack of any objective criteria for selection of a study groups consisting of persons that are truly either insensitive or hypersensitive to EMF (although this has yet to be proven).

For the first time, the data obtained here clearly show that MWs from GSM mobile phones affect simultaneously the formation of 53BP1 and γ-H2AX foci in human lymphocytes as function of carrier frequency. This result obtained in lymphocytes from both healthy and hypersensitive persons is of great importance. Such frequency dependence suggests a mechanism that does not deal with thermal heating. Investigation

of this mechanism and the molecular targets of the frequency-dependent effects of MWs in the frequency range of mobile communication is a fundamental problem.

Another aspect of this finding is that criteria other than “thermal”, based on SAR and power density in acute exposures may be needed for accurate safety standards. In particular, these safety standards certainly cannot be based on data obtained at one specific frequency.

Conclusions

Non-thermal microwaves from GSM mobile phones at lower levels than the ICNIRP safety standards affect 53BP1 and γ-H2AX foci and chromatin conformation in human lymphocytes. These effects suggest induction of stress response and/or DNA damage. For the first time, we report that mobile phone MWs affect 53BP1 and γ-H2AX foci dependent on carrier frequency. We also show that heat shock induces similar responses. The same responses were observed in lymphocytes from healthy subjects and from subjects reporting hypersensitivity to electromagnetic fields.

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Tables

Table 1. Information on hypersensitive subjects and matched control healthy persons.

Subject	Gender	Age (years)	Duration of hypersensitivity (years)
301[1]	M	32	5
302	M	33	-
403[1]	M	33	2
406	M	29	-
501[1]	M	47	8
502	M	44	-
606	M	45	-
607[1]	M	45	1
707	M	59	-
708[1]	M	60	2

[1] designates cases of reported hypersensitivity to electromagnetic fields.

Table 2. Relative changes in chromatin conformation in response to MWs as analyzed by the AVTD assay immediately after exposure and normalized to sham (normalised relative viscosity, NRV). Lymphocytes from 5 hypersensitive subjects and 5 matched control healthy persons were exposed to MWs at 905 MHz or 915 MHz during 1 h. Mean of 3 measurements and standard deviation (SD) is shown along with p-values, Mann-Whitney U-test. Asterisks designate statistically significant effects at $p<0.05$* and $p<0.01$**.

Subject	905 MHz			915 MHz			41°C			43°C		
	NRV	SD	p	NRV	SD	p	NRV	SD	p	NRV	SD	p
301[1]	1.08	0.15	0.6122	0.45	0.04	0.0039**	0.75	0.10	0.0937	1.02	0.11	0.8485
302	1.33	0.18	0.1374	0.39	0.08	0.0018**	0.94	0.09	0.5422	1.31	0.10	0.0314*
403[1]	1.51	0.20	0.0256*	1.77	0.21	0.0034**	1.26	0.17	0.1486	2.43	0.39	0.0105*
406	0.72	0.15	0.1338	0.66	0.02	0.0003**	0.58	0.13	0.0304*	0.49	0.02	0.0001**
501[1]	0.81	0.29	0.5851	0.96	0.23	0.8613	0.84	0.20	0.6278	1.49	0.34	0.1008
502	0.78	0.14	0.2566	0.62	0.12	0.0844	0.56	0.12	0.0618	0.92	0.15	0.6411
606	0.90	0.07	0.2282	0.71	0.06	0.0137*	-			0.67	0.12	0.0617
607[1]	0.68	0.08	0.0200*	0.83	0.05	0.0322*	-			0.90	0.09	0.3154
707	1.16	0.14	0.3193	1.12	0.06	0.0990	1.10	0.03	0.0323**	0.80	0.03	0.0025**
708[1]	0.83	0.10	0.1814	0.97	0.12	0.8313	0.83	0.23	0.5085	0.77	0.10	0.1019
All subjects	0.98	0.28	0.4812	0.85	0.40	0.0232*	0.86	0.24	0.0831	1.08	0.56	0.4812

[1] designates cases of reported hypersensitivity to electromagnetic fields.

Table 3. Changes in 53BP1 foci in response to 1 h MW exposure. Lymphocytes from 5 subjects reporting hypersensitivity to EMF (HE) and 5 matched healthy control subjects (CS) were exposed to MWs at 905 MHz and 915 MHz or heat shocked. For each subject, mean of measurements in 300-600 cells and SD is shown along with p-values for differences as compared to sham-exposure by the Mann-Whitney U test. Asterisks designate statistically significant effects at $p<0.05$* and $p<0.01$**. Arrows ↓ and ↑ designate direction of effects, decrease or increase, respectively.

