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Mobile phone specific radiation disturbs cytokinesis and causes cell death but not acute chromosomal damage in buccal cells: Results of a controlled human intervention study

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ABSTRACT

Several human studies indicate that mobile phone specific electromagnetic fields may cause cancer in humans but the underlying molecular mechanisms are currently not known. Studies concerning chromosomal damage (which is causally related to cancer induction) are controversial and those addressing this issue in mobile phone users are based on the use of questionnaires to assess the exposure. We realized the first human intervention trial in which chromosomal damage and acute toxic effects were studied under controlled conditions. The participants were exposed via headsets at one randomly assigned side of the head to low and high doses of a UMTS signal (n = 20, to 0.1 W/kg and n = 21 to 1.6 W/kg Specific Absorption Rate) for 2 h on 5 consecutive days. Before and three weeks after the exposure, buccal cells were collected from both cheeks and micronuclei (MN, which are formed as a consequence of structural and numerical chromosomal aberrations) and other nuclear anomalies reflecting mitotic disturbance and acute cytotoxic effects were scored. We found no evidence for induction of MN and of nuclear buds which are caused by gene amplifications, but a significant increase of binucleated cells which are formed as a consequence of disturbed cell divisions, and of karyolitic cells, which are indicative for cell death. No such effects were seen in cells from the less exposed side. Our findings indicate that mobile phone specific high frequency electromagnetic fields do not cause acute chromosomal damage in oral mucosa cells under the present experimental conditions. However, we found clear evidence for disturbance of the cell cycle and cytotoxicity. These effects may play a causal role in the induction of adverse long term health effects in humans.

1. Introduction

Billions of humans are using mobile phones worldwide and the exposure to telecommunication specific high frequency electromagnetic fields (HF-EMF) has raised global concerns about their potential adverse health effects. It was repeatedly reported that exposure to HF-EMFs is associated with neoplasms (in particular with gliomas and schwannomas and possibly also with leukemia) (Brabant et al., 2023; Carlberg et al., 2017; Coureau et al., 2014; Hardell and Carlberg, 2015; Hardell et al., 2013; IARC, 2013; Jalilian et al., 2022). Therefore, this form of radiation exposure was categorized by IARC as group 2B ("possibly carcinogenic to humans") on the basis of the Interphone study and studies of the Hardell group on glioma and neuroma (IARC, 2013).

Numerous studies have been performed to investigate the molecular mechanisms which lead to neoplastic transformation of cells by HF-EMF (Al-Serori et al., 2019; Panagopoulos et al., 2021; Yang et al., 2012). One of the modes of action which was frequently studied *in vivo* and *in vitro* is damage of the genetic material (Jagetia, 2022; Panagopoulos, 2019), which is a hallmark of human cancer (Cairns, 1975).

The results of *in vitro* experiments with mammalian cells are highly controversial and it was emphasized that the outcome depends strongly on the design, i.e. studies that had a high quality, yielded only weak or negative results (Vijayalaxmi and Prihoda, 2019). Also human biomonitoring studies lead to conflicting findings. The most frequently used

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markers were micronuclei (MN), which were monitored either in lymphocytes or in exfoliated buccal cells. The findings of individual studies are summarized in recent reviews (Al-Serori et al., 2019; Jagetia, 2022; Revanth et al., 2020).

MN are formed as a consequence of structural and chromosomal aberrations (Norppa and Falck, 2003; Stopper and Muller, 1997). A variety of chromosome and chromatid type alterations, which are caused by DNA reactive chemicals and also by ionizing and non-ionizing radiation, lead to formation of these structures representing DNA containing bodies in the cytoplasm which consist of entire chromosomes and/or fragments (Fenech, 2019). Standardized protocols for *in vitro* and *in vivo* MN experiments have been developed for the routine testing of chemicals (OECD, 2016a,b). Furthermore, MN experiments with lymphocytes and exfoliated cells are widely used in studies with humans that are exposed at workplaces and in the environment to chemicals and radiation (Nersesyan et al., 2022). MN formation was frequently studied in occupational and lifestyle studies in blood cells (Knasmueller et al., 2016) and in exfoliated epithelial cells from different organs (mouth, cervix and bladder) (Wultsch et al., 2019). It is known that MN in lymphocytes are a reliable biomarker for increased human cancer risks (Bonassi et al., 2007, 2011b). The good correlations between MN rates in lymphocytes and buccal cells suggest that also MN frequencies in epithelial mouth cells are predictive for cancer (Ceppi et al., 2010; Fenech et al., 2024). This observation is also valid for exposure to HF-EMF since chromosomal alterations which are detected in MN experiments play a causal role in neoplastic transformation of cells (Holzel et al., 2020; Kloeber and Lou, 2022).

