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Author(s): L. Manti, H. Braselmann, M. L. Calabrese, R. Massa, M. Pugliese, P. Scampoli, G. Sicignano, and G. Grossi
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Effects of Modulated Microwave Radiation at Cellular Telephone Frequency (1.95 GHz) on X-Ray-Induced Chromosome Aberrations in Human Lymphocytes *In Vitro*

L. Manti,[a,1] H. Braselmann,[b] M. L. Calabrese,[c] R. Massa,[c] M. Pugliese,[a] P. Scampoli,[a] G. Sicignano[a] and G. Grossi[a]

[a] *Department of Physical Sciences, University of Naples Federico II, Naples, Italy;* [b] *Institute of Molecular Radiation Biology, Helmholtz Zentrum München, German Research Center for Environmental Health (GmbH); and* [c] *Department of Electronic and Telecommunication Engineering, University of Naples Federico II, Naples, Italy*

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The case for a DNA-damaging action produced by radiofrequency (RF) signals remains controversial despite extensive research. With the advent of the Universal Mobile Telecommunication System (UMTS) the number of RF-radiation-exposed individuals is likely to escalate. Since the epigenetic effects of RF radiation are poorly understood and since the potential modifications of repair efficiency after exposure to known cytotoxic agents such as ionizing radiation have been investigated infrequently thus far, we studied the influence of UMTS exposure on the yield of chromosome aberrations induced by X rays. Human peripheral blood lymphocytes were exposed *in vitro* to a UMTS signal (frequency carrier of 1.95 GHz) for 24 h at 0.5 and 2.0 W/kg specific absorption rate (SAR) using a previously characterized waveguide system. The frequency of chromosome aberrations was measured on metaphase spreads from cells given 4 Gy of X rays immediately before RF radiation or sham exposures by fluorescence *in situ* hybridization. Unirradiated controls were RF-radiation- or sham-exposed. No significant variations due to the UMTS exposure were found in the fraction of aberrant cells. However, the frequency of exchanges per cell was affected by the SAR, showing a small but statistically significant increase of 0.11 exchange per cell compared to 0 W/kg SAR. We conclude that, although the 1.95 GHz signal (UMTS modulated) does not exacerbate the yield of aberrant cells caused by ionizing radiation, the overall burden of X-ray-induced chromosomal damage per cell in first-mitosis lymphocytes may be enhanced at 2.0 W/kg SAR. Hence the SAR may either influence the repair of X-ray-induced DNA breaks or alter the cell death pathways of the damage response.

[1] Address for correspondence: Department of Physical Sciences, University of Naples Federico II, Via Cinthia, Complesso Universitario di Monte S. Angelo, 80126-Naples Italy; e-mail: manti@na.infn.it.

INTRODUCTION

The question of whether electromagnetic fields in the radiofrequency (RF) range constitute a health hazard in exposed individuals has gained broad public interest because of the widespread applications of RF-radiation-based technology (*1–3*). Although the majority of the published results from *in vivo* and *in vitro* studies point to the absence of a significant DNA-damaging effect in mammalian somatic cells as evaluated using a variety of biological end points, some reports have supported the opposite view, as reviewed elsewhere (*4–7*). Research to assess the cellular response to the Global System of Mobile Communication (GSM) signal has been active (*5, 8–16*). In contrast, the number of studies on the cytotoxic and genotoxic potential of the recently developed Universal Mobile Telecommunication System (UMTS) standard, which differs greatly in frequency band and modulation, is limited despite its wide use (*4, 17–18*). Moreover, even assuming that subthermal exposure to electromagnetic radiation does not damage the DNA directly, it is possible that it may have epigenetic effects, for instance by altering repair of the damage induced by other environmental agents (*10, 11, 14*). There is a need for studies to examine the possibility that UMTS modifies the cytogenetic damage induced by ionizing radiation (*5*). Chromosomal aberrations are induced efficiently by ionizing radiation and are known to result from unrepaired or misrepaired DNA damage (*19*), and an increased chromosome aberration frequency in human lymphocytes from healthy subjects is widely regarded as a biomarker of cancer risk (*20, 21*).

In this work, we studied the effects of an *in vitro* 24-h exposure to a 1.95 GHz UMTS signal on the yield of chromosome aberrations induced by 4 Gy of X rays in unstimulated G_0 human lymphocytes. Recent studies have found no difference in the cytogenetic response of either G_0- or phytohemagglutinin (PHA)-activated lymphocytes to RF radiation (*4*). Lymphocytes are normally quiescent under physiological conditions and have levels of repair enzymes similar to those in proliferating lymphocytes (*22*)

and therefore can convert radiation-induced damage to chromosome aberrations. The 24-h RF-radiation exposure time allows for the slow repair component to come to completion (*19*).

