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DNA damage in Molt-4 T-lymphoblastoid cells exposed to cellular telephone radiofrequency fields in vitro

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Abstract

Molt-4 T-lymphoblastoid cells have been exposed to pulsed signals at cellular telephone frequencies of 813.5625 MHz (iDEN® signal) and 836.55 MHz (TDMA signal). These studies were performed at low SAR (average = 2.4 and 24 μW g^{-1} for iDEN® and 2.6 and 26 μW g^{-1} for TDMA) in studies designed to look for athermal RF effects. The alkaline comet, or single cell gel electrophoresis, assay was employed to measure DNA single-strand breaks in cell cultures exposed to the radiofrequency (RF) signal as compared to concurrent sham-exposed cultures. Tail moment and comet extent were calculated as indicators of DNA damage. Statistical differences in the distribution of values for tail moment and comet extent between exposed and control cell cultures were evaluated with the Kolmogorov–Smirnoff distribution test. Data points for all experiments of each exposure condition were pooled and analyzed as single groups. It was found that: 1) exposure of cells to the iDEN® signal at an SAR of 2.4 μW g^{-1} for 2 h or 21 h significantly decreased DNA damage; 2) exposure of cells to the TDMA signal at an SAR of 2.6 μW g^{-1} for 2 h and 21 h significantly decreased DNA damage; 3) exposure of cells to the iDEN® signal at an SAR of 24 μW g^{-1} for 2 h and 21 h significantly increased DNA damage; 4) exposure of cells to the TDMA signal at an SAR of 26 μW g^{-1} for 2 h significantly decreased DNA damage. The data indicate a need to study the effects of exposure to RF signals on direct DNA damage and on the rate at which DNA damage is repaired. © 1998 Elsevier Science S.A.

Keywords: Radiofrequency radiation; Cellular telephones; Comet assay; Single cell gel electrophoresis; DNA damage; Single strand breaks

1. Introduction

There is growing concern about possible relationships between human pathology and exposure to electric and magnetic fields produced by power lines, electric appliances, and other devices, such as cellular telephones, radio towers, and radar apparatus. This concern has been driven by the results of numerous epidemiological studies, which have demonstrated an association between various disorders, including cancer and Alzheimer's disease, and either occupational or residential exposure to electromagnetic fields (EMFs) generated by high voltage power lines as well as by microwave generating devices [1–3]. Indeed, in an attempt to understand how EMF exposure may be linked with human disease, numerous in vitro studies have been performed and their results reported in the scientific literature. Recently, Repacholi et al. [4] reported that Eμ-*pim1* transgenic mice exposed to a pulse-modulated radiofrequency (RF) field similar to those used in digital mobile telecommunications exhibited a statistically significant 2.4-fold increase in lymphomas. However, there has been little insight into how electromagnetic signals couple with biological systems and, once such coupling has occurred, by what series of biochemical and molecular steps (i.e., a biological mechanism) pathology may result. This is not to say, however, that there is no information about effects produced in biological systems exposed to electromagnetic signals. Such exposures have produced, for instance, changes in gene transcription [5], enzyme activities [6–12], calcium status [13], and other key cellular parameters [reviewed in Ref. [14]]. Nonetheless, there is a lack of understanding of how, if at all, these alterations in cellular biochemistry may fit together and cooperate to change the course of cellular physiology.

Recently, there has been increased interest in the effect of exposure to various electromagnetic signals on the