Subject	Sham		905 MHz			915 MHz			41°C			43°C		
	53BP1	SD	53BP1	SD	p	53BP1	SD	p	53BP1	SD	p	53BP1	SD	p
301[1]	0.95	0.73	1.54	0.65	0.0652	0.03↓	0.06	0.0099**	0.06↓	0.04	0.0013**	0.14↓	0.09	0.0030**
302	1.45	0.81	2.44↑	0.63	0.0020**	0.17↓	0.27	0.00001**	1.80	0.93	0.3460	0.08↓	0.09	0.0000**
403[1]	0.42	0.47	2.87↑	1.48	0.0002**	0.00↓	0.00	0.0015**	0.01↓	0.03	0.0372*	0.00	0.00	0.0712
406	0.62	0.13	0.28↓	0.23	0.0031**	0.08↓	0.17	0.0002**	0.06↓	0.09	0.0000**	0.04↓	0.04	0.00001**
501[1]	1.06	0.22	1.67↑	0.50	0.0028**	0.15↓	0.11	0.0007**	0.18↓	0.14	0.0000**	0.01↓	0.01	0.00001**
502	0.66	0.25	1.35↑	0.45	0.0079**	0.25	0.23	0.0556	0.24↓	0.08	0.0066**	0.01↓	0.01	0.0003**
606	0.84	0.26	0.08↓	0.07	0.0079**	0.20↓	0.14	0.0079**	-			0.00↓	0.00	0.0001**
607[1]	1.33	0.39	0.18↓	0.08	0.0079**	0.35↓	0.18	0.0079**	-			0.01↓	0.01	0.0001**
707	0.88	0.19	1.68↑	0.44	0.0228**	0.52	0.25	0.0556	0.56↓	0.08	0.0089**	0.11↓	0.09	0.0000**
708[1]	1.62	0.39	1.09↓	0.17	0.0159**	0.70↓	0.13	0.0079**	0.85↓	0.10	0.0028**	0.10↓	0.04	0.0000**
CS	0.89	0.10	1.17	0.98	0.4728	0.24↓	0.17	0.0176**	0.67	0.78	0.3235	0.05↓	0.05	0.0037**
HE	1.08	0.10	1.47	0.98	0.5575	0.25↓	0.29	0.0013**	0.28↓	0.39	0.0072**	0.05↓	0.07	0.0061**
all	0.98	0.10	1.32	0.94	0.3150	0.24↓	0.22	0.0001**	0.47↓	0.61	0.0266**	0.05↓	0.05	0.00001**

[1] designates cases of reported hypersensitivity to electromagnetic fields.

Table 4. Changes in γ-H2AX foci in response to 1 h MW exposure. Lymphocytes from 5 subjects reporting hypersensitivity to EMF (HE) and 5 matched healthy control subjects (CS) were exposed to MWs at 905 MHz and 915 MHz or heat shocked. Mean of measurements in 300-600 cells and SD is shown along with p-values for differences as compared to sham by the Mann-Whitney U test. Asterisks designate statistically significant effects at $p<0.05$* and $p<0.01$**. Arrows ↓ and ↑ designate direction of effects, decrease or increase, respectively. In cells from subjects 301 and 302 the γ-H2AX foci were not stained and analyzed.