The RF-radiation exposures were carried out using a waveguide system at two specific absorption rates (SARs), 0.5 and 2.0 W/kg, under strictly controlled dosimetric and environmental conditions. To assess the possible impairment of the repair efficiency of the damage induced by ionizing radiation by exposure to the UMTS signal, we analyzed the first cell generation after RF-radiation exposure. After RF-radiation treatment and 48 h PHA-induced stimulation, metaphase spreads were harvested using conventional cytogenetic protocols. Structural chromosome aberrations were scored in fluorescence *in situ* hybridization (FISH)-stained chromosomes 1 and 2, which account for about 16% of the human genome. Most of the existing data on the genotoxicity of RF radiation have been obtained using solid staining; this is the first time that the effect of UMTS RF radiation on ionizing radiation-induced damage has been assessed by FISH, which detects a wider array of chromosome alterations, including non-lethal, potentially heritable cytogenetic alterations (*23*). Lymphocytes exposed to X rays followed by RF radiation or sham exposure were used to investigate the RF-radiation-associated modification of the damage induced by ionizing radiation; unirradiated lymphocytes that were exposed to RF radiation or were sham-exposed were used as controls.

MATERIALS AND METHODS

Exposure System Set-up

This study was carried out in a coordinated research program on ''Wireless Technology Health Risks (WITHER)'', where several research groups joined to evaluate, using the same exposure conditions, cancer-related end points in mammalian cells after *in vitro* exposure to wireless RF signals.[2] A standardized exposure device was used; a detailed description can be found elsewhere (*17, 24*). A working frequency of 1.95 GHz was chosen that corresponds to a frequency of the uplink UMTS systems, and the wideband code-division multiple-access (WCDMA) standard, according to the 3GPP 3.5 2001-03 specifications, was used (five power-controlled user data channels + 1 control channel). Briefly, the UMTS signal (Agilent E4432B ESG-D source) was amplified (Microwave Amplifiers, LtdAM38A-092S-40-43) and fed through a bidirectional power sensor (Rohde & Schwarz, NRT-Z43) into the exposure chamber. As shown in Fig. 1A, this consisted of a rectangular thermostated waveguide (WR 430: 109.2 mm × 54.6 mm), whose feeding end was a coaxial waveguide adapter (Maury Microwave R213A2, VSWR<1.05); the other end terminated with a fixed short circuit.

The power sensor and signal generator were connected to a dedicated computer. Software developed in-house based on a commercially available program (LabVIEW 7, National Instruments) provided a user-friendly system interface and continuous control of the power level that was adjusted to the required SAR. The SAR was evaluated as the ratio between the power absorbed by the sample (Pa) and its mass (m), with Pa being the difference between the incident (Pi) and the reflected (Pr) powers, both measured by the bidirectional power sensor. For each experiment, the biological samples were placed in four 35-mm-diameter petri dishes (BD Biosciences Europe), each filled with 3 ml of cell suspension and held by a plastic stand. Numerical and experimental dosimetry showed that the best tradeoff between efficiency (Pa/Pi) and SAR uniformity was achieved when the center of the samples was at a distance from the short circuit of $|z| \approx 0.5\ \lambda_z$. The distance between the samples allowed simultaneous exposure at two different SARs, the mean SARs 2.0 W/kg and 0.5 W/kg (Fig. 1A). These values are within the currently accepted safety limit (2.0 W/kg) for cellular telephone microwave emission (*25*). With this configuration, a satisfactory uniformity was achieved in the spatial distribution of the electric field along the vertical direction and across the samples, because the induced electric field was essentially parallel to the sample surface (*17*). Measurements of local SAR and power efficiency showed good agreement with the calculated values, thus ensuring reliable information about power deposition patterns and degree of non-uniformity. The latter was evaluated numerically as the ratio between the SAR standard deviation and the average SAR (coefficient of variation, CV) and was 0.33 in all four samples. Two identical waveguides were housed in a commercial incubator at 37°C in a 95% air/5% CO_2 atmosphere. One waveguide was used for actual exposures and the other for sham exposures (samples inserted in the same position as in the former but without the microwave signal). Local temperature measurements were carried out using a multichannel thermometer with fiber-optic temperature probes (FISO Technologies, FOT-M/2m) inserted vertically in the culture medium. Measurement points were located at the sites of maximum and minimum local SAR and at 0.5 mm from the bottom, e.g., at the fifth layer of the simulated sample, although almost the same field distribution occurs in all sample layers (*24*). Temperature readings were taken at 4-s intervals and recorded by computer. The measured increase in temperature during the 24-h RF-radiation exposure at the high SAR was 0.2 ± 0.1°C, which can be regarded as sufficiently low to exclude damage induced by thermal processes.