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damage and repair of nuclear DNA. This attention is certainly justified from several perspectives. In a recent minireview, for instance, Cleaver stated, ''DNA repair processes are now indispensable actors in any script for spontaneous and environmentally induced cancers'' [15a,15b]. The bioelectromagnetics literature dealing with DNA damage and repair is relatively limited and deals more with damage than with repair. Furthermore, investigators studying the effects of exposure to electromagnetic signals on DNA damage have taken two different approaches, one looking at exposure-induced effects on chromosomal aberrations, sister chromatid exchange, or micronuclei formation, and the other measuring exposure-induced single- and double-strand breaks in nuclear DNA. Unfortunately, the results of studies reported to date have been conflicting, leaving us with an unclear picture of how exposure to ELF/EMF, RF, and microwave radiation may affect the integrity of a biological system's genetic information. For instance, Cohen et al. [16,17], Khalil and Qassem [18], and Paile et al. [19] reported negative findings on the chromosomal aspects of EMF exposure. Additionally, Juutilainen and Liimatainen [20] reported that EMF exposure was negative in Ames' Salmonella mutagenicity testing, while Frazier et al. [21] found no EMF-induced effect on the mutation rate of a genetic locus known to be responsive to various genotoxic mechanisms. On the other hand, others have reported chromosomal abnormalities in cells exposed to both ELF/EMF [22–24] and microwave radiation [25–27]. Most recently, Sarkar et al. [28] exposed mice to 2.45 GHz microwave radiation at a power density of 1 mW cm^{-2} for 2 h day^{-1} for 120, 150 and 200 days. Isolated nuclear DNA from exposed and control animals was cleaved with the restriction enzyme Hin fI, electrophoresed, and hybridized with a synthetic oligonucleotide probe. It is intriguing that the DNA from brain and testes of exposed animals exhibited a distinctly different band pattern in the 7–8 kilobase range, although the mechanism underlying this chromosomal rearrangement is unknown.

Two groups have investigated DNA strand breaks using the comet, or single cell gel electrophoresis, assay. This technique is the most sensitive available for measuring DNA single-strand breaks, and can detect one break per 2×10^{10} daltons of DNA in lymphocytes [29]. Indeed, Singh et al. [30] have reported that the comet assay is more than twice as sensitive as other chromatid abnormality assays when assessing DNA damage produced by ionizing radiation. The assay is performed by embedding cells in agarose, lysing the cells, and then performing electrophoresis under alkaline or neutral conditions to detect and quantitate DNA single- or double-strand breaks, respectively. Lai and Singh [31,32] exposed rats to pulsed and continuous wave 2450 MHz radiation (SAR 1.2 W kg^{-1}). These investigators reported increased single- and double-strand DNA breaks in brain cells either immediately after a 2 h exposure or after a 4 h post-exposure period, as compared to brain cells from sham-exposed, control rats.

We now report the results of comet assays performed to detect DNA single-strand breaks in Molt-4 T-lymphoblastoid cells exposed for short (2 and 3 h) and long (21 h) periods to pulsed signals at cellular telephone frequencies of 813.5625 MHz and 836.55 MHz. These studies were performed at low SAR (average = 2.4 and 24 μW g^{-1} and 2.6 and 26 μW g^{-1}, respectively) in studies designed to look for athermal RFR effects.

2. Materials and methods

2.1. Cells

Molt-4 cells were the generous gift of Dr. Narendra Singh, University of Washington. These cells were chosen because of their sensitivity to agents which produce DNA damage (N.P. Singh, personal communication). Cells were maintained in RPMI-1640 tissue culture medium (Cellgro), which was supplemented with 10% fetal calf serum (Gemini Bioproducts), and kept in a Forma Model 3158 incubator in a humidified atmosphere at 37°/5% CO_2. Cells were seeded into 60 mm Petri dishes the day before experimentation and were at a cell density of approximately 1×10^6 cells ml^{-1} at the time of experimentation. Medium depth in the dishes was 2.4 mm.

2.2. RF exposure

The system environmental control and physical arrangement was the same as that reported previously [33]. Two TEM cells (CS-110S, Instruments for Industry, Ronkonkoma, NY) were used and were housed in a single Napco Model 4300 water-jacketed incubator maintained at 37° in a humidified atmosphere of 5% CO_2 in air. The incubator was fitted with a heat exchanger and inline humidifier to precondition the atmosphere before its entry directly into the interior of the TEM cells. By this means, equilibration time has been reduced significantly. In exposure experiments, one TEM cell was powered (exposed) and one was unpowered (sham). In sham/sham experiments, both TEM cells were unpowered.

