Nonthermal GSM Microwaves Affect Chromatin Conformation in Human Lymphocytes Similar to Heat Shock

Ruslan Sarimov, Lars O.G. Malmgren, *Member, IEEE*, Eva Marková, Bertil R. R. Persson, and Igor Y. Belyaev

***Abstract*—Here we investigated whether microwaves (MWs) of Global System for Mobile Communication (GSM) induce changes in chromatin conformation in human lymphocytes. Effects of MWs were studied at different frequencies in the range of 895–915 MHz in experiments with lymphocytes from seven healthy persons. Exposure was performed in transverse electromagnetic transmission line cell (TEM-cell) using a GSM test-mobile phone. All standard modulations included 2 W output power in the pulses, specific absorbed rate (SAR) being 5.4 mW/kg. Changes in chromatin conformation, which are indicative of stress response and genotoxic effects, were measured by the method of anomalous viscosity time dependencies (AVTD). Heat shock and treatment with the genotoxic agent camptothecin, were used as positive controls. 30-min exposure to MWs at 900 and 905 MHz resulted in statistically significant condensation of chromatin in lymphocytes from 1 of 3 tested donors. This condensation was similar to effects of heat shock within the temperature window of 40°C–44 °C. Analysis of pooled data from all donors showed statistically significant effect of 30-min exposure to MWs. Stronger effects of MWs was found following 1-h exposure. In replicated experiments, cells from four out of five donors responded to 905 MHz. Responses to 915 MHz were observed in cells from 1 out of 5 donors, $p < 0.002$. Dependent on donor, condensation, 3 donors, or decondensation, 1 donor, of chromatin was found in response to 1-h exposure. Analysis of pooled data from all donors showed statistically significant effect of 1-h exposure to MWs. In cells from one donor, this effect was frequency-dependent ($p < 0.01$). Effects of MWs correlated statistically significantly with effects of heat shock and initial state of chromatin before exposure. MWs at 895 and 915 MHz affected chromatin conformation in transformed lymphocytes. The conclusion—GSM microwaves under specific conditions of exposure affected human lymphocytes similar to stress response. The data suggested that the MW effects differ at various GSM frequencies and vary between donors.**

***Index Terms*—Biological effects of electromagnetic radiation, biological cells, genetics, mobile phones.**

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I. Introduction

THE GROWING concern about effects of microwave (MW) exposure from mobile phones have been discussed in many countries because of increasing usage of wireless communication systems. It was also reported that increased incidence of brain tumors correlated with exposure to mobile phone MWs [1], [2]. There is evidence of nonthermal effects of MWs that suggest a possible relationship of MW exposure with permeability of brain blood barrier [3] and stress response [4]. Recent data by Salford *et al.* provided evidence for neuronal damage in the brains of exposed rats [5]. Proteomic analysis of human endothelial cells in culture have been performed and indicate that mobile phone MWs activate a variety of cellular signal transduction pathways; among them, the hsp27/p38MAPK stress response pathway [6]. In other studies, no effects of nonthermal MWs were observed [7]. However, experimental evidence suggested that MW effects occur only under specific parameters of exposure depending on several physical and biological variables [8]–[12]. Nonthermal effects of MWs in extremely high frequency range (millimeter waves) have been reported to be dependent on frequency [9], [12], [13]. Strong dependence of MW effects on several physical parameters, including frequency, and biological variables could explain various outcomes of studies with nonthermal MWs [9].

It has been described that MWs, under specific conditions of exposure, induced DNA strand breaks in rat brain cells as measured by the single cell electrophoresis [14]. The mechanisms of this effect are not understood, but could be related to the induced changes in interaction of DNA with proteins rather than DNA damage [15]. In this paper, we analyze these possibilities using the AVTD method. Both chromatin decondensation, which is induced by DNA damage, and chromatin condensation which is observed during stress response, can be measured by this technique [9], [15], [16].

There is substantial lack of knowledge in biophysical modeling of MW-induced nonthermal biological effects. Resonance-like interactions of MWs with such targets as cellular membranes, DNA, and ions in protein cavities have been proposed [8], [10], [17], [18]. Recently published data suggests that MWs of low power levels from mobile phones alter protein conformation [19].

Nonthermal effects of GSM MWs on the conformation of chromatin, and the tumor suppressor p53 binding protein 1 (53BP1) in human lymphocytes, have been recently described [16]. Based on comparative analysis of responses of

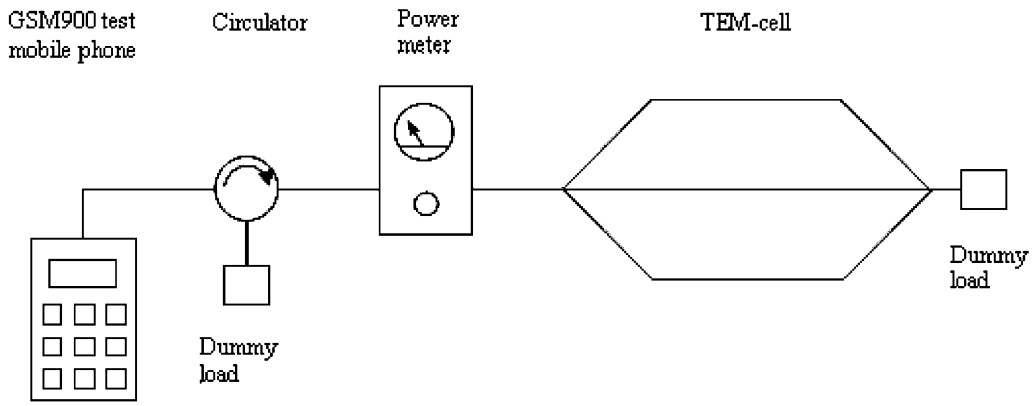

Fig. 1. Block-scheme for the exposure installation. The output of the GSM900 test-mobile phone was connected by coaxial cable to a TEM-cell where exposure or sham-exposure of human lymphocytes was performed.