Lymphocyte Exposure and Evaluation of Cytogenetic Damage

Peripheral blood lymphocytes were collected as described previously (*23*) using buffy coats from four healthy nonsmoking male donors aged between 23 and 30 who gave informed consent and were chosen on the basis of their medical history and lifestyle habits. Buffy coats were drawn into heparinized Vacutainer™ tubes (Becton Dickinson) and centrifuged at 1000*g* for 30 min. Lymphocyte-rich suspensions were removed from the resin interface and washed twice with phosphate-buffered saline (PBS) solution (Cambrex BioSciences, Belgium). Freshly isolated lymphocytes were then resuspended in 20% bovine serum-enriched RPMI 1640 medium (Gibco, Invitrogen Life Technologies). Immediately after separation, aliquots were given to a collaborating group (*17*) for related studies. Four experiments were performed, one for each donor, according to the following scheme (Fig. 1B). Eight dishes were inoculated with lymphocytes in each experiment; two dishes were given 4 Gy of X rays and then immediately exposed to RF radiation (one dish for each SAR); two dishes were irradiated with 4 Gy of X rays but were then subjected to sham RF-field exposure. In addition, two sets of dishes, consisting of one dish per SAR and respective sham exposure, were used to evaluate the genotoxicity of the UMTS signal itself. Each 35-mm petri dish was seeded with 2×10^6 cells, which were allowed to settle for 2 h before the experiment. The X rays were produced by a Thomson tube (TR 300F; 250 kVp, 0.8 Gy min^{-1}, Stabilipan, Siemens) and filtered by 1-mm-thick copper foil. The irradiation room was adjacent to that hosting the RF-radiation generator and the thermostated incubator (37°C, 95% air/5% CO_2). The dishes were placed inside the two waveguides and kept for 24

[2] M. L. Calabrese, G. Castello, G. d'Ambrosio, F. Izzo, G. F. Grossi, R. Massa, M. Napolitano, G. Petraglia, A. Sannino, M. Sarti, P. Scampoli, M. R. Scarfì and O. Zeni, A project in the framework of the Campania EU-Region Center of Competence on Information and Communication Technologies, related to the evaluation of cancer related endpoints in mammalian cells following *in vitro* exposures to UMTS radiofrequency signal. Presented at 26th Annual Meeting of The Bioelectromagnetics Society, Washington, DC, June 2004. [Available online at http://bioelectromagnetics.com/doc/bems2004-abstract.pdf].