2.2.1. Exposure to North American Dual-Mode Cellular (NADC) fields with Time Domain Multiple Access (TDMA) modulation

This exposure apparatus has been described in detail [33], and was used without modification. Briefly, the output of a prototype NADC transmitter (provided by Motorola) was connected through a directional coupler to the TEM cell input. The TEM cell output was terminated with a high-quality 50 Ω resistive load. A power meter/chart recorder combination connected to the directional coupler provided constant monitoring of forward power.

For experiments at +10 dB, the transmitter output was attenuated to drive a power amplifier (Microwave Power Equipment PAS-53-0-800/1000). The amp output was passed through a directional coupler (Narda 3001-30) to the TEM cell input. Forward power was monitored and recorded as described above. In all cases, exposure timing was controlled by an electronic timer.

2.2.2. Exposure to iDEN® cellular phone fields

The iDEN® system utilizes time-domain multiplexing in which each second of time is broken down into a series of about 22 frames, each of 45 ms duration. Each frame is further divided into three 15 ms slots. Thus, the incident field at the head of a phone user is a series of RF carrier bursts, each 15 ms duration and repeating at 45 ms intervals. The iDEN® system signal also includes a brief amplitude training pulse at the leading edge of the slot. Normally this pulse is of approximately the same amplitude as the average power during the burst (slot-average power), but once every 200 frames (every 9 s), one training pulse is transmitted at approximately 10 times the slot average power. The peak power of this pulse in a 600 mW handheld radio is thus about 6 W, for a duration of a few hundred μs. It was therefore necessary to utilize a linear amplifier with at least 10 dB headroom to accommodate the high-power training pulse without clipping.

The RF carrier was generated by a Motorola-supplied iDEN® transmitter (configured by Motorola to operate at a carrier frequency of 813.5625 MHz) driving a 200 W linear power amplifier (MPE PAS-53-0-800/1000) through an adjustable attenuator (Merrimac AU-45ASN) used to set power levels. The amplifier output was measured by a Narda 3001-30 directional coupler, HP 8482A power sensor, and an HP 435B power meter. The analog output from the power meter was connected to a chart recorder and was recorded at all times. The powered TEM cell was terminated with a high quality 50 Ω RF resistive load. Two power levels were used: the nominal exposure (450 mW slot-average input) and 10 dB higher (4.5 W). Before each series of experiments, the power at the TEM cell input was confirmed with an HP 8431B power sensor (with calibrated 30 W attenuator) and an HP 437B digital power meter. As stated above, the forward power was recorded throughout each experiment.

In conformity with our previous TDMA exposure protocols, the carrier was turned on and off at 20 min intervals by a Chrontrol electronic timer. Total incubation times of 2, 3, and 21 h, therefore, represented total RF exposure periods of 1, 1.67, and 10.67 h, respectively.

2.3. Dosimetry

We used a CC-110s TEM cell (inside dimensions of 18 cm W × 18 cm D × 9 cm H, both above and below the septum) for RF exposures and placed the dishes on a 1.5 cm platform of styrene plastic that supported the 60 mm Petri dish containing the cell culture within a region of reasonably uniform field ($\bar{E}$ normal to the dish). The platform was placed on the TEM cell septum. This decision was based on dosimetric assessments for this system performed by Prof. Dr. Niels Kuster and colleagues at the Swiss Federal Institute of Technology, Zurich, Switzerland [34]. The specific conditions used in this experiment were as follows: 1) 60 mm Petri dishes with 5 ml of medium, $\varepsilon = 77$, and $\sigma = 1.8$ mho m^{-1}; 2) 1 dish was placed centrally along the longitudinal axis on the septum in each TEM cell. These conditions were simulated using the MAFIA electromagnetic simulation tool (The Mafia Collaboration, User's Guide Mafia Version 3.x; CST GmbH, Lautenschlaegerstr. 38, D64289 Darmstadt, Germany). Calculations yielded the following slot average SAR values for exposure to the iDEN® signal at 0.8 mW cm^{-2} (input power of 450 mW): 1) average SAR = 2.4 μW g^{-1}; 2) standard deviation = 0.3 μW g^{-1}. We have reported previously SAR values of 2.6 μW g^{-1} (average) and 1.9 μW g^{-1} (SD) for exposure to the TDMA signal at 0.9 mW cm^{-2} (input power of 510 mW). For experiments performed at +10 dB, therefore, average SAR values were 24 μW g^{-1} for the iDEN® signal (input power of 4.5 W) and 26 μW g^{-1} for the TDMA signal (input power of 5.1 W). Importantly, there was no detectable rise in temperature at any power density used in these experiments. Media temperature was measured using a microprocessor-controlled thermometer developed in our laboratory. This instrument is based on a Vitek-type probe (BSD Medical Devices, Salt Lake City, UT), and can resolve temperature changes as small as 0.002°C.