Since human buccal cells are directly exposed to EMFs from mobile phones, they were frequently used in HF-EMF studies. In total, more than 20 studies have been realized with this technique. Supplementary Table 1 summarizes these studies. Overall, the findings are inconsistent and many earlier studies have methodological shortcomings, i.e. the number of cells counted was lower than recommended in international guidelines (Thomas et al., 2009) and/or DNA unspecific stains were used, which may cause misleading (false positive) results (Nersesyan et al., 2006). Another shortcoming concerns the inadequate assessment of the exposure. Some investigations analyzed effects in users and non-users of mobile phones, while others assessed the exposure on the basis of questionnaires in which the daily use and the overall duration of the exposure (in years) were recorded (Banerjee et al., 2016; Daroit et al., 2015; Hintzsche and Stopper, 2010; Menezes et al., 2022; Ros-Llor et al., 2012; Shaikh et al., 2016; Souza Lda et al., 2014). This approach enables only a vague assessment of the actual exposure, which also depends on the length of the calls, the locations with respect to the serving base-stations and on the models of mobile phones, which were used. The SAR values of the phones are indicated in five investigations (see Supplementary Table 1) but it was criticized that such studies are not reliable and may be biased by several factors (Vijayalaxmi et al., 2007). All studies which have been published so far had a cross-sectional design, i.e. different exposure groups were compared with unexposed or less exposed individuals. To avoid confounding by demographic factors, it was suggested to match the groups in human MN studies in regard to gender, age, nutrition, smoking and alcohol consumption (Bonassi et al., 2011a; Thomas et al., 2009); however, this criterion was not adequately taken into consideration in most of the trials. Furthermore, it was repeatedly postulated that (apart from MN) other nuclear anomalies should be recorded in experiments with exfoliated cells as they provide additional relevant information about genetic instability, mitotic disturbances, acute cytotoxicity and about the division rate of the oral basal membrane (Bolognesi et al., 2013; Thomas et al., 2009), but only in few investigations these anomalies were scored. Only in two studies the numbers of basal cells, which provide information about the mitotic activity of the mucosa, were evaluated (for details see Supplementary Table 1).

This article describes the results of the first intervention trial with mobile phone specific HF-EMF (UMTS signal), which was realized according to international guidelines for MN experiments with buccal cells (Bolognesi et al., 2013; Thomas et al., 2009). In order to minimize confounding and to provide reliable results, we realized the first controlled intervention trial concerning the impact of HF-EMF on buccal cells, with defined duration and exposure conditions under laboratory conditions. This experimental setup reduces inter-individual variations, which were encountered in earlier studies. Genotoxic as well as acute cytotoxic effects were monitored immediately before and three weeks after exposure separately in cells from both cheeks. We scored all nuclear anomalies that are recommended in the international guideline published by Thomas et al. (2009) and we applied a DNA specific stain (Feulgen-Fast Green) to avoid misinterpretation of keratine bodies as micronuclei (Nersesyan et al., 2006). A special headset developed for this trial allowed us to expose the participants to defined HF-EMF intensities. Their "pre-exposure" (due to regular use of mobile phones) was taken into consideration by use of questionnaires. All participants were asked to use hands-free devices when they made or received phone calls three weeks before the start of the experiment, during the exposure phase and three weeks after the last radiation.

2. Materials and methods

2.1. Enrollment of the participants

The study was performed with the consent of the Ethics Committee of the Medical University of Vienna (1004/2013). Participants were recruited by advertisement at public places in Vienna and informed consent was obtained from all participants before the begin of the intervention.

In total, 42 individuals were enrolled. One did not complete the study due to personal reasons. The remaining 41 individuals (n = 20 in the 0.1 W/kg exposure group, n = 21 in the 1.6 W/kg group) had an average age of 29 ± 10 years, 21 were males and 20 females, 6 of them were left and 34 were right handed, 33 had a college education, 6 a

Table 1
Demographic characteristics of the participants.

Parameters			Group	
			0.1 W/kg (n = 20)	1.6 W/kg (n = 21)
Age (years)		Mean ± SD [range]	29 ± 11 [22–56]	29 ± 10 [22–56]
Gender	males	n (%)	9 (45%)	12 (57%)
	females	n (%)	11 (55%)	9 (43%)
Education	technical school	n (%)	0 (0%)	2 (10%)
	college	n (%)	15 (75%)	18 (86%)
	university	n (%)	5 (25%)	1 (5%)
Profession	student	n (%)	15 (75%)	19 (90%)
	employee	n (%)	5 (25%)	2 (10%)
BMI (kg/m^2)		Mean ± SD [range]	21.9 ± 2.9 [18.1–28.4]	21.8 ± 2.5 [18.3–26.3]
non-smoker		n (%)	17 (85%)	16 (76%)
Smoker		n (%)	3 (15%)	5 (24%)

university degree and two had graduated from technical schools. All of them were healthy, consumed a mixed diet and drank no or only moderate amounts of alcohol (max. 3 glasses of wine (150 ml/glass) or 4 glasses of beer (330 ml/glass) per week). The demographic characteristics of the two study groups are shown in Table 1. The participants were asked to use hands-free sets three weeks before and during the intervention and three weeks after the last treatment. If they did not possessthem, they were provided with such a device suitable for their mobile phone.