Subject	Sham		905 MHz			915 MHz			41°C			43°C		
	H2AX	SD	H2AX	SD	p	H2AX	SD	p	H2AX	SD	p	H2AX	SD	p
301[1]	-		-			-			-			-		
302	-		-			-			-			-		
403[1]	0.91	0.69	7.24↑	1.54	0.00001**	0.10↓	0.25	0.0015**	0.10↓	0.25	0.0105**	0.00↓	0.00	0.0130*
406	1.06	0.57	1.05	1.11	0.4173	0.10↓	0.14	0.0002**	0.52↓	0.40	0.0003**	0.02↓	0.03	0.0003**
501[1]	1.30	1.11	0.09↓	0.08	0.00001**	0.00↓	0.00	0.0007**	0.00↓	0.00	0.0231**	0.02↓	0.02	0.0248*
502	0.06	0.02	0.03	0.04	0.0992	0.00↓	0.00	0.0079**	0.00↓	0.00	0.0002**	0.00↓	0.00	0.0001**
606	0.53	0.32	0.01↓	0.01	0.0079**	0.03↓	0.02	0.0079**	-			0.00↓	0.00	0.0059**
607[1]	0.36	0.04	0.03↓	0.03	0.0079**	0.04↓	0.02	0.0079**	-			0.00↓	0.00	0.00001**
707	0.52	0.12	1.22↑	0.38	0.0079**	0.12↓	0.10	0.0079**	0.18↓	0.18	0.0068**	0.68	0.34	0.3402
708[1]	1.34	0.14	1.09	0.34	0.4206	0.18↓	0.05	0.0079**	0.80↓	0.20	0.0011**	0.25↓	0.05	0.00001**
CS	0.54	0.11	0.58	0.65	0.9033	0.06	0.06	0.0808	0.23	0.26	0.1546	0.18	0.34	0.2637
HE	0.98	0.11	2.11	3.45	0.5613	0.08↓	0.08	0.0256*	0.30	0.43	0.0587	0.07↓	0.12	0.0192*
all	0.76	0.11	1.34	2.44	0.5737	0.07↓	0.06	0.0019**	0.27↓	0.32	0.0426*	0.12↓	0.24	0.0029*

[1] designates cases of reported hypersensitivity to electromagnetic fields.

Table 5. MW effects on formation of 53BP1 and γ-H2AX foci as analyzed by the Kruskal-Wallis ANOVA by ranks (multiple comparisons of sham, 905 MHz and 915 MHz) in cells from hypersensitive subjects and matched control healthy persons. For each experimental condition, 300-600 cells were analyzed. Asterisks designate statistically significant effects at $p<0.05$* and $p<0.01$**. In cells from subjects 301 and 302 the γ-H2AX foci were not stained and analyzed (NA).

Subject	Number of images	p-value 53BP1	p-value γ-H2AX
301[1]	24	0.0011**	NA
302	32	0.00001**	NA
403[1]	30	0.00001**	0.00001**
406	27	0.0002**	0.0003**
501[1]	25	0.0002**	0.00001**
502	20	0.0011**	0.0155*
606	16	0.0052**	0.0051**
607[1]	15	0.0034**	0.0075**
707	15	0.0098**	0.0019**
708[1]	15	0.0032**	0.0075**

[1] designates cases of reported hypersensitivity to electromagnetic fields.

Table 6. Comparison of MW effects on 53BP1 and γ-H2AX foci at different frequencies, 905 MHz and 915 MHz in cells from hypersensitive subjects and matched control healthy persons, Mann-Whitney U-test. For each experimental condition, 300-600 cells were analyzed. Asterisks designate statistically significant effects at $p<0.05$* and $p<0.01$**.

Subject	Number of images		p-value	p-value
	905 MHz	915 MHz	53BP1	γ-H2AX
301[1]	10	5	0.0007**	0.00001**
302	10	10	0.00001**	0.4173
403[1]	10	10	0.00001**	0.00001**
406	10	10	0.0029**	0.0992
501[1]	10	5	0.0006**	0.0079**
502	10	5	0.0007**	0.0079**
606	5	5	0.1508	0.0079**
607[1]	5	5	0.0317*	0.4206
707	5	5	0.0159*	0.00001**
708[1]	5	5	0.0159*	0.4173

[1] designates cases of reported hypersensitivity to electromagnetic fields.

Figure legends

Fig 1. Panels show typical images of fixed human lymphocytes (counterstained in blue with ToPro-3-iodide) with 53BP1 foci (stained in green with Alexa fluor 488) and γ-H2AX foci (stained in red with Cy3) as revealed by immunostaining and confocal laser microscopy of cells from subject 501. Significantly fewer foci were observed after 1-h exposure to 915 MHz and heat shock, 41^{o}C, than in control cells. Exposure to 905 MHz resulted in statistically significant increase in number of 53BP1 foci in cells from this subject (Table 3). Scale bar is 10 μm.

Fig. 2 53BP1 and γ-H2AX foci in human lymphocytes of matched controls (A) and hypersensitive subjects (B) after exposure to 905 MHz, 915 MHz and heat shock at 41^{o}C and 43^{o}C, as measured by immunostaining and confocal laser microscopy following 1 h treatment. Mean values for amounts of foci per cell from five subjects in each panel and standard deviations are shown. Similar reduction of foci level was seen after 915 MHz exposure and after heat shock. Exposure to 905 MHz led to either reduction or induction of foci dependent on subject resulting in larger standard deviation for this treatment as compared to 915 MHz.