2.4. Local static and 60 Hz magnetic fields

The local static field in the incubator used in this series of experiments was measured with a MAG-03 3-axis fluxgate magnetometer (Bartington Instruments, Oxford, England). Because of size limitations (i.e., size of the probe vs. size of the TEM cells), static field measurements were not made inside the TEM cells. Rather, measurements were made at nine locations on a square 10 cm grid on a shelf at the approximate center of the incubator. The magnitude of the local static field was 31 ± 12 μT at an inclination angle of $-9 \pm 28°$ relative to the horizontal. The ambient 60 Hz magnetic field was measured at each TEM cell location using a Monitor Industries 42B gaussmeter, and was found to be 0.13 ± 0.02 μT_{rms} at the location of one Crawford cell (used for RF exposure) and 0.20 ± 0.04 μT_{rms} at the location of the second Crawford cell (used for the sham exposure).

2.5. Comet assay

DNA single-strand breaks were measured with the alkaline comet, or single cell gel electrophoresis, assay, which

Table 1
Mean values for tail moment (TM) and comet extent (CE) from experiments in which Molt-4 populations, one placed in the upper TEM cell and the other in the lower TEM cell, were both sham exposed

Time[a]	TM (upper)	TM (lower)	p[b]	CE (upper)	CE (lower)	p
2	4.11 ± 0.42[c]	4.29 ± 0.44	0.53	96.4 ± 2.1	96.7 ± 1.9	0.43
3	2.90 ± 0.41	2.61 ± 0.55	0.54	72.8 ± 2.0	78.0 ± 2.4	0.44
21	2.96 ± 0.42	3.09 ± 0.55	0.52	82.2 ± 1.8	81.4 ± 2.1	0.52

[a] Values are the total incubation times in hours for cell cultures (see Section 2).
[b] The p value was derived from analysis of data using Kolmogorov–Smirnoff distribution test.
[c] Values given are the mean $\pm$ SE; the number of experiments pooled for each exposure condition were: 2 h, $n = 5$; 3 h, $n = 3$; 21 h, $n = 3$.

was performed using a modification of the technique reported by Singh et al. [29]. At the conclusion of each experiment, cells were collected by centrifugation in a microfuge (500 rpm, 5 min) at room temperature. Care was taken not to overspin the cells, since this resulted in increased DNA damage. The supernatant was discarded, and the pellet was suspended in 40 μl complete tissue culture medium. Slides were prepared by pipetting 120 μl agarose solution (0.5% 3:1 high resolution blend agarose, Amresco, Solon, OH) containing 5 mg ml^{-1} proteinase K (Amresco, Solon, OH) onto fully frosted glass slides, which were covered immediately with a #1 coverglass. Slides were kept on ice for 30–60 s and the coverglass was then removed. Seventy five μl of cell suspension in agarose (10 μl suspended cell pellet + 200 μl agarose; mixed gently) was then pipetted onto the slide and again covered immediately with a coverglass. After 30–60 s on ice, the coverglass was removed, and a final 100 μl aliquot of agarose/proteinase K solution was pipetted onto the slide, which was covered with a coverglass. After 30–60 s on ice, the slides were immersed for 15 min in ice-cold lysis solution (2.5 M NaCl, 1% Na lauryl sarcosinate, 100 mM Na_2EDTA, 10 mM Tris (pH 10.0), plus Triton X-100 added freshly each experiment to a final concentration of 1%). Slides were then transferred to a Coplin jar containing lysis solution at 37°, and incubation was continued for 2 h at this temperature. After lysis, slides were transferred to the horizontal slab of an electrophoresis unit and covered with electrophoresis buffer (300 mM NaOH, 0.1% 8-hydroxyquinoline, 2% dimethyl sulfoxide, 10 mM Na_4EDTA). After allowing 20 min for DNA to unwind, electrophoresis was performed at 10 V for 60 min. Buffer circulation was accomplished by stirring. After electrophoresis, slides were neutralized 3×30 min in 0.4 M Tris · HCl, pH 7.4. Subsequently, slides were dehydrated 3×30 min in absolute ethanol and air dried. All procedures were performed with either minimal direct lighting or red light.