cells to electromagnetic fields in different frequency ranges, resonance-like dependencies of microwave effects in the GSM900 frequency range with half-widths of resonance of the order of 1–10 MHz were predicted [20]. Here, effects of GSM MWs were investigated at different frequencies in replicated experiments with normal lymphocytes from seven healthy persons and transformed cells obtained by immortalization of human lymphocytes with Epstein–Barr virus (EBV).

Stress response, induced by heat shock, was used as a positive control for chromatin condensation. Treatment with a topoisomerase I inhibitor camptothecin (CPT) was used as a positive control for chromatin decondensation. CPT is known to stabilize the topoisomerase I—DNA cleavable complex introducing protein-linked single-strand breaks in DNA [21].

II. Materials and Methods

A. Chemicals

Reagent grade chemicals were obtained from Sigma (St. Louis, MO) or Merck (Germany).

B. Cells

Blood samples were obtained from seven healthy volunteers. The cells were transferred to basal medium (BM); RPMI 1640 medium supplemented with glutamax-I, 10% fetal bovine serum, 12.5 IU/ml penicillin, 12.5 μg/ml streptomycin (ICN Pharmaceuticals, Inc., USA) at 5% CO_2 and 37 °C in a humidified incubator. Adherent monocytes were removed by overnight incubation of the cell suspension in culture flasks (Falcon) at the cell density of 3×10^6 cells/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 pre-incubated for 1 h before exposure. The viability of cells was always above 95% as measured with trypan blue exclusion assay.

Immortal lymphoblastoid cell line (ELI) was obtained by infection of human lymphocytes of healthy donor with EBV. EBV was obtained from the cell line B95/8.

C. Cell Exposure

All exposures were performed at room temperature in 5-ml round-bottom tubes (Falcon); 0.5 ml of cell suspension per tube. Duration of exposures was 30 min or 1 h. Lymphocytes were exposed to MWs using a GSM900 test-mobile phone (model GF337, Ericsson, Sweden) (Fig. 1). The output of the phone was connected by coaxial cable to TEM-cell, which has previously been described [3], [5]. There are 124 different channels/frequencies, which are used in GSM900 mobile communication. They differ by 0.2 MHz in the frequency range between 890.2 MHz and 914.8 MHz. Frequency is supplied randomly to mobile phone users. The test-mobile phone was programmed to use a pre-set frequency. We used the channels 24, 49, 74, 99, and 124 with the frequencies of 895, 900, 905, 910, and 915 MHz, respectively. The signal included standard GSM modulations. GSM signal is pulsed by 577-μs pulses (time slots), 2 W power in pulse, with the waiting time of 4039 μs (seven time slots) between pulses. No voice modulation was applied. Discontinuous transmission mode (DTX) was off during all exposures.

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 during exposures, at 33 dBm, as monitored online using a power meter (Bird 43, USA). The SAR-value was determined by measurements and calculations. Transmitted and reflected power was measured at continuous wave (CW) exposure (0.25 W, 915 MHz), with and without sample tubes in the TEM-cell. This was done by measurement of input, reflected and output power 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. Because the absorbed power in the 0.5-ml sample was too small to be measured, the measurements were performed using 5-ml sample instead. The absorbed power in the sample ($\boldsymbol{P}_{\mathrm{abs,sample}}$) was estimated to be 0.186 mW using the following equation:

$$\begin{aligned}\boldsymbol{P}_{\mathrm{abs,sample}} &= \boldsymbol{P}_{\mathrm{abs,TEM+sample}} - \boldsymbol{P}_{\mathrm{abs,TEM}} \\ &= \boldsymbol{P}_{\mathrm{refl,TEM}} + \boldsymbol{P}_{\mathrm{out,TEM}} - P_{\mathrm{refl,TEM+sample}} \\ &\quad - \boldsymbol{P}_{\mathrm{out,TEM+sample}}\end{aligned}$$

where reflected power in the TEM-cell without sample, $\boldsymbol{P}_{\mathrm{refl,TEM}}$, was 4.983 mW, output power from the TEM-cell without sample, $\boldsymbol{P}_{\mathrm{out,TEM}}$, was 242 mW, reflected power from the TEM-cell with a sample, $\boldsymbol{P}_{\mathrm{refl.,TEM+sample}}$, was 4.797 mW, and output power from the TEM-cell with a sample,

TABLE I
FREQUENCY TABLE BELOW GIVES THE DISTRIBUTION FOR EACH OF THE SEVEN HORIZONTAL LAYERS OF THE EXPOSE SAMPLE AS WELL AS THE DISTRIBUTION FOR ALL OF THE CELLS