Figures

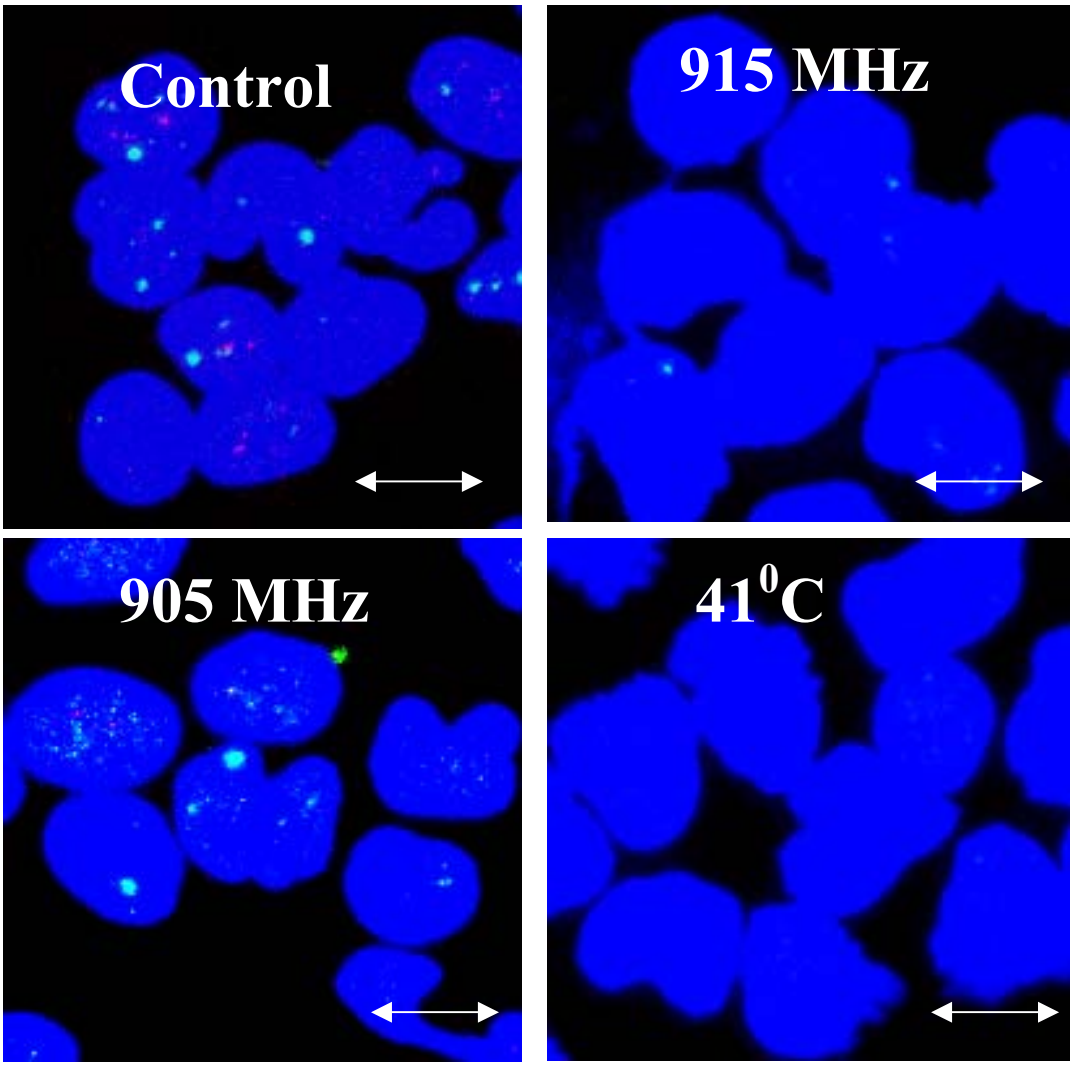

Figure 1

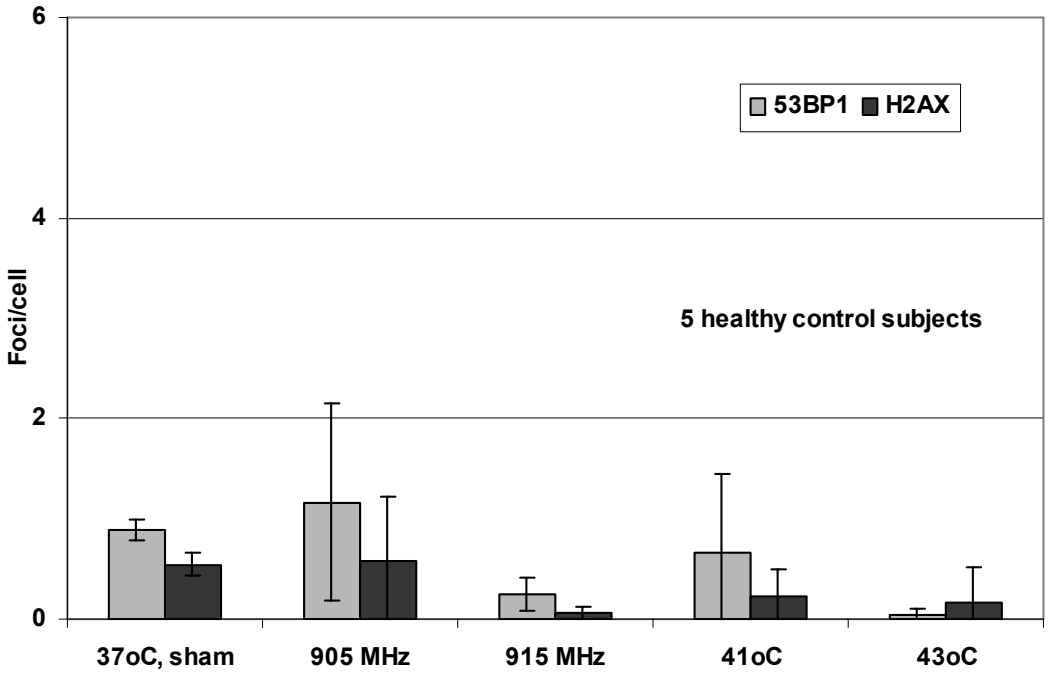