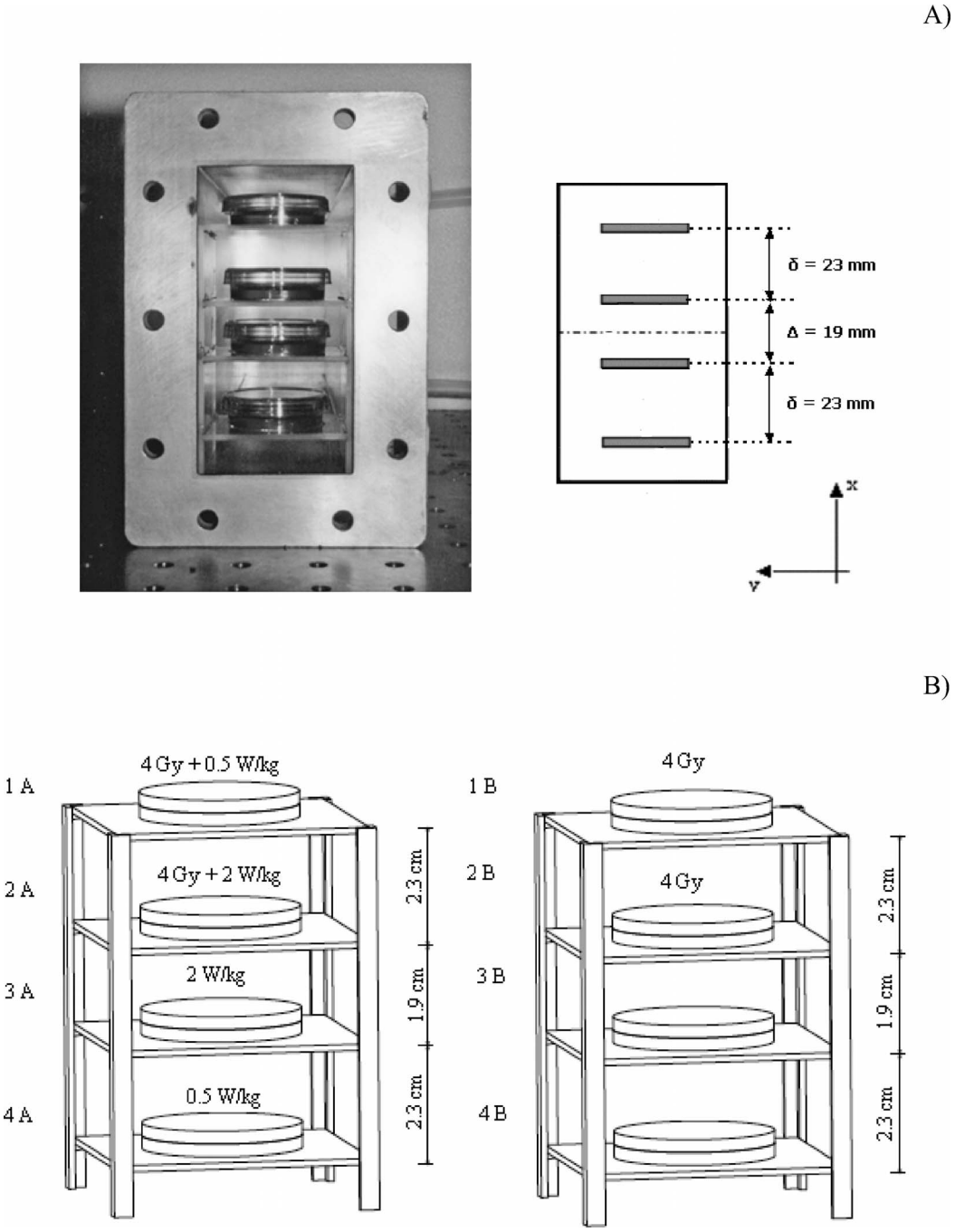

FIG. 1. Panel A: Arrangement of the petri dishes and plastic stand in the waveguide. Panel B: Sketch of the configuration adopted for the positioning of the biological samples inside the waveguide. The samples denoted as B are placed in the second waveguide in the absence of the RF signal.

h in the presence or absence of the RF signal; cells were then gently resuspended in PHA-containing growth medium (1% in RPMI 1640 medium; Gibco) and transferred to standard 25-cm^2 tissue culture flasks (Becton Dickinson). The final volume was brought to 10 ml to prevent medium depletion. After 46 h, cultures were treated for 2 h with the spindle inhibitor colcemid (0.2 μg/ml; Irvine Scientific), and metaphase chromosomes were collected by swelling in 75 m*M* KCl at 37°C and fixation at 4°C in freshly prepared Carnoy solution (3:1 v/v methanol/acetic acid). Air-dried slides were FISH-painted with whole-chromosome probes (MetaSystems, Germany) specific for human chromosome 1 (spectrum green) and chromosome 2 (spectrum red); unlabeled chromosomes were counterstained with 4,6-diamidino-2-phenylindole (DAPI, Olympus, Italy). No centromere probe was used, but centromeres were clearly distinguishable as bright bands under DAPI illumination Slides were scanned at a computerized Zeiss epifluorescence microscope controlled by the Metafer 4 metaphase finder and three-color image acquisition software (MetaSystems). Chromosome analysis was carried out blind on stored images, with all aberrations being scored by the same individual. All kinds of structural chromosome aberrations detectable by FISH hybridization of two painted chromosomes were scored: interchanges, both symmetrical and asymmetrical, centric rings and acentric fragments (terminal or interstitial deletions). The fraction of aberrant cells and the fre-

TABLE 1
Measured Aberrant Cells and Yield of Total Chromosome Exchanges per Donor

Donor	X-ray dose (Gy)	SAR (W/kg)	Number of cells scored	Number of aberrant cells (fraction ± standard error)	Number of total exchanges (frequency ± standard error)
1	4	2	437	306 (0.70 ± 0.02)	428 (0.98 ± 0.05)
1	4	0	219	146 (0.67 ± 0.03)	167 (0.76 ± 0.06)
1	0	2	417	1 (0.002 ± 0.002)	1 (0.002 ± 0.002)
1	0	0	390	1 (0.003 ± 0.003)	1 (0.003 ± 0.003)
2	4	2	560	336 (0.60 ± 0.02)	419 (0.75 ± 0.04)
2	4	0.5	334	215 (0.64 ± 0.03)	189 (0.57 ± 0.04)
2	4	0	511	299 (0.59 ± 0.02)	336 (0.66 ± 0.04)
2	0	2	1100	6 (0.005 ± 0.002)	5 (0.004 ± 0.002)
2	0	0.5	381	0	0
2	0	0	1056	0	0
3	4	2	340	297 (0.87 ± 0.02)	489 (1.44 ± 0.06)
3	4	0.5	247	188 (0.76 ± 0.03)	279 (1.13 ± 0.07)
3	4	0	793	659 (0.83 ± 0.01)	1017 (1.28 ± 0.04)
3	0	2	397	3 (0.007 ± 0.004)	2 (0.005 ± 0.004)
3	0	0.5	413	6 (0.014 ± 0.006)	5 (0.012 ± 0.005)
3	0	0	626	10 (0.016 ± 0.005)	10 (0.016 ± 0.005)
4	4	2	382	291 (0.76 ± 0.02)	434 (1.13 ± 0.05)
4	4	0.5	233	167 (0.72 ± 0.03)	258 (1.11 ± 0.07)
4	4	0	738	569 (0.77 ± 0.02)	819 (1.11 ± 0.04)
4	0	2	504	2 (0.004 ± 0.003)	1 (0.002 ± 0.002)
4	0	0.5	480	2 (0.004 ± 0.003)	2 (0.004 ± 0.003)
4	0	0	1050	6 (0.006 ± 0.002)	6 (0.006 ± 0.002)