SAR (mW/kg)	Slice 1	Slice 2	Slice 3	Slice 4	Slice 5	Slice 6	Slice 7	All
10-20	0	0	0	0	13	53	0	66
20-30	0	0	0	4	103	102	166	375
30-40	0	0	0	92	40	44	51	227
40-50	0	0	12	29	23	26	34	124
50-60	0	0	65	18	14	18	11	126
60-70	0	6	17	10	7	13	20	73
70-80	0	36	13	4	11	1	8	73
80-90	0	15	3	6	8	5	8	45
90-100	2	4	9	5	1	3	5	29
100-110	4	4	3	2	0	0	3	16
110-120	0	2	0	2	0	0	3	7
120-130	1	0	0	0	0	0	0	1
130-140	1	0	0	0	0	0	0	1
140-150	1	0	0	0	0	0	0	1

$\boldsymbol{P}_{\mathrm{out,TEM+sample}}$, was 242 mW. From these measurements, the SAR was calculated to be 37 mW/kg.

Numerical calculations were performed using the finite different time domain (FDTD) method. The TEM-cell and a test tube with 0.5 ml of sample were modeled using (0.75 mm $\times$ 0.75 mm $\times$ 0.75 mm) size cells and run with Remcom, Inc. XFDTD 6.0 software for 912 MHz CW, 2 W, as input to the TEM-cell. The amplitude of the applied signal was 14-V peak which produced an E-field of 175-V/m peak in the central part of the TEM-cell. The routine was run for seven cycles to assure that the results have converged. The distribution for each of the seven horizontal layers of sample, as well as the distribution for all of the cells, is given in Table I. The average SAR for the 0.5-ml sample was 43 ± 23 mW/kg with the minimum in any one cell of 17 mW/kg and a maximum of 142 mW/kg. Fifty-two percent of the cells had SAR values between 20 and 40 mW/kg. The extrapolation to the pulsed GSM signal with a duty factor of 0.125 and 2 W during the pulses resulted in the average SAR of 5.4 mW/kg under our conditions of exposure. This is referred herein as the SAR value. The difference between the FDTD-derived value and the measured SAR should be explained by different coupling of the samples, 0.5 ml and 5 ml, to the field in the exposure system. Numerical calculations for a 5-ml sample resulted in the average SAR of 0.28 W/kg for the 2 W output power. This corresponded to the SAR of 35 mW/kg for 250 mW of real GSM exposure, and was very similar to 37 mW/kg as calculated above from direct measurements. These SAR values are well below thermal effects.

At the place of exposure, static magnetic field (SMF) was 58 ± 6 μT as measured by means of a magnetometer (Sam3, Dowty Electronics Ltd., England), and background extremely low frequency (ELF) magnetic field was not more 50 nT, rms, as measured with three-dimensional microteslameter (Field dosimeter 3, Combinova, Sweden).

Simultaneously with the exposures to MWs, the control cells were kept under the same conditions as the exposed cells. In each experiment, control cells were analyzed in duplicate, in the beginning and the end of exposures. In the middle of experiments, sham exposures were performed in the GSM unit with MWs off. The sham-exposed cells were kept under the same conditions as the exposed ones. No significant differences were observed between control and sham-exposed cells. Therefore, the data from sham-exposures and controls were pooled to compare with effects of MWs. The heat treatment in a water bath in the range of 40–46 °C was used as a positive control for chromatin condensation. Deviations of temperature during the heat treatments were no more than 0.3 °C. As a positive control for chromatin decondensation, the cells were treated with CPT, 1–50 μg/ml, 1 h at the temperature of 37 °C. CPT was diluted in DMSO. Final concentration of DMSO in media was 1%.

D. Cell Lysis and AVTD Measurements

Lymphocytes were lyzed as described previously by addition of 3 ml of lysis solution (0.25 M Na_2EDTA, 2% sarcosyl, 10 mM Tris-base, pH 7.4) to 0.1 ml of a cell suspension [15]. Under these conditions of lysis, histones are released from DNA, and the resulting DNA-protein complexes (nucleoids) represented nuclear matrixes with DNA-loops attached. DNA integrity is preserved and DNA-loops can be either relaxed or condensed by appropriate concentrations of ethidium bromide [15]. The AVTD method was used as previously described [15]. Briefly, this method is based on radial migration of large DNA-protein complexes such as nucleoids in a high-gradient hydrodynamic field of a rotary viscometer [22], [23]. Non-nucleoid particles such as proteins, protein aggregates, apoptotic cells with fragmented DNA, and also nucleoids with significantly condensed DNA-loops do not contribute to AVTD [15], [24], [25]. Radial migration of nucleoids toward the rotating rotor causes anomalous changes of viscosity that can be registered by measuring the rotor rotation period as a function of time. This anomalous viscosity time dependence strongly depends on conformation of nucleoids, molecular weight, and the number of proteins bound to DNA [22], [23]. Each AVTD is a set of experimental points (period of rotation versus time of measurement), which are recorded by a personal computer (Fig. 2). The AVTDs were measured in the cell lysates at a shear rate of 5.6 s^{-1} and a shear stress of 0.007 N m^{-2} using an AVTD-analyzer (Archer-Aquarius, Ltd., Russia). The maximum period of rotation ($\boldsymbol{T}_{\max}$) corresponds to the maximum viscosity and has previously been shown to be the most sensitive AVTD parameter [23]. Each version of the experiment included no less than three AVTD measurements in different tubes. To characterize the condensation of chromatin, we used normalized maximal relative viscosity

$$\mathbf{NRV} = (\boldsymbol{T}_{\max \exp} - \boldsymbol{T}_{\mathrm{sol}})/(\boldsymbol{T}_{\max \mathrm{con}} - \boldsymbol{T}_{\mathrm{sol}})$$