2.2. Study plan and exposure

Twenty-one participants were allocated randomly to the low exposure group (one did not complete the study and was excluded from the evaluation); nine individuals in this group were exposed to radiation on the left side and 11 on the right side. The exposure in the first group was 0.1 W/kg in terms of spatial average SAR inside the buccal mucosa ($SAR_{avg,\ mucosa}$) in the region of interest from where the buccal cells were collected. Twenty-one participants (group two) were placed in the high exposure group ($SAR_{avg,\ mucosa}$ 1.6 W/kg), 10 were exposed on the left side and 11 were exposed on the right side. The value of 1.6 W/kg for $SAR_{avg,\ mucosa}$ in the high exposure group was chosen to achieve a maximal possible SAR level inside the mucosa, at the same time ensuring that the exposure limit for the general public, i.e. 2 W/kg in terms of peak spatial 10 g-average SAR ($psSAR_{10g}$, averaged over all tissues in the exposed region) was not exceeded for ethical reasons. Starting from 1.6 W/kg for $SAR_{avg,\ mucosa}$ for the high exposure group, a level of 0.1 W/kg used for the low exposure group ensured sufficient contrast between high and low exposure when taking into account the expected variability of SAR due to individual differences between participants and unavoidable uncertainties of the exposure system (small variations of orientation and distance between antennas and cheek surface) and the applied dosimetric methodology (for details, see section "2.3 Exposure System").

All participants filled in a diary for 47 days beginning three weeks before the start of intervention until three weeks after the last exposure. They were asked to use only hands-free sets during this time period and were provided with these devices when needed. The use of mobile phones and potential confounding factors (gingival bleeding, consumption of spicy meals, visits at dentists) were recorded in the diary. Furthermore, also the duration of in- and outgoing calls was recorded as well as the use of hands-free equipment.

The design of the intervention study is depicted in Fig. 1; buccal cells were collected immediately before the start of the intervention and 3 weeks after the last day of the intervention (exposure to HF-EMF with the headsets). This schedule was chosen on the basis of earlier

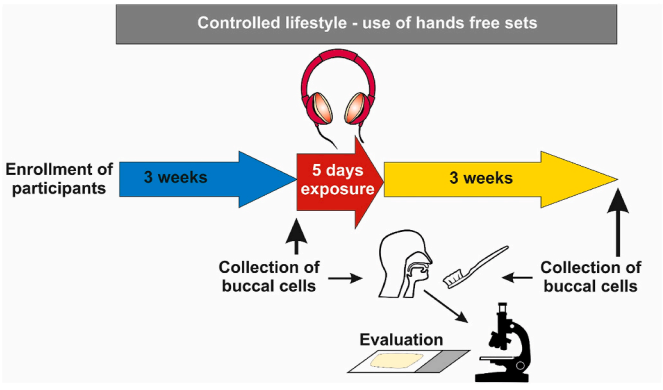


Fig. 1. Schematic illustration of the study design. The participants of the study were asked to use hand free sets three weeks before, during and three weeks after the intervention. Cells were collected before exposure to radiation (with headphones), and three weeks after the end of the exposure period.

experiments indicating that MN rates reach maximal values 27 days after exposure (Kassie et al., 2001). After an acclimatization phase (20 min) and positioning of the antennas, the exposure session was started (via the user interface of the control computer enabling double blind application of the exposure condition).

MN can be only seen when the damaged indicator cells have undergone one cell division after the exposure (Fenech, 2019). Therefore, the scoring time depends on the mitotic activity of the cells differing strongly in epithelial tissues from various organs (Nersesyan et al., 2022). The turnover rate in buccal cells is in the range between 14 and 21 days (Brizuela and Winters, 2024). We sampled the cells in the present study 3 weeks after the last exposure as acute cytotoxic effects may slow down their mitotic activity. Several investigations concerning the fate of MN have been realized. It was found that they are either degraded, incorporated into the main nucleus or lost as a consequence of cell death (Hintzsche et al., 2017; Stopper and Hintzsche, 2019). However, the results of many *in vitro* studies with cultivated cells and live cell imaging showed that the loss of MN is a slow process and that these structures persist in the cells till the next mitosis takes place (Hintzsche et al., 2017; Stopper and Hintzsche, 2019). Therefore, earlier MN formation (e.g. 2 weeks after the exposure) would have been detected with the present study design. In this context it is notable that we found in an earlier small intervention trial with khat chewers that the MN rates in buccal cells reached maximal values after 27 days (Kassie et al., 2001).

Exposure started on Monday and continued daily until Friday for 2 h per day. The participants were exposed to a HF-EMF (1950 MHz, UMTS modulation) under controlled conditions by use of a headset (Fig. 2a). The duration of the exposure period we used (2 h) reflects the time spent using mobile phones per day in most Middle European countries (Howart, 2024). During the exposure, participants performed standardized tasks inside a shielded chamber. Double blind exposure of the right and left cheek was accomplished by use of a random generator before the start of the experiments. After entry of the names of the subjects via the user interface of the control software, the exposure was automatically assigned to the pre-defined side.