2.6. Data acquisition and analysis

One slide at a time was stained with 50 μl 1 mM YOYO-1 (Molecular Bioprobes, Eugene, OR) and covered with a 24×50 mm coverglass. Fluorescently-stained DNA was detected with an Olympus BX40F3 fluorescent microscope equipped with a Dage SIT-68 camera and a Dage model DSP-200 image enhancer. Data was acquired for 50 randomly chosen comets per group with Komet 3.0 software (Integrated Laboratory Systems, Research Triangle Park, NC) and transferred to Excel spreadsheets for analysis. Two parameters were used to assess the extent of DNA damage in individual cells: 1) tail moment (TM), which is defined as tail length $\times$ tail intensity or percent migrated DNA; and 2) comet extent (CE) (i.e., comet length). Descriptive statistics (i.e., mean, standard error) were calculated to provide: 1) some characterization of the population of cells used for each experiment; and 2) an indication of the direction of change (i.e., increase or decrease) in cases where statistically significant differences in DNA damage were observed. Statistical differences in the distribution of TM and CE values in control vs. exposed groups

Table 2
Mean values for TM and CE for Molt-4 cells exposed to the iDEN® RFR signal (SAR of 2.4 μW g^{-1}; power density of 0.8 mW cm^{-2}) vs. sham-exposed cells and the TDMA RFR signal (SAR of 2.6 μW g^{-1}; power density of 0.9 mW cm^{-2}) vs. sham-exposed cells

	Time[a]	Control TM	Exposed TM	p[b]	Control CE	Exposed CE	p
iDEN®	2	6.24 ± 0.62[c]	3.93 ± 0.33	$< 0.0001*$	112.5 ± 1.7	105.3 ± 1.5	$< 0.0001*$
	3	3.14 ± 0.36	3.41 ± 0.35	$< 0.0001*$	97.9 ± 1.9	100.8 ± 1.6	$< 0.0001*$
	21	4.22 ± 0.41	2.74 ± 0.37	$< 0.0001*$	90.4 ± 1.7	88.3 ± 1.4	$< 0.0001*$
TDMA	2	3.77 ± 0.38	3.50 ± 0.41	$< 0.0001*$	90.3 ± 1.5	91.5 ± 1.5	$< 0.0001*$
	3	2.91 ± 0.71	2.11 ± 0.50	0.68	84.5 ± 3.5	92.1 ± 2.3	$0.001*$
	21	2.86 ± 0.26	1.68 ± 0.17	$< 0.0001*$	75.7 ± 1.0	67.1 ± 0.9	$< 0.0001*$

[a,b] See Table 1.
[c] Values given are the mean $\pm$ SE; the number of experiments pooled for each exposure condition were: iDEN®: 2 h, $n = 5$; 3 h, $n = 6$; 21 h, $n = 6$; TDMA: 2 h, $n = 5$; 3 h, $n = 1$; 21 h, $n = 8$.