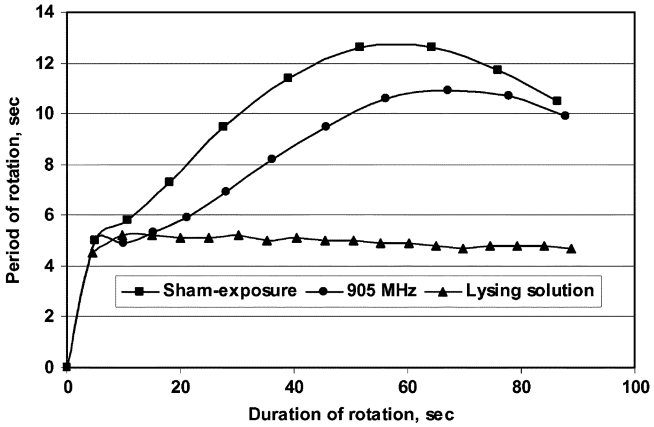

Fig. 2. Anomalous viscosity time dependence in the lysates of human lymphocytes, donor 6: (-▲-) cell-free lysing solution; (- • -) sham-exposed cells were lyzed at a density of 2×10^6 cell ml^{-1}; (-■-) cells were lyzed at a density of 2×10^6 cell ml^{-1} immediately after exposure to MWs (905 MHz, 60 min) at the same cell density.

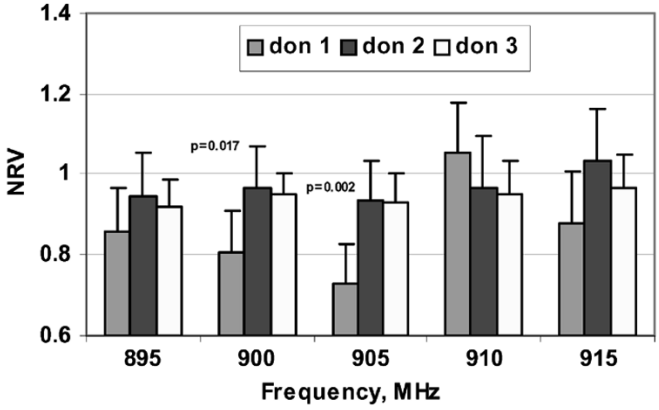

Fig. 3. Normalized maximum relative viscosity following 0.5 h exposure of human lymphocytes to MWs at the frequencies of 895, 900, 905, 910, and 915 MHz. Cells from donors 1, 2, and 3 were exposed to MWs, SAR = 5.4 mW/kg, and lyzed immediately after exposure. The error bars represent standard deviations of mean of six-twelve measurements in two-four independent experiments. Statistical analysis was performed with the Mann–Whitney U test.

where $(\boldsymbol{T}_{\max \exp})$ is a mean values in exposed samples, $(\boldsymbol{T}_{\max \mathrm{con}})$ is a mean values in control (sham-exposed) samples, and $\boldsymbol{T}_{\mathrm{sol}}$ is a period of rotation in cell-free lysis solution. Per-cell normalized maximal relative viscosity

$$\mathbf{NRV}_c = (\boldsymbol{T}_{\max con} - \boldsymbol{T}_{\mathrm{sol}})/(\boldsymbol{C} \times \boldsymbol{T}_{\mathrm{sol}})$$

where $\boldsymbol{C}$ is a cell density, was used to characterize condensation of chromatin in control cells.

E. Statistical Analysis

The data were analyzed using Kolmogorov–Smirnov test. Most data did not fulfill the normal distribution. Therefore, the data were compared by Mann–Whitney U-test or Kruskal–Wallis test. A correlation analysis was performed using Spearman R test. Results were considered as significantly different at $\mathrm{p} < 0.05$.

III. Results

In the first set of experiments, lymphocytes from three different donors were exposed to MWs at various frequencies: 895, 900, 905, 910, and 915 MHz. The duration of exposure was 30 min in these experiments. Statistically significant condensation of chromatin was observed in cells from donor 1 at frequencies of 900 and 905 MHz, $\mathrm{p} < 0.017$ and 0.002, respectively, Mann–Whitney U-test (Fig. 3). These data suggested that effects of MWs might be frequency dependent and various responses might be also observed in cells from different individuals. We tested hypothesis that MWs had an effect on cells performing multiple comparison between all experimental groups by Kruskal-Wallis test. This analysis has shown a statistically significant effect of MWs in cells from donor 1 (Table II). Analysis of pooled data from donors 1–3 has shown significant effect of 30-min exposure on chromatin conformation in human lymphocytes (Table II). Using the Kruskal-Wallis test, we tested a hypothesis that the effect of MWs, 30-min exposure, was dependent on frequency, based on multiple comparisons of the effects at different frequencies. This hypothesis was not confirmed (Table II). The cells from all donors were sensitive to heat shock induced by 30-min heating (Fig. 4). Condensation of chromatin was observed in response to heating in the range of 40 °C–44 °C. At the temperature 46 °C, significant increase in AVTD was observed (data not shown). Therefore, condensation of chromatin was observed only in a specific temperature window. In the second set of experiments, lymphocytes from five different donors were exposed to MWs at the frequencies of 895, 905, and 915 MHz. Duration of exposure was 1 h. In these experiments, 905 MHz was a most "effective" frequency. Cells from four out of five donors responded to the frequency of 905 MHz.