2.3. Exposure system

For the exposure a generic radio frequency signal according to the Universal Mobile Telecommunication System (UMTS) standard with a center frequency of 1950 MHz and a signal bandwidth of 5 MHz was used. The time course of the signal was defined in order to reflect realistic worst case temporal fluctuations of the transmit power of UMTS MPs (Schuderer 2005). After generation, this signal was amplified (power amplifier BLMA 1921–50, Bonn Elektronik, Germany) and fed via a directional power sensor (NRT-Z44, Rohde & Schwarz, Germany) and a switch to either the antenna close to the left cheek or the antenna close to the right cheek of the participant. The antennas (specially designed patch antennas, with a 95 × 55 mm^2 backplane and a 70 × 36.5 mm^2 patch) were held in place in the cheek region by a special headset worn by the participant, which allowed free movement of the head (Fig. 2a). Exposure of the participants took place in a cabin with walls covered by radio frequency absorbing material in order to minimize reflections and unwanted propagation paths. The signal amplitude and the switch were computer-controlled by a corresponding control and recording software enabling a double blinded application of the four different exposure conditions (low exposure left cheek, high exposure left cheek, low exposure right cheek, high exposure right cheek). As explained in the last section, target exposure levels for high and low exposure group of 0.1 W/kg and 1.6 W/kg for $SAR_{avg,\ mucosa}$ were applied, respectively. All relevant exposure data (forward and reverse power) were measured and recorded in 10 s intervals and stored with a timestamp in encrypted form on a control computer. The developed antenna models had been validated by SAR-measurements in a homogeneous phantom filled with tissue simulating liquid and corresponding numerical computations using the Simulation platform SEMCAD X

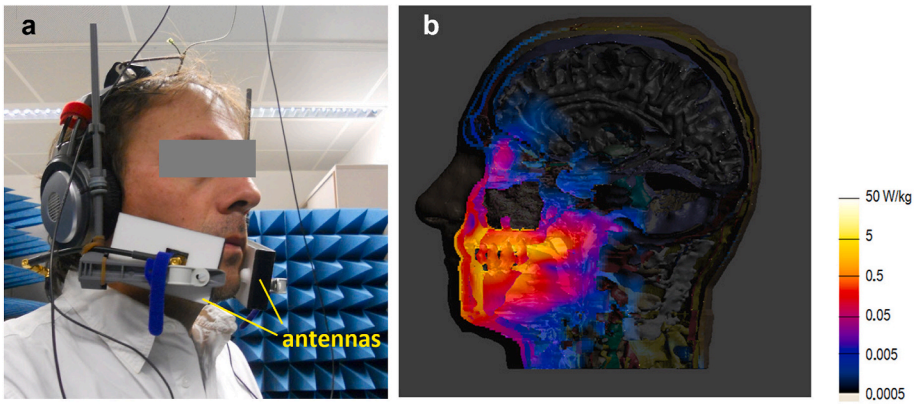


Fig. 2. Photographic image of a headset (2A) and spatial distribution of (unaveraged) SAR in the mucosa at the side of the active antenna (2B).

(Version 14.4, Schmid & Partner Engineering AG, Zurich, Switzerland). A detailed dosimetric analysis of radio frequency absorption inside the oral mucosa was carried out using generic planar layered tissue models as well as three different anatomical body models (Fats, Duke, Louis) from the Virtual Population (IT'IS Foundation, https://itis.swiss/virtual-population/virtual-population). Generic planar tissue models were used for a detailed variation analysis of absorption due to different individual tissue layering in the cheek region, considering the following tissue layers (and thicknesses): skin (1.5 mm ± 0.5 mm), fat (6.0 mm ± 3.0 mm), muscle (5.0 mm ± 3.0 mm), buccal mucosa (50.0 μm ± 30.0 μm). The anatomical models allowed for an analysis of spatial distribution of absorption inside the buccal mucosa taking into account the realistic non-planar geometry of the oral cavity (Fig. 2b). Moreover, expected variability of SAR due to unavoidable uncertainties of the exposure system (small variations of orientation and distance between antennas and cheek surface) and the applied dosimetry methodology were analyzed. The analysis revealed that an antenna input power of 90 mW lead to a $SAR_{avg,\ mucosa}$ of 1.6 W/kg used for the high exposure group. Hence, an antenna input power of 6 mW (leading to a $SAR_{avg,\ mucosa}$ of 0.1 W/kg) was considered reasonable for the low exposure group, ensuring sufficient exposure contrast between high and low exposure groups. The minimal exposure contrast in terms of $SAR_{avg,\ mucosa}$ between ipsilateral and contralateral mucosa (with respect to the active antenna) was at least 19.2 dB (factor 83).