Notes. Errors on counts are given by standard errors of the mean (SEM). In the case of donor no. 1 it was not possible to carry out the experiment at 0.5 W/kg SAR.

quency of total chromosome exchanges per cell (i.e. simple exchanges, either complete or incomplete, plus complex interchanges) were calculated by dividing the number of aberrant cells and the sum of all chromosome exchanges by the total number of scored cells, respectively.

Statistical Analysis

For each experiment, standard errors of the mean (SEM) for the fraction of aberrant cells or the frequency of exchange aberrations were calculated using binomial statistics or Poisson variances, respectively (*26*). The influence of RF-radiation exposure on the frequency of aberrant cells or on the frequency of exchanges was examined by two-way analysis of variance (ANOVA), the donors being taken into account as a second variable. ANOVA was performed by generalized linear modeling (*26*) using a binomial distribution model for aberrant cells and a Poisson model for the number of exchanges. This approach takes into account the varying numbers of cells and the dependence between mean and variance. For calculation of standard errors the model dispersion parameter (i.e. mean Pearson residual χ^2), which was greater than 1.0, was taken into account (quasi-likelihood modeling). Correspondingly, differences between model estimates were evaluated by *F* or *t* tests. X-irradiated samples contained at least 200 cells to ensure that differences between aberrant cells or exchange frequencies of 20% or lower could be detected with a statistical power of 0.80. Unirradiated samples were used as qualitative controls. Differences between pairs of unirradiated samples were evaluated by Fisher's exact test. Statistical analysis was carried out using R, which is open source (http://www.r-project.org/); *P* values less than 0.05 were regarded as statistically significant.

RESULTS

Table 1 shows the raw data obtained for all experiments. X-irradiated samples had a high incidence of aberrant cells (between 59 and 87%), and a frequency of exchanges between 0.57 and 1.44 exchanges per cell was found, as was expected for this dose.

The bar charts in Fig. 2 suggest differences between donors. For donors 1–3, the fractions of aberrant cells observed at 2.0 W/kg SAR appear to be slightly higher than those in samples exposed to X rays alone. However, the differences are not significant (binomial homogeneity test, $P > 0.05$ for each donor). No clear trend is observable for 0.5 W/kg SAR.

Table 2 shows the results of the ANOVA used to test the effect of SAR including the donor as a factor in the analysis. Comparison of the fractions of aberrant cells for the three SARs after X irradiation by two-way ANOVA indicated no significant variation due to RF-radiation exposure (*F* test, $P = 0.46$). The overall differences between donors were significant ($P = 0.0025$), in particular the difference between donors 1 and 3 ($P = 0.008$) and consequently between donors 2 and 3, possibly as a result of individual X-ray sensitivity (Table 2a).

Differences between donors also existed in the frequencies of measured exchanges per cell (Fig. 1B). The ANOVA for exchange frequencies revealed significant overall differences between SARs (*F* test, $P = 0.029$). Particularly, the frequencies recorded at 2.0 W/kg SAR showed a significant shift of 0.11 exchanges per cell ($P = 0.036$) compared to SAR 0 (intercept in Table 2b).

The frequencies of fragments per cell were determined

for X-irradiated samples (Table 3). The overall mean at 0 W/kg SAR was 0.18 fragments per cell. Poisson-based ANOVA showed a significant effect ($P = 0.001$) due to SAR after correcting for donors (no data were available for donor 1) resulting in −0.05 fragments per cell between 0 and 2.0 W/kg SAR.

For unirradiated samples, no significant overall differences were found after correcting for donors (residual deviance $\chi^2 = 11.9$, $P = 0.10$, for both aberrant cells and exchange frequencies). Even for donor 3, who exhibited the greatest incidence of background chromosomal damage (see Table 1), the difference between SAR 0 and SAR 2 is not significant (Fisher's exact test, $P = 0.39$ for aberrant cells and $P = 0.14$ for exchanges).