Figure 2A

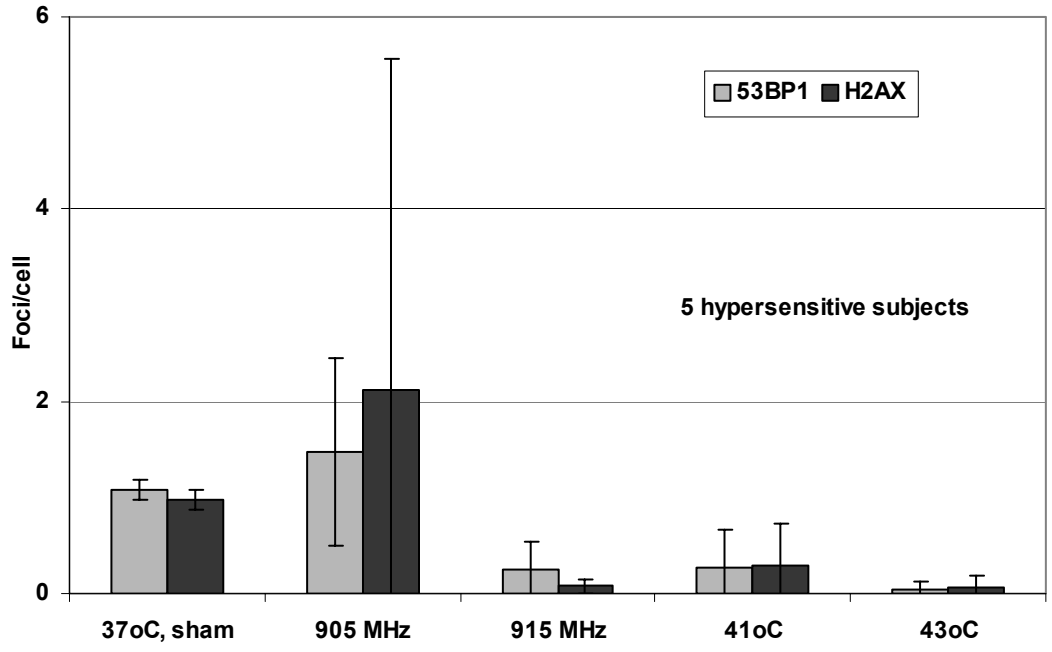

Figure 2B