Table 3
Mean values for TM and CE for Molt-4 cells exposed to the iDEN® RFR signal (SAR of 24 μW g^{-1}; power density of 8 mW cm^{-2}) vs. sham-exposed cells, and the TDMA RFR signal (SAR of 26 μW g^{-1}; power density of 9 mW cm^{-2}) vs. sham-exposed cells

	Time[a]	Control TM	Exposed TM	p[b]	Control CE	Exposed CE	p
iDEN®	2	6.09 ± 0.41[c]	11.10 ± 0.58	< 0.0001 *	105.6 ± 1.3	121.0 ± 1.4	< 0.0001 *
	3	2.44 ± 0.38	1.93 ± 0.37	0.33	71.8 ± 1.4	69.3 ± 2.0	0.002 *
	21	4.31 ± 0.42	6.35 ± 0.60	< 0.0001 *	92.5 ± 1.9	93.6 ± 2.3	< 0.0001 *
TDMA	2	4.03 ± 0.41	3.44 ± 0.37	< 0.0001 *	109.4 ± 1.5	99.7 ± 1.5	< 0.0001 *
	3	3.49 ± 0.50	2.93 ± 0.50	0.65	106.7 ± 2.0	103.8 ± 1.7	0.18

[a,b] See Table 1.
[c] Values given are the mean ± SE; the number of experiments pooled for each exposure condition were: iDEN®: 2 h, $n = 7$; 3 h, $n = 2$; 21 h, $n = 5$; TDMA: 2 h, $n = 6$; 3 h, $n = 2$.

were determined using the Kolmogorov–Smirnoff distribution test.

3. Results

Table 1 presents the results of our sham/sham experiments for total incubation times of 2, 3, and 21 h (RF exposure times of 1, 1.67, and 10.67 h, respectively; see Materials and Methods). These experiments were not performed as a continuous block, but were performed at random intervals during the entire course of this study. It is seen that for all conditions, the distribution of values for TM and for CE were not statistically different for cells incubated concurrently in the unpowered upper and lower TEM cells.

Table 2 presents the results of studies in which Molt-4 cells were exposed to either the iDEN® or the TDMA signal at SARs of 2.4 or 2.6 μW g^{-1}, respectively. Both signals induced a statistically significant shift in the distribution of TMs to lower values after incubation times of 2 and 21 h. On the other hand, after 3 h incubation time, the iDEN® signal produced a slight, although significant, shift in TMs upward. Although the TDMA signal appeared to shift TMs to higher values after 3 h total incubation time, this result is based on only a single experiment. CE values generally follow the same trends, although means are not shifted upward or downward to the same extent as seen with TM values.

Table 3 presents the results of studies in which Molt-4 cells were exposed to either the iDEN® or the TDMA signal at SARs of 24 or 26 μW g^{-1}, respectively. After total incubation times of 2 and 21 h, the iDEN® signal produced large and statistically significant shifts in the distribution of TMs to higher values. Interestingly, after 3 h total incubation time, the iDEN® signal shifted the distribution of TMs to lower values, although this result was not significant and based only on two experiments. In contrast, after 2 h total incubation time, the TDMA signal induced a statistically significant shift in the distribution of TMs to lower values. After 3 h total incubation time, TMs were again shifted to lower values although this result was not statistically significant and was based on only two experiments. Here, as with the data of Table 2, CE values generally follow the same trends, although means are not shifted upward or downward to the same extent as seen with TM values. Regrettably, it was not possible to perform experiments at 9 mW cm^{-2} for 21 h total incubation time.

4. Discussion

In this study, we have assessed the damage produced in cells exposed in vitro to two different RF signals by employing the comet, or single cell gel electrophoresis, assay under alkaline conditions. We have analyzed two endpoints, tail moment (TM) and comet extent (or length; CE). The latter is the simplest parameter available to measure DNA damage and is that reported by Lai and Singh in their studies [31,32,35]. The concept of TM to assess DNA damage was introduced by Olive et al. [36], since this parameter increases linearly over a wider range of ionizing radiation doses than does CE. In fact, some consider TM to be a superior metric for assessing DNA damage, since TM incorporates a measure of both the smallest DNA fragment detectable (reflected in comet length) and the number of DNA fragments (represented by the amount of DNA in the tail). Indeed, our data indicate TM to be a more sensitive measure of DNA damage than CE, as judged by the greater magnitude of change demonstrated in TM as compared to CE for any given experiment or condition. It has been observed by others that there is an upper limit to CE for a given set of experimental conditions that is reached rapidly. Additional damage, therefore, increases the proportion of DNA in the tail, but it does not increase the CE. It is for this reason that we concentrate on RF signal-induced changes in TM in this discussion.