TABLE II
COMPARISON OF MULTIPLE GROUPS USING KRUSKAL-WALLIS TEST

Donor	Multiple comparison of groups: sham, 895, 900, 905, 910 and 915 MHz				Multiple comparison of groups: 895, 900, 905, 910 and 915 MHz			
	Amount of		H value	p-level	Amount of		H value	p-level
	groups	samples			groups	samples		
1	5	46	15.7	0.008*	4	29	8.8	0.066
2	5	78	3.3	0.648	4	45	2.3	0.686
3	5	99	4.0	0.544	4	54	1.2	0.879
all	5	223	16.3	0.006*	4	128	6.64	0.156

Cells from donors 1-3 were sham-exposed or exposed to MWs (895, 900, 905, 910 and 915 MHz) during 0.5 h in 11 experiments. Statistically significant effects of MWs were found in cells from donor 1 and by analysis of pooled data from all donors. No statistically significant differences between effects of 0.5-h exposure to MWs at different frequencies were found. P-values of statistically significant effects are shown with asterisk.

At this frequency, statistically significant condensation of chromatin was seen in cells from donors 3, 5, and 6, $\mathrm{p} < 0.009, 0.04$, and 0.002, respectively, Mann–Whitney

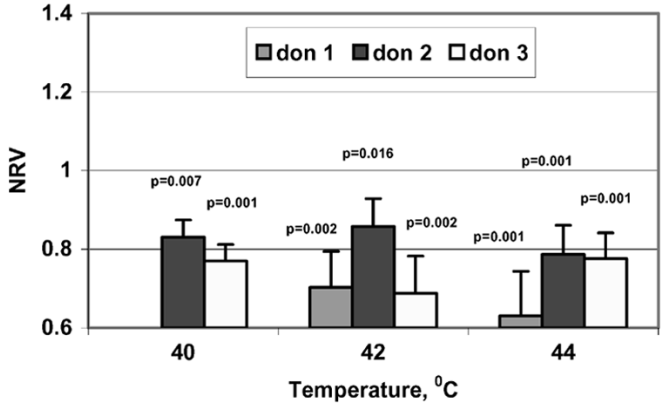

Fig. 4. Dependence of normalized maximum relative viscosity on temperature after 0.5 h heat shock. The cells from donors 1, 2, and 3 were exposed to heat at 40 °C, 42 °C, and 44 °C and lyzed immediately after exposure. Two samples with control cells were used in each experiment. One sample was incubated at room temperature and another at 37 °C. The AVTD were similar in both control samples. For calculations of NRV the data obtained at room temperature were used. The error bars represent the standard deviations of mean of six-twelve measurements in two-four independent experiments. Significance level was calculated with Mann–Whitney U test.

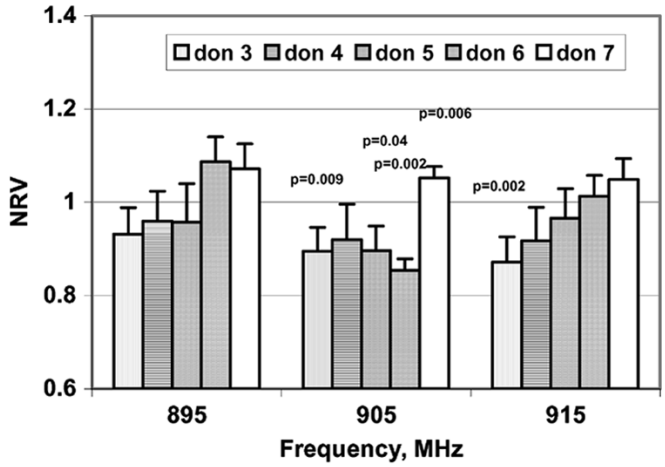

Fig. 5. Normalized maximum relative viscosity following 1 h exposure of human lymphocytes to MWs at the frequencies of 895, 905, and 915 MHz. The cells from donors 3, 4, 5, 6, and 7 were exposed to MWs, $\mathrm{SAR} = 5.4$ mW/kg, and lyzed immediately after exposure. The error bars represent standard deviations of mean of 9–15 measurements in 3–5 independent experiments. Statistical analysis was performed with the Mann–Whitney U test.

U-test (Fig. 5). The relaxation of chromatin was observed following exposure to 905 MHz in cells from donor 7, $p < 0.006$, Mann–Whitney U-test (Fig. 5). MWs at 915 MHz induced statistically significant condensation of chromatin in cells from donor 3, $p < 0.002$, Mann–Whitney U-test (Fig. 5). We tested hypothesis that MWs had an effect on cells by multiple comparison between all experimental groups using the Kruskal-Wallis test. This analysis has shown statistically significant effect of MWs in cells from donors 3, 6, and 7 (Table III). The analysis of pooled data from donors 3–7 has shown significant effect of MWs, 1 h exposure, on chromatin conformation in human lymphocytes (Table III). Thereafter, we tested a hypothesis that the effect of MWs was frequency dependent by multiple comparisons of effects at different frequencies using Kruskal-Wallis