2.4. Collection of the cells and evaluation of the samples

Participants were asked to rinse their mouth with water before the sampling. Cells were collected from both cheeks with soft tooth brushes immediately before the start of the exposure and after 3 weeks. Subsequently, the material was placed into 50 ml tubes with 20 ml buccal cell buffer (Tris–HCl, EDTA and sodium chloride in distilled water). The brushes were placed into the tubes and rotated to dislodge the cells and release them into the suspensions. Tubes were centrifuged at 581×*g* at room temperature for 10 min. Subsequently, new buffer was added to the pellets; after vortexing, the suspensions were centrifuged for 10 min. This procedure was repeated twice. Cells were spread on slides with a cytospin (Shandon Cytocentrifuge, Thermo Electron Corporation, UK), air-dried in the dark, fixed with chilled (−20 C°) 80% methanol and stained with Schiff's reagent (Sigma–Aldrich) for 90 min. Subsequently, they were washed with running tap water for 5 min and then counterstained with 0.2% (w/v) Fast Green (Sigma–Aldrich) for 30 s. All slides were coded and scored under 400-fold magnification (bright light). MN were verified under 1000-fold magnification with fluorescence (Nikon Photophot-FXA, Tokyo, Japan). All chemicals were purchased from Carl Roth (Karlsruhe, Germany), except otherwise mentioned.

Cytome MN assays were performed as described by Thomas et al. (2009). The frequencies of different types of nuclear anomalies were scored in 2000 buccal cells per sample according to the criteria defined by Bolognesi et al. (2013). Apart from MN, which reflect chromosomal breakage and loss, also nuclear buds (NB) indicating gene amplifications and binucleated cells (BN) were recorded; this type of anomalies reflects disturbed mitosis (Thomas et al., 2009). Furthermore, anomalies, which are indicative for cytotoxic effects (karyolytic (KL), karyorrhectic (KR) and pyknotic (P) cells), as well as cells with condensed chromatin (CC) were evaluated. Additionally, the numbers of basal cells (BC) were scored, they provide information about the mitotic activity of the basal membrane (Thomas et al., 2009). A schematic illustration of the different endpoints can be found in Supplementary Fig. 1.

2.5. Statistical methods

Nuclear anomalies were statistically evaluated by generalized estimating equation models with Poisson distribution and a log link and unstructured covariance matrix. Potential over-dispersion and zero inflation were tested by chi-square tests. Experimental factors were group (low/high exposure) as between subjects factor and time (baseline/3 weeks) and exposed vs. contralateral side as within subject factors. Age, gender, smoking and minutes of private mobile phone use without headset during three weeks before collection of cells were included as covariates. All analyses were performed using Stata 17.0 (StataCorp, College Station, TX) and figures were made using Statistica 10.0 (StatSoft, Tulsa, OK). The level of significance was set to 5%. For illustrative purposes, levels of significance were also reported as <0.01 if applicable.

3. Results

The results of the evaluation of the questionnaires are summarized in Supplementary Table 2. It is evident that the time the participants phoned without hands-free sets was minimal. Consumption of spicy meals was recorded only by two individuals (one consumption during the entire period), furthermore, gingival bleeding was extremely rare (it happened two times during the entire study period).

The results of the cytome MN assays are summarized in Figs. 3 and 4. Fig. 3 depicts the results of comparisons of the number of nuclear anomalies before and 3 weeks after the intervention. Numbers indicate the alterations of the frequencies that were seen after exposure to low (0.1 W/kg) and high (1.6 W/kg) doses of the UMTS signal. The number of KR was significantly increased after exposure to the higher dose (by 57%). Furthermore, we observed also clear increase of BN cells by 28%.

Comparisons of the differences between the exposed (ipsilateral) and unexposed (contralateral) cheeks are shown in Fig. 4. We observed a clear difference in the frequencies of KR cells and of cells with CC in material that was collected from the exposed sides. Also the numbers of BN cells was higher in the cheeks that were directly exposed (however