We have chosen to focus on the *distribution* of damage among the cells of a given population rather than on changes in group mean response, which can be altered easily by only a very few comets at a measurement extreme. Consequently, rather than analyze our data using analysis of variance as other investigators do, we have employed the Kolmogorov–Smirnoff distribution test to assess differences between our two cell populations. Group

mean values have been calculated so that the direction in which changes in TM or CE distribution have occurred could be determined. Furthermore, we have pooled the data from all experiments at each specific exposure condition. This procedure increased the sensitivity with which we could detect shifts in the distribution of TM and CE values since group size was increased over that of the individual experiments, and has been employed by others to achieve the same purpose [37,38].

Our results are of interest for several reasons. First, the data indicate that two different RF signals were capable of interacting with a biological system in vitro and altering the extent to which damaged DNA could be observed. These results confirm, at least in concept, the reports by Lai and Singh [31,32] and by Sarkar et al. [28] of increased DNA damage in the organs of animals exposed in vivo to microwave radiation. Second, the iDEN® and TDMA RF signals produced generally similar decreases in DNA damage after exposure to SARs of 2.4 and 2.6 μW g^{-1}, respectively, for 2 h and 21 h total incubation time. In contrast, exposure of Molt-4 cells to the iDEN® signal at an SAR of 24 μW g^{-1} produced a substantial increase in DNA damage after 2 h total incubation time, while exposure to the TDMA signal at an SAR of 26 μW g^{-1} for 2 h total incubation time resulted in decreased DNA damage compared to control cell cultures. The decreased DNA damage in TDMA-exposed cell cultures is of particular interest, since it has been reported by us that long-term in vivo exposure of rats treated in utero with the chemical carcinogen, ethylnitrosourea, to the TDMA RF signal resulted in significantly fewer central nervous system tumors as compared to unexposed animals [39]. Indeed, even rats not treated with carcinogen but exposed to the TDMA signal demonstrated fewer spontaneous central nervous system tumors than control animals [39]. Furthermore, it appears that the apparent 'protective' effect of the TDMA signal may be related to the signal's modulation, since ethylnitrosourea-treated rats exposed to an FM (continuous wave) signal at the same frequency and power density and with the same exposure regimen demonstrated no difference in tumor incidence between control and exposed groups [40].

How is it that exposure of cells to the same signal under different conditions (i.e., of time and/or intensity) or to different RF signals can produce both increases and decreases in detectable DNA damage? We believe the key to interpreting such data lies in understanding the balance that exists between DNA damage and the repair of that damage. For instance, an overall increase in DNA damage may be caused by: 1) increased damage with no effect on repair mechanisms; 2) no effect on damage per se, but decreased capacity for repair; or 3) increased damage and decreased repair. Similarly, an overall decrease in DNA damage may be caused by: 1) decreased DNA damage with no effect on repair mechanisms; 2) no effect on damage per se, but increased capacity for repair; or 3) decreased damage and increased repair. Furthermore, it must be borne in mind that our model system, Molt-4 cells, is unsynchronized and dynamic. Depending on the state of the cells at the start of each experiment (e.g., cell cycle distribution, growth rate), one response may be favored over another. Using the data of Table 2 as an example, we offer the following interpretation. After 2 h total incubation time, cells exposed to the iDEN® RF signal have, compared to unexposed control cells, either decreased DNA damage, increased damage repair capacity, or both. However, by 3 h total incubation time, either damage has increased so that the capacity of the repair systems have been exceeded, or the repair systems have become impaired or otherwise less active, or both of these. After 21 h total incubation time, we observe a situation similar to that seen at 2 h. A different situation is seen with the data of Table 3. After 2 or 21 h total incubation time, net DNA damage is greater in iDEN®-exposed as compared to control cultures because RF exposure has produced greater damage to DNA, or repair mechanisms have become less active, or both of these. Finally, we must stress that, because of the consistent results derived from our sham/sham exposure experiments, we believe our data to indicate a real effect of RF exposure on DNA damage detectable in the comet assay.