TABLE III
COMPARISON OF MULTIPLE GROUPS USING KRUSKAL-WALLIS TEST

Donor	Multiple comparison of groups: sham, 895, 905, and 915 MHz				Multiple comparison of groups: 895, 905, and 915 MHz			
	Amount of		H value	p-level	Amount of		H value	p-level
	groups	samples			groups	samples		
3	3	112	12.7	0.005*	2	66	1.26	0.53
4	3	82	2.47	0.48	2	53	0.48	0.79
5	3	84	4.86	0.18	2	54	1.43	0.49
6	3	54	11.67	0.009*	2	27	8.73	0.01*
7	3	54	8.31	0.04*	2	27	0.34	0.84
all	3	386	16.17	0.001*	2	227	3.79	0.15

In 17 experiments, cells from donors 3-7 were sham-exposed or exposed to MWs (895, 905, and 915 MHz) during 1 h. Statistically significant effects of MWs were found in cells from donors 3, 6, and 7 and by analysis of pooled data from all donors. The differences in effects of MWs at different frequencies were found in cells from donor 6. P- values of statistically significant effects are shown with asterisk.

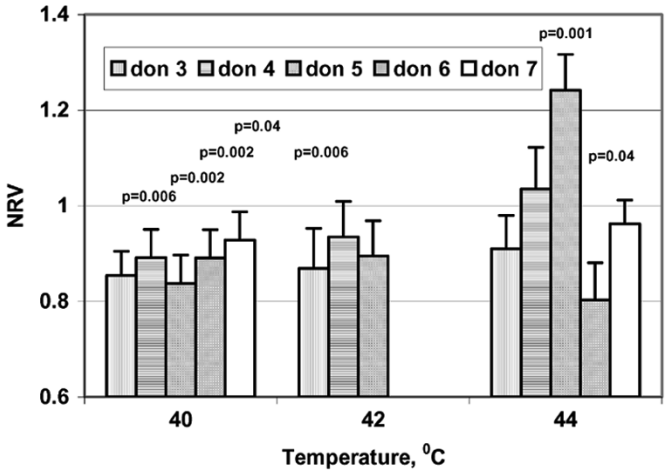

Fig. 6. Dependence of maximum relative viscosity on temperature of heat shock. The cells from donors 3, 4, 5, 6, and 7 were exposed to heat at 40 °C, 42 °C, and 44 °C during 1 h and lyzed immediately after exposure. Two samples with control cells were used in each experiment. One sample was incubated at room temperature and another at 37 °C. The AVTD were similar in both control samples. For calculations of NRV the data obtained at room temperature were used. The error bars represent the standard deviations of mean of 9–15 measurements in 3–5 independent experiments. Significant level was calculated with Mann–Whitney U test.

test. This hypothesis was found to be correct for cells from donor 6 (Table III).

Significant condensation of chromatin was observed after 1 h heat shock in the range of 40 °C–42 °C (Fig. 6). Contrary to 30 min heat shock at the same temperature, and similar to 30 min heat shock at 46 °C, decondensation of chromatin was observed in cells from donor 5 (Fig. 6). We assumed that heat shock could

TABLE IV
CORRELATION BETWEEN EFFECTS OF MWS AND HEAT SHOCK IN HUMAN LYMPHOCYTES AS ANALYZED BY SPEARMAN R TEST

Exposure	40 °C		42 °C		44 °C	
	R	p	R	p	R	p
895 MHz	-0.30	0.624	0.56	0.192	-0.11	0.819
900 MHz	NA	NA	0.30	0.624	0.70	0.188
905 MHz	NA	NA	0.90	0.015*	0.49	0.329
910 MHz	-0.50	0.667	-0.80	0.104	-0.20	0.747
915 MHz	-0.60	0.400	0.957	0.03*	0.77	0.072

Cells from donors 1-3 were exposed to MWs (895, 900, 905, 910 and 915 MHz) or heat shock (40, 42 and 44°C) during 0.5 h in 11 experiments. Changes in chromatin conformation were measured with AVTD technique. Statistically significant correlation was found between effects of heat shock at 42°C and MW exposure at 905 MHz. P- values of statistically significant effects are shown with asterisk. NA – data were not analyzed because the data arrays were limited and Spearman R test could not be applied.

TABLE V
RESULTS OF CORRELATION ANALYSIS (SPEARMAN R TEST) BETWEEN EFFECTS OF MWS AND HEAT SHOCK ON CHROMATIN CONFORMATION IN HUMAN LYMPHOCYTES AS MEASURED WITH AVTD TECHNIQUE

Exposure	40 °C		42 °C		44 °C	
	R	p	R	p	R	p
895 MHz	0.30	0.277	0.81	0.015*	0.26	0.348
905 MHz	0.41	0.144	0.69	0.058	0.31	0.265
915 MHz	0.40	0.140	0.83	0.005*	0.44	0.092

In 17 experiments, cells from donors 3-7 were exposed to microwaves (895, 905 and 915 MHz) or heat (40, 42 and 44°C) during 1 h. Statistically significant correlation was found between effects of heat shock at 42°C and MW exposure (895 and 915 MHz). P- values of statistically significant effects are shown with asterisk.