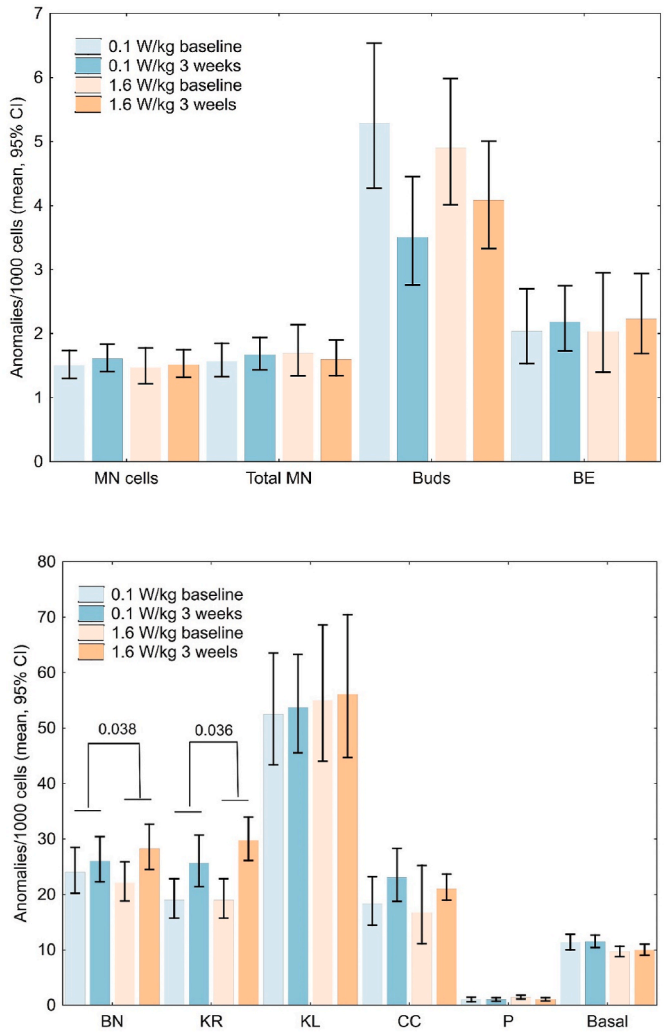


Fig. 3. Comparisons of the number of nuclear anomalies before and after the radiation (MN cells, number of micronucleated cells , total MN- total number of micronuclei , Buds –nuclear buds, BE – broken eggs, BN – binucleated cells, KR – karyorrhectic cells, KL – karyolitic cells, CC – cells with condensed chromatin, P – pyknotic cells, Basal – basal cells, for schematic images of the morphology see Supplementary Fig. 1). The Y-axes indicate the numbers per 1000 cells.

this effect did not reach significance).

In this context it is notable that several earlier investigations showed that condensation of the chromatin plays a key role in different form of cell death (apoptosis and necrosis) (Bianchi and Manfredi, 2004; Charriaut-Marlangue et al., 1996; Rose et al., 2022; Ziegler and Groscurth, 2004). Therefore, this observation supports the assumption of induction of acute cytotoxicity, which is reflected by formation of KR cells.

4. Discussion

In order to control the exposure to HF-EMF, to minimize confounding factors encountered in questionnaire-based investigations and to provide reliable results, we realized the first controlled intervention trial concerning the impact of HF-EMF on buccal cells, with defined duration and exposure conditions under laboratory conditions. This experimental setup reduces the inter-individual variations, which were encountered in earlier cross-sectional studies. Genotoxic as well as acute cytotoxic effects were monitored immediately before and three weeks after exposure separately in cells from both cheeks. We scored all nuclear anomalies that are recommended in the international guideline published by Thomas et al. (2009) by use of a DNA specific stain (Feulgen-Fast Green) to avoid misinterpretation of keratin bodies as MN (Nersesyan et al., 2006). A special headset was developed for this trial and allowed us to expose the participants to defined HF-EMF intensities in a double-blind fashion. Their pre-exposure (due to regular use of mobile phones) was taken into consideration by use of questionnaires to control additional exposures due to possible calls. All participants were asked to use hands-free devices when they made or received phone calls three weeks before the start of the experiments, during the exposure phase and three weeks after the last radiation.

The results of the present study indicate that exposure to the UMTS signal causes acute cytotoxic effects, namely KR. Furthermore, we observed also a significant increase of BN cells, which is indicative for disturbed cytokinesis. As shown in Supplementary Fig. 1, these anomalies are formed sequentially. Anomalies other than MN were not evaluated in the majority of earlier studies with buccal cells (see Supplementary Table 1). An increase of several different nuclear anomalies (KL, P, CC, “broken egg”, NB) was reported in three studies from India (Gandhi, 2015; Shaikh et al., 2016; Srujana Aravinda et al., 2022) while no such effects were found in other trials (Daroit et al., 2015; Ros-Llor et al., 2012; Yadav and Sharma, 2008). Elevated BN frequencies, which were observed in the present investigation, were detected only in one study (Yadav and Sharma, 2008) (4-fold increase, aceto-orcein stain).

Numerous *in vitro*, animal and human studies have been published concerning the induction of genetic damage by mobile phone specific HF-EMF. The results are controversial and depend strongly on the cell type and on the intensity and frequencies (Franzellitti et al., 2010; Lai et al., 2021; Misik et al., 2023; Tiwari et al., 2015). An important finding in the review by Lai et al. (2021) was that of 361 studies on genetic effects of RF-EMF, the majority (66%) reported positive evidence of genotoxic effects.

No evidence was observed in the present investigation in regard to induction of MN and NB, which are indicative for genetic instability and chromosomal aberrations. As mentioned in the introduction, MN formation in buccal cells as a consequence of exposure to mobile phone specific EMF was investigated in a number of earlier studies (results are summarized in Supplementary Table 1). All previous investigations were performed by use of questionnaires, which do not allow a precise assessment of the exposure. The present study is the first intervention trial in which the exposure was strictly controlled. In some earlier investigations, attempts were made to define the individual exposure by recording the SAR values of the cell phones (Gandhi et al., 2014; Gandhi and Singh, 2005; Revanth et al., 2021) but this approach was criticized as users may change their phones in short time intervals and the SAR values obtained during compliance tests before marketing do not reflect the exposure precisely. It depends on several conditions including distance to the base-station and position of the phone with respect to the head (Vijayalaxmi et al., 2007).