DISCUSSION

Chromosome aberrations as revealed by FISH of human chromosomes 1 and 2 were used to assess the effect of UMTS RF radiation at two mean SARs on the occurrence of ionizing radiation-induced DNA damage in human lymphocytes. This is of interest for a number of reasons. First, most of the studies available in the peer-reviewed literature did not use the UMTS technology. UMTS, albeit similar to previous digital technology regarding carrier frequency and exposure strength, differs widely in the signal modulation technique (*27*). It implements the WCDMA standard, which allows multiple users to transmit simultaneously and at varying data rates; the overlay of five user data channels (data rate of 960 kb/s per channel) with a typical control signal and a quadrature phase-shift keying (QPSK) modulation was employed. The power control signal, operating at 1.5 kHz, is an important feature of UMTS. It compensates for the fading of the mobile channel and guarantees that all user signals have nearly equal power levels at the base station receiver (*28*). These low-frequency variations of the RF-radiation envelope should be taken into account since the hypothetical influence of electromagnetic fields on biological systems is often attributed to amplitude modulation (*29*). In addition, the wide band spectrum (about 5 MHz) of the UMTS signal has been suggested to give rise to interactions with biological materials because of potential frequency windows (*30*). Second, the majority of cytogenetic data were derived from solid staining of metaphases. This restricts analysis to mainly lethal aberrations such as dicentrics, overlooking most exchange-type aberrations that are of relevance since they can be inherited by progeny of exposed cell populations. Such aberrations are revealed using FISH-based techniques as in our study. Third, the preponderant evidence accumulated on the genotoxicity of RF radiation in lymphocytes does not support a direct, non-thermal DNA-damaging action in the microwave region (*4, 9, 10, 13, 15–17, 27, 31*), while early studies reporting an increased incidence of DNA damage (*32, 33*) have been questioned because thermal effects may have occurred (*5*). Few studies have specifically addressed the possibility that exposure to RF radiation may enhance the cytogenetic damage caused by known carcinogens. One group studied the epigenetic effects of GSM RF radiation on G_0 human lymphocytes, examining the frequency of chromosome aberrations in combined-exposure experiments (*34*). They found a strong synergistic effect of sequential exposure to RF radiation and mitomycin C (MMC), although a later study by the same group did not reproduce such a response using either MMC or 1 Gy of X rays (*14*). Another laboratory has reported enhancement of DNA damage in human lymphocytes as assessed by the comet assay in combination studies with a 3 W/kg SAR 1.8 GHz signal and either MMC (*11*) or UVC radiation (*35*). As Vijayalaxmi and Obe (*5*) pointed out, *in vitro* studies of RF radiation in combination with other chemical or physical mutagens have generally proven inconclusive.

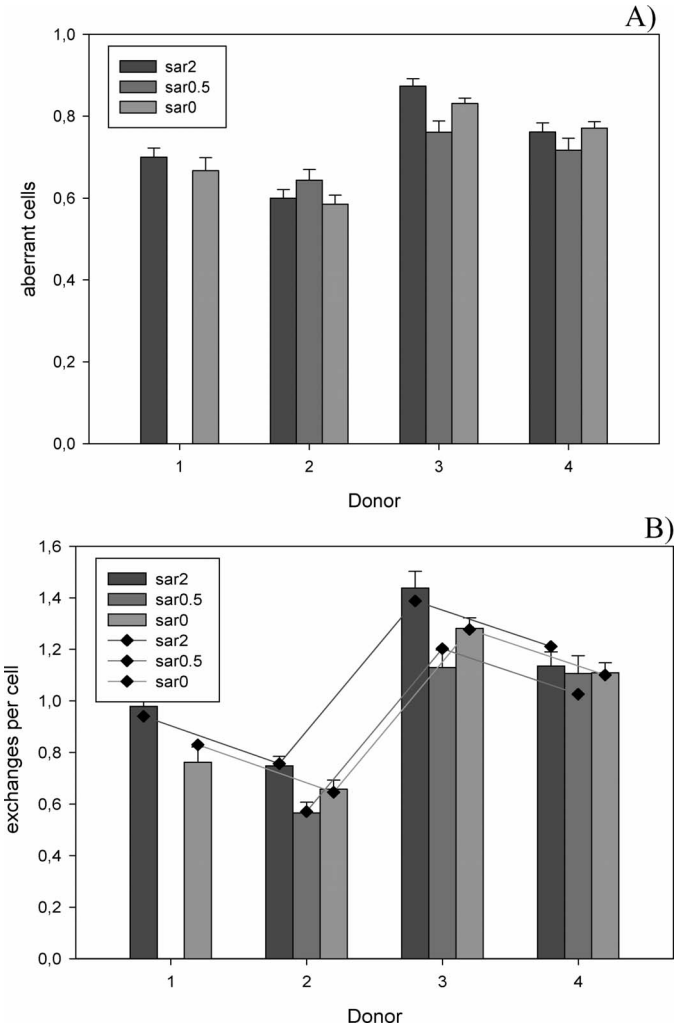

FIG. 2. Panel A: Bar chart showing the fraction of aberrant cells after 4 Gy X irradiation and RF-radiation exposure at various SARs as in Table 1. A difference due to donors can be observed. Panel B: Observed frequencies of exchanges per cell; diamonds represent the fitted frequencies (maximum likelihood for Poisson distribution). The fitted frequencies are connected by straight lines as a guide for the eye. Because of the lack of overall significance, fitted values were not shown for data panel A.