TABLE VI
ANALYSIS OF CORRELATION (SPEARMAN R TEST) BETWEEN PER-CELL NORMALIZED MAXIMUM RELATIVE VISCOSITY AND CHANGES IN CHROMATIN CONFORMATION AS MEASURED WITH AVTD TECHNIQUE AFTER EXPOSURE OF HUMAN LYMPHOCYTES TO MWS

Exposure	Correlation of NRV with NRV_c	
	Correlation, R	Significance level, p
895 MHz	-0.58	0.023*
905 MHz	-0.23	0.420
915 MHz	-0.55	0.023*
40°C	-0.28	0.321
42°C	-0.78	0.013*
44°C	-0.18	0.499

Cells from donors 3-7 were exposed to MWs (895, 905, and 915 MHz) or heat shocked (40, 42 and 44°C) during 1 h in 17 experiments. Significant negative correlation between initial level of chromatin condensation and MW/heat shock effects under specific conditions of treatment was found. P- values of statistically significant effects are shown with asterisk.

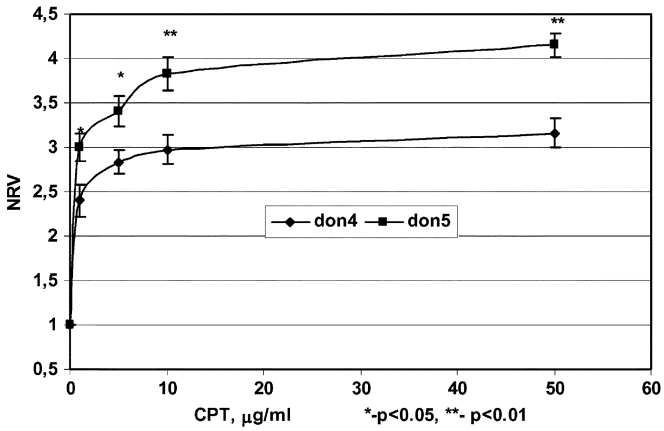

Fig. 7. Dependence of maximum relative viscosity on concentration of CPT. Cells from donors 4 and 5 were treated with CPT at 0, 1, 5, 10, and 50 μg/ml at 37 °C during 1 h. The cells were lyzed immediately after treatment and the AVTD measurements were performed. The error bars represent standard deviations of mean of six measurements in two independent experiments.

result in both chromatin condensation and decondensation dependent on temperature/time of heating, and individual traits of lymphocytes.

The correlation between effects of MWs and heat shock was analyzed using Spearman R test. Statistically significant correlation between condensation of chromatin induced by 42 °C heat shock and effect of MWs at the frequency of 905 MHz and 915 MHz was found in experiments with 0.5-h treatment (Table IV). Spearman R analysis has also shown statistically significant correlation between condensation of chromatin induced by 42 °C heat shock and effects of MWs at the frequencies of 895 and 915 MHz when duration of treatments was 1 h (Table V). Coefficient of correlation between the 42 °C heat shock and the 905 MHz effect was 0.69; however, this correlation was not statistically significant, $p = 0.058$.

Per-cell normalized maximum relative viscosity varied in experiments, indicating variations in conformation of chromatin in control cells. Using the Spearman R test, we analyzed a hypothesis that the effects of MWs depend on initial conformation of chromatin in the exposed cells. By analysis of the pooled data with 1-h exposure, we found statistically significant negative correlation of NRV_c with effects of MWs at 895 and 915 MHz, and heat shock at 42 °C (Table VI). No such correlation was obtained with shorter time of exposure. Treatment of lymphocytes with CPT resulted in dose-dependent increase in NRV (Fig. 7). The dose response obtained, saturated at the concentration of 10 μg/ml, was higher in cells from donor 5 as compared to cells from donor 4 at the concentrations of 5, 10, and 50 μg/ml, $p < 0.04, 0.01, 0.006$, respectively, the Mann–Whitney U test. Concentrations of 10 and 50 μg/ml were chosen to analyze CPT-induced DNA-loop relaxation in lymphocytes from different donors. Similar relaxation was observed in cells from all tested donors except for donor 5, where relaxation was higher (Fig. 8).

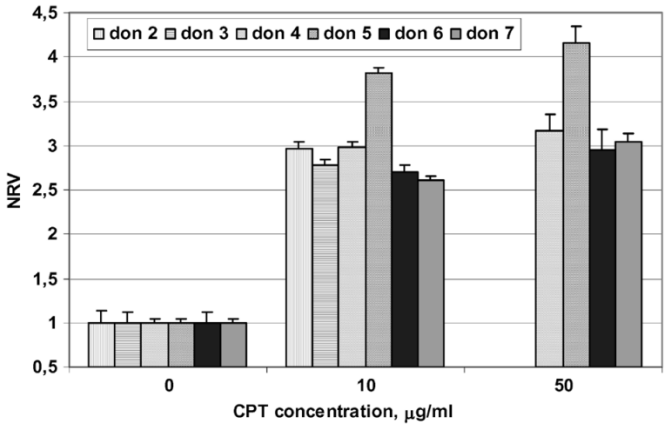

Fig. 8. Relaxation of chromatin by CPT in human lymphocytes from different donors. The cells from don 3, 4, 5, 6, and 7 were treated with CPT at the concentrations of 0, 10, and 50 μg/ml during 1 h and lyzed immediately after the treatment. The error bars represent standard deviations of mean of six measurements in two independent experiments.