At this point, there are two key questions. First, is it possible for RF exposure to produce an increase in DNA damage directly (i.e., without affecting the rate of DNA repair)? Second, is it possible for RF exposure to alter, either by increasing or by decreasing, the rate at which DNA repair occurs? Each of these questions will be considered in turn.

There is continued study of the relationships between free radicals and human pathology. This is of interest in bioelectromagnetics research, since it has been proposed that electromagnetic signals may 'couple' to biological systems through effects on chemical reactions involving the formation of free radicals [41,42]. There is mounting evidence that reactive oxygen species (such as $O_2^{\cdot -}$, $HO^{\cdot}$, and H_2O_2) and reactive nitrogen species (such as $NO^{\cdot}$, $NO_2^{\cdot}$, and $ONO_2^{\cdot}$) contribute to human tumorigenesis through the production of genetic mutations that are associated with the initiation and progression of cancer and with changes in cell proliferation associated with chronic inflammation. Additionally, many neurological disorders may be derived from free radical-induced injury, simply because the high lipid content and high energy requirements of the brain make that organ especially sensitive to damage mediated by free radicals and oxidative stress. In this regard, it is of interest that long-term exposure to low-level extremely low frequency EMFs appears to be associated with increased incidences of cancer [43,44] and Alzheimer's disease in humans [45].

The production of free radicals is a natural consequence of aerobic metabolism and cellular biochemistry, and DNA damage produced by oxidation appears to be the most

significant endogenous damage [46]. It has been estimated that the 'hits' to DNA from endogenous oxidants are normally 10^5 per cell per day in the rat and 10^4 in the human [47,48]. Oxidative damage is repaired effectively, although not perfectly, and lesions that escape repair have a certain probability of producing mutations when the cell divides, a situation which may ultimately lead to disease. In proliferating cells, several mechanisms exist which allow damaged DNA to be repaired and the number of spontaneous or exogenously-induced genetic alterations to be minimized. These include: a) DNA excision repair pathways (repair single strand breaks, base damage, adduct formation); b) postreplication repair; c) repair of DNA double-strand breaks; and 4) delayed progress through the cell cycle, thus providing added time for DNA repair either prior to DNA replication (S phase of cell cycle) or to mitosis (M phase). As indicated above, the question is whether or not RF exposure or other EMF exposure can alter the rate at which DNA repair occurs. Unfortunately, this is an area that has not yet been investigated, although exposure to various electromagnetic signals has been reported to affect the activity of a variety of enzymes, such as protein kinases [6,49,50] acetylcholinesterase [51], and ornithine decarboxylase [7,9,52]. Additionally, studies from our lab have indicated that exposure of Molt-4 cells to a 1 G sinusoidal MF at 60 Hz decreased the activity of the repair enzyme, poly(ADP-ribose) polymerase, and increased the number of etoposide-treated cells that are destroyed by apoptosis (J.L. Phillips, unpublished data). Also, it is of interest that Lai and Singh [53] have recently demonstrated that free radicals may indeed play a part in RFR-induced DNA damage.

In summary, our data indicate that exposure of Molt-4 T-lymphoblastoid cells in vitro to two different RF signals under athermal conditions altered the amount of DNA single-strand breaks detected by the alkaline comet assay. Depending on the signal and the time of exposure, DNA damage was observed to both increase and decrease. It is of interest to determine whether or not differences in the modulation of the TDMA and iDEN® signals have an effect on the direction of change. Indeed, Penafiel et al. [54] have reported recently that modulation of an 835 MHz RF signal played a role in determining the effect of RF exposure on ornithine decarboxylase activity in L929 murine fibroblasts. Furthermore, in order to more fully understand the underlying mechanism(s) responsible for these changes, it will be necessary in future studies to distinguish between RFR effects on DNA damage and RFR effects on DNA repair.

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