It is interesting that most investigations using DNA unspecific stains reported higher MN rates in exposed groups (see Supplementary Table 1). These results are probably due to the use of inadequate stains. In studies with DNA specific stains mainly negative findings were obtained. It was postulated that DNA-unspecific stains can cause misleading results as keratohyalin bodies (granules) formed as a consequence of keratinization of the epithelium may be misinterpreted as MN (Nersesyan et al., 2006). Keratinization is induced as a consequence of chemical and mechanical cell damage (Rice et al., 1989). The increase of the rates of cells with KR and CC, which we found in the present study, indicates that HF-EMF exposure causes acute cytotoxic effects in the buccal mucosa, which may lead to formation of these structures.

Taken together, the results of the present study indicate that exposure of buccal cells to HF-EMF does not cause acute chromosomal damage but causes formation of nuclear anomalies, which are indicative for acute cytotoxic effects (KR, CC) and disturbed cytokinesis (BN cells). Numerous earlier studies investigated the impact of mobile phone

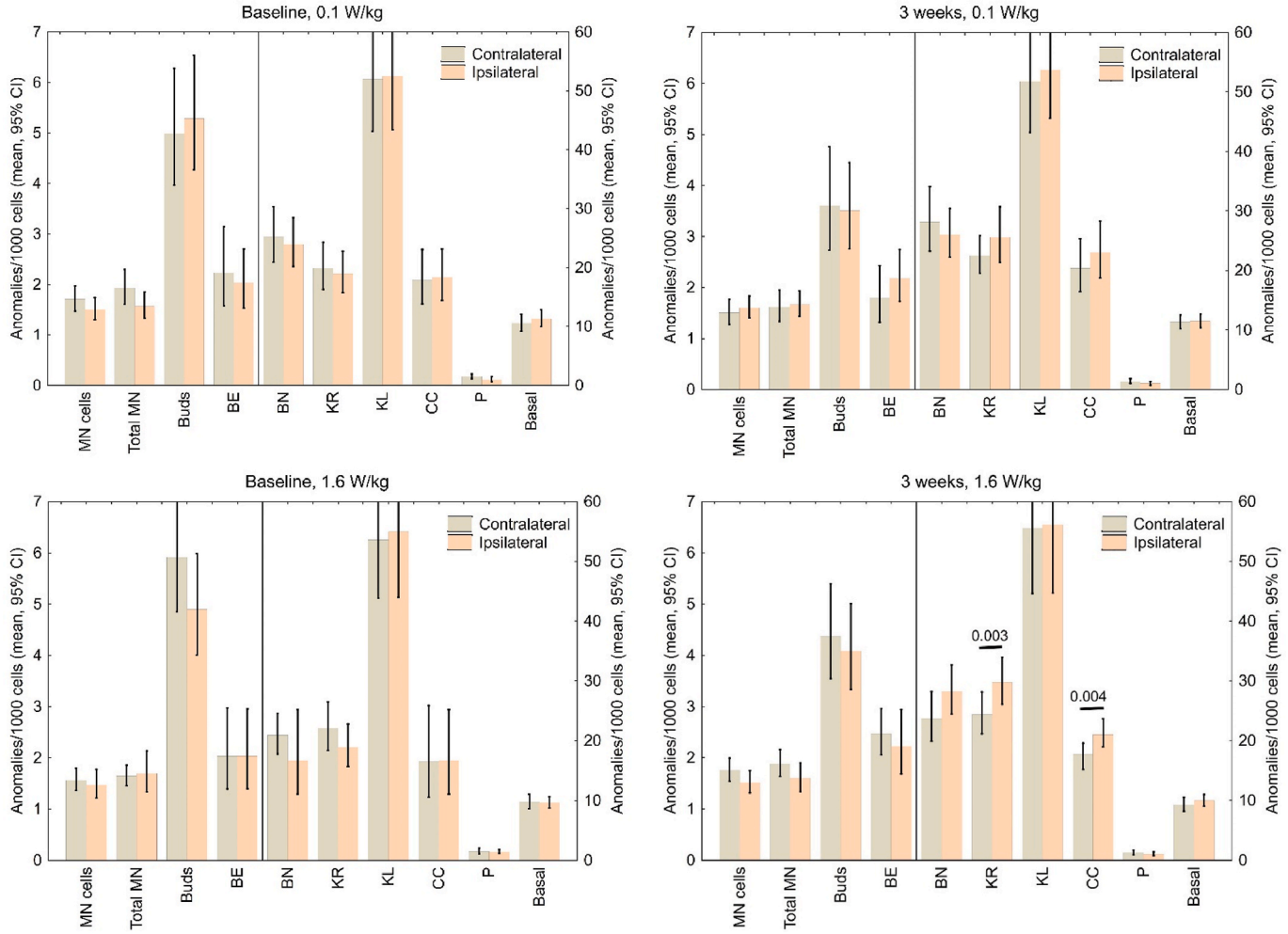


Fig. 4. Impact of low and high dose radiation with 1950 MHz (UMTS modulated) for 5 days (2 h/day) on the numbers of different nuclear anomalies in buccal cells recorded before and after radiation on the exposed and less exposed cheeks (ipsi- and contralateral exposure). Abbreviations see legend of Fig. 3. The Y-axis indicates the numbers per 1000 cells.