TABLE VII
NORMALIZED MAXIMUM RELATIVE VISCOSITY AS MEASURED WITH AVTD TECHNIQUE AFTER EXPOSURE OF ELI CELLS TO MWS

Exposure	NRV±SD	Significance level. p
Sham	1.00+0.13	
895 MHz	0.88+0.11	0.0190*
915 MHz	0.82+0.12	0.0005*
44^0C	1.01+0.28	0.8805

P-values of statistically significant effects are shown with asterisk.

It is interesting to note that heat shock at 40 °C and 42 °C, 1 h, resulted in maximal condensation of chromatin in cells of donor 5. In addition, only in cells from donor 5, 1 h treatment with 44 °C resulted in chromatin decondensation (Fig. 6).

Finally, we addressed the question of whether MW affect transformed human lymphocytes. ELI cells were exposed to MWs (895 and 915 MHz) or heat shocked (44 °C) during 0.5 h in three experiments. Significant chromatin condensation following MW-exposure was found (Table VII). In contrast, heat shock did not condense chromatin.

IV. DISCUSSION

Recent study provided evidence that 2-h exposure to MWs at 915 MHz induced condensation of chromatin in human lymphocytes [16]. In this investigation, we confirmed and extended this finding. The MW effect was statistically significant both after 30-min and 1-h exposure, as analyzed in human lymphocytes of seven healthy donors at five different frequencies from the GSM900 band (Tables II and III). MWs also effected transformed lymphocytes (Table VII). The SAR value was very low in the samples exposed to MWs. Therefore, the MW effects could not be attributed to heating induced by the exposure system. It should be mentioned that the exposure level in this study was far below the ICNIRP value.

The analysis of individual data has shown that in cells from three out of seven donors, the effect of MWs was not statistically significant (Tables II, III). This data suggests individual sensitivity of human lymphocytes to MW exposure.

Frequency dependent response of rat thymic lymphocytes and in *E. coli* cells to MWs has previously been reviewed [9]. Here, we tested whether response of human lymphocytes depends on frequency within GSM900 band, exposing cells at the frequencies of 985, 900, 905, 910, and 915 MHz. We observed statistically significant effects at three out these five frequencies: 900, 905, and 915 MHz. The frequency of 905 MHz was most effective in experiments. Multiple comparisons of effects obtained at different frequencies have shown statistically significant differences between the effects in cells from one out seven tested donors (Table III). Altogether, the data suggested that the effects of MWs in the range of 895–915 MHz might be individual and might depend on the frequency. These results are in line with previously published data which demonstrate how various individuals respond to individual frequencies in wide frequency range of electromagnetic fields [26]. It should be noted here that eventual individual sensitivity to specific frequencies is used in the former Soviet Union countries for treatment of various human diseases by MWs as has recently been reviewed [12].

Contrary to increase in the NRV, which was observed immediately after genotoxic impacts, such as ionizing radiation [9], [16] or CPT (Figs. 7 and 8), NRV usually decreased following exposure to MWs (Figs. 2, 3, 5, and Table VII). Several experimental observations have suggested that an increase in the NRV is caused by relaxation of DNA-loops, and a decrease of NRV is caused by DNA-loop condensation. Single cell gel electrophoresis and halo assay confirmed this suggestion [15], [25]. A decrease in NRV has also been induced in normal lymphocytes by heat shock at 41 °C for 2 h [16]. In this paper, we confirmed and extended this finding. Dependent on the time and temperature of treatment, both condensation and decondensation of chromatin was observed in human lymphocyte upon heat shock (Figs. 4 and 6). Based on recent data [16] and results obtained herein, we conclude that condensation of chromatin is a typical response of normal human lymphocyte to heat shock at 0.5–2 h in the temperature window of 40 °C–42 °C, as measured with the AVTD technique. These AVTD results correlate with some literature data showing condensation of chromatin in mammalian cells in response to mild heat shock [27]. Statistically significant correlation was observed between effects of MWs and heat shock at 42 °C. Our data suggested that AVTD measurements might provide a new tool to analyze stress response. The heat shock protein hsp60 accumulated in nuclear matrix in response to heat shock and 915 MHz exposures as was observed in preliminary experiments [28]. This accumulation needs to be confirmed in additional studies.

The correlations of MW effects with different physiological parameters, characterizing for the initial state of exposed cells, have been previously reviewed [9], [12], [13]. A novel finding of this study is a negative correlation of MW effects with initial chromatin condensation in cells before exposure (Table VI). These new data provide evidence that stress factors such as heat

shock and MWs that condense chromatin under specific conditions of exposure affect stronger those lymphocytes, which have more condensed chromatin.

Contrary to the chromatin condensation that was induced in normal cells by heat shock at 40 °C–42 °C, 0.5–2 h treatment, chromatin decondensation could be caused either by heat shock with longer time of exposure and higher temperature or by DNA damage. Heat shock at 44 °C did not induce chromatin condensation in transformed cells (Table VII), indicating that this effect may be cell-type dependent.

As far as MWs induced statistically significant effects in chromatin conformation, both in normal and transformed lymphocytes, further study of biophysical and molecular mechanism behind effects described here is desirable in order to analyze possible health effects of exposure to MWs from mobile communication systems.

V. Conclusion

The data has shown that MWs from GSM mobile phone, at the level of exposure that is below the ICNIRP safety standards, affected chromatin conformation in human normal and transformed lymphocytes. The MW effects depended on carrier frequency, varied between individuals, correlated with the initial state of chromatin as measured before exposure, and were similar to stress response induced by heating.

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