TABLE 2a
ANOVA Results for Frequency of Aberrant Cells Based on Generalized Linear Modeling and the Binomial Distribution

Model terms[a]	Residual deviance	Residual degrees of freedom (*df*)	*F* value (nd/dd)[b]	*P* value
Overall mean	208.1	10		
Donors	19.1	7	22.5 (3/7)	0.0025
SAR	14.0	5	0.90 (2/5)	0.46
Estimated levels Level	Estimate[d] ± SE (in fraction of aberrant cells)		*t* value (*df* = 5)	*P* value
Intercept[c]	0.680 ± 0.034			
Donor 2	−0.074 ± 0.038		−1.94	0.11
Donor 3	0.150 ± 0.036		4.27	0.008
Donor 4	0.081 ± 0.037		2.18	0.081
SAR 0.5	−0.025 ± 0.030		−0.84	0.44
SAR 2.0	0.017 ± 0.024		0.72	0.51

[a] Added sequentially from top to bottom.
[b] nd = numerator *df*, dd = denominator *df*.
[c] Intercept is donor 1 and SAR 0.
[d] Additive differences relative to intercept for rows 2–6.

However, RF-radiation-induced modifications of the efficiency and fidelity of repair of damage caused by ionizing radiation remain poorly investigated. Recently, Stronati *et al.* (*10*) used G_0 human lymphocytes and a GSM RF signal (1.0 and 2.0 W/kg SAR) in combination with 1 Gy of 250 kVp X rays delivered either immediately before or after RF-radiation exposure and did not find a genetic or epigenetic effect of RF radiation based on several standard *in vitro* DNA damage assays. Chromosome aberration frequency was determined by solid staining. As stated above, this represents a limitation; nevertheless, the large number of donors screened, i.e. 14, is a strong feature of this study.

Our data indicate that 24 h exposure of X-irradiated human lymphocytes to a 1.95 GHz UMTS signal did not increase the fraction of aberrant cells (Table 1). The bar chart in Fig. 2A highlights the differences that occurred between donors; hence we tested the effects of SAR, taking into account the donor factor according to a model that considers main effects only. This showed that influence of SAR was not significant (asymptotic *F* test, $P = 0.46$) after correction for donors (Table 2a). Pairwise comparisons shown in Table 2a indicate that 0.5 W/kg and 2.0 W/kg SAR are not significantly different from the intercept (0 W/kg SAR). The difference between 0.5 W/kg and 2.0 W/kg SAR also was not significant. This supports the view that the yield of DNA damage caused by X radiation is not influenced by the UMTS RF signal. However, the Poisson distribution-based ANOVA of the frequency of total exchanges per cell revealed a significant enhancement by SAR, albeit the magnitude of the shift due to RF-radiation exposure is small, i.e. 0.11 exchanges per cell (Table 2b). This is to say that, because exchanges have a total mean of roughly 1.0 exchanges per cell, the estimated effect of 2.0 W/kg SAR is then +11%.The influence of SAR on the exchange frequency is statistically significant after correction for donor variability (Fig. 2B). In particular, the exchanges found in the metaphases from X-irradiated lymphocytes that were treated with 2.0 W/kg SAR exhibited the highest occurrence compared to 0 W/kg SAR ($P = 0.036$).

There may be a number of reasons for the apparent higher frequency of exchanges after SAR exposure, which necessarily involves some degree of speculation. Ionizing radiation at the dose used in our study is very effective at inducing double-strand breaks (DSBs), which, if unrepaired or misrepaired, lead to formation of chromosome aberrations. In eukaryotes, DSB rejoining is a biphasic process, mainly under the control of the non-homologous end-joining molecular machinery, which has a fast and a slow kinetics whose half-times are of the order of minutes and hours, respectively. It has been hypothesized that the fast component is highly efficient, thereby suppressing aberration formation (*36*). Conversely, the slow component is more error-prone and is thus more likely to result in exchange aberrations. Pivotal in this model is the availability of DNA-PK or its accessibility through changes in chromatin conformation (*36*). Therefore, one possibility is that the SAR, which in our study operated on a time scale that is comparable to that of the slow-order repair process, may induce chromatin changes through which the slow component is affected, which results in a higher likelihood of misrejoining at high SAR and thus a larger number of aberrations per cell. SAR-dependent chromatin modifications of the accessibility of repair enzymes to damaged sites have been discussed elsewhere (*5, 10*). The lack of a detectable difference in the fraction of aberrant cells would instead argue against an effect on the fast-order break rejoining kinetics, which processes the bulk of the ionizing radiation-induced lesions. Thus the initial yield of cells with aberrations would not be influenced by RF radiation.

The frequency of fragments tends to decrease as the SAR

TABLE 2b
ANOVA Results for Frequency of Exchanges Based on Generalized Linear Modeling and the Poisson Distribution

Model terms[a]	Residual deviance	Residual degrees of freedom (*df*)	*F* value (nd/dd)[2]	*P* value
Overall mean	332.6	10		
Donors	29.5	7	70.6 (3/7)	0.0002
SAR	7.18	5	7.8 (2/5)	0.029
Estimated levels Level	Estimate[4] ± SE (in exchanges per cell)	*t* value (*df* = 5)		*P* value
Intercept[c]	1.100 ± 0.038			
Donor 1	−0.270 ± 0.056	−4.67		0.0054
Donor 2	−0.460 ± 0.043	−10.47		0.0001
Donor 3	0.180 ± 0.050	3.52		0.017
SAR 0.5	−0.075 ± 0.046	−1.63		0.16
SAR 2.0	0.110 ± 0.034	2.85		0.036

[a] Added sequentially from top to bottom.
[b] nd = numerator *df*, dd = denominator *df*.
[c] Intercept is donor 4 and SAR 0.
[d] Additive differences relative to intercept for rows 2–6.

increases (Table 3). At 2.0 W/kg, this frequency is lower by about 28% compared with that at 0 W/kg. This effect qualitatively complements that on exchanges (+11%). It is believed that fragments arise mainly from defects in DSB rejoining, i.e. from non-rejoined breaks, whereas DSB misrejoining is responsible for exchange-type aberrations (*37*). Thus a greater portion of the initially induced DSBs may remain unrejoined with no or low SAR and/or break rejoining could be favored at high SAR. On the other hand, acentric fragments are likely to lag and be lost at mitosis, while chromosomal fragments are associated with ionizing radiation-induced G_2/S-phase arrest in lymphocytes (*38*). Analysis by calyculin A-induced premature chromosome condensation may be considered in future investigations to take into account the differentially transmissible aberrations and possible effects of cell cycle delay (*23*).

The lack of an increased susceptibility to apoptosis induced by non-thermal RF radiation alone (*39, 40*) or in the presence of other stressors such as UVB radiation and heat shock (*41*) has been reported. It is plausible that rapid interphase death (apoptosis) as triggered by X radiation occurs preferentially in heavily damaged cells at no or low SAR resulting in more damaged cells reaching the first postirradiation mitosis at high SAR. According to this scenario, increasing SARs do not interfere with repair processes but act by rescuing X-ray-damaged cells from death.

TABLE 3
Frequency of Acentric Fragments

Donor	X-ray dose (Gy)	SAR (W/kg)	Fragment frequency
2	4	2	0.079 ± 0.012
2	4	0.5	0.081 ± 0.015
2	4	0	0.115 ± 0.015
3	4	2	0.14 ± 0.02
3	4	0.5	0.18 ± 0.03
3	4	0	0.22 ± 0.02
4	4	2	0.19 ± 0.02
4	4	0.5	0.21 ± 0.03
4	4	0	0.24 ± 0.02

Notes. Data from donor no. 1 not available. The occurrence of fragments is consistently lower at 2.0 W/kg SAR compared to other SARs after 4 Gy of X rays. Errors are SEMs.

Our data from the controls indicate no qualitative differences in the fractions of aberrant cells or in the frequencies of exchange aberrations per cell and thus agree with the notion that RF-radiation exposure itself does not cause cytogenetic damage, as recently confirmed by other lymphocyte studies using RF radiation with similar characteristics (*17, 27*).

We conclude that, under our experimental conditions, there is no evidence for a significant enhancement of the yield of ionizing radiation-induced chromosomal damage in peripheral blood human lymphocytes by non-thermal action of UMTS RF radiation. However, our data on the frequency of chromosome exchange aberrations cannot rule out epigenetic modifications of repair of radiation-induced lesions such as DSBs. At this stage, it can only be tentatively speculated as to whether such modifications impair correct joining of X-ray-induced breaks, leading to an increase in exchange-type aberrations, or favor rejoining processes, thereby lowering the incidence of fragments. RF-radiation-induced differential expression (*42, 43*) of gene(s) involved in the selection of repair pathways or chromatin modifications (*5*) might be the pathway for the action of RF radiation on repair processes at high SAR in X-irradiated lymphocytes. Alternatively, exposure to RF radiation may affect X-ray-induced interphase death by suppressing apoptosis and allowing damaged cells to reach cell division.

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