specific radiation on the transcription of genes and on proteins, which are involved in damage of the genetic material and cell death (Gaestel, 2010; Jagetia, 2022; Nylund and Leszczynski, 2006; Sekijima et al., 2010; Vanderstraeten and Verschaeve, 2008). It was found in some studies that the radiation causes changes of the transcription and methylation of genes involved in DNA repair (Leszczynski et al., 2012). In this context it is notable that we found earlier transient induction of DNA damage (comet formation) in glioblastoma cells but no evidence for formation of MN after exposure to the UMTS signal, which was also used in the present experiments (Al-Serori et al., 2017, 2018). The formation of comets reflects single- and double-strand breaks of DNA as well as apurinic sites (Collins et al., 2008). This discrepancy could be explained by induction of DNA repair processes (BER and NER), which eliminate primary DNA damage (Al-Serori et al., 2018). This observation is supported by a results of a proteome analysis indicating that several proteins involved in NER are up-regulated after exposure to the UMTS signal (Al-Serori et al., 2018).

As described above several human biomonitoring studies with buccal cells found evidence for acute cytotoxic effects and for disturbed cell division. The observation for increased numbers of BN cells is indicative for incomplete cytokinesis (Thomas et al., 2009). In this context it is notable that earlier studies with cultivated cells showed that HF-EMF has an impact on components of the cytoskeleton, e.g. on actin stress fibers (Leszczynski et al., 2004; Nylund and Leszczynski, 2004). Furthermore, it was found that p38 *MAPK* kinase is activated in human cells by mobile phone specific radiation (Han et al., 2020). p38 *MAPK* is known to fulfil checkpoint functions during mitotic entry and proper spindle formation and regulation of the timely dynamics of kinetochore microtubule formation (Lee et al., 2010). KR (chromatin fragmentation), which was increased in the present study in cells of exposed cheeks, is associated with two forms of cell death, i.e. apoptosis and necrosis (Elmore, 2007). Both forms of cell death are preceded by condensation of chromatin. Notably in the present study we found also an increase of CC. Several earlier studies reported an increase of apoptosis and pro-apoptotic proteins as a consequence of HF-EMF irradiation of cells (Cig and Naziroglu, 2015; Tohidi et al., 2021). It is also notable that p38 *MAPK* plays not only a key role in cytokinesis but also in the regulation of apoptosis and necrosis (Maslov et al., 2022; Yue and Lopez, 2020). Mitotic disturbance indicated by formation of BN leads to karyorrhectic effects and also to necrosis and apoptosis (Rello-Varona et al., 2010).

One of the limitations of the present study is that the daily exposure period was restricted to 2 h. This period reflects the daily use of mobile phones in most Middle European countries, much longer periods (4–5 h) are common in South East Asian and South American countries (Howart, 2024) and it cannot be excluded that these long exposure periods may cause more pronounced effects and genetic damage. The participants of the present study were not willing to extend the use of headphones to such long time intervals.

The rationale for the selection of the sampling time is explained in section Materials and methods. It was chosen on the basis of the

available data concerning the mitotic activity of the buccal mucosa. The time kinetics of formation of other nuclear anomalies additionally evaluated in our study is not known at present. Therefore, it cannot be excluded that also other anomalies reflecting cytotoxic effects are induced by the UMTS signal at earlier or later time points. Indeed, some studies with cross-sectional design found evidence of such effects (see Supplementary Table 1). However, our present results show clearly that the number of KR cells is increased after the exposure. This effect was dose-dependent, i.e. it was more pronounced in the exposed checks and was only seen with the higher dose. Further investigations will clarify if other anomalies are induced at earlier or later time points and can elucidate the underlying molecular mechanisms.

As mentioned in the introduction, evidence is accumulating that exposure to HF-EMF is associated with specific brain tumors (Brabant et al., 2023; Carlberg et al., 2017; Coureau et al., 2014; Hardell and Carlberg, 2015; Hardell et al., 2013; IARC, 2013; INTERPHONE Study Group, 2010). The results of the present investigation indicate that molecular mechanisms other than chromosomal damage may cause neoplastic transformation of the cells as a consequence of exposure to mobile phone specific HF-EMF. As described in the result section, we found in the present study clear evidence for induction of acute toxicity and disturbance of the cell cycle (cytokinesis) as a consequence of exposure to a high radiation dose (1.6 W/kg). It is possible that these effects cause inflammatory responses and/or release of ROS, which were seen in a number of laboratory studies (e.g. Alipour et al., 2022; Benavides et al., 2023; IARC, 2013; Yakymenko et al., 2016). These processes may possibly lead to formation of neoplastic cells.

CRediT authorship contribution statement

Michael Kundi: Writing – review & editing, Writing – original draft, Validation, Supervision, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Armen Nersesyan:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Formal analysis. **Gernot Schmid:** Resources, Methodology, Investigation. **Hans-Peter Hutter:** Supervision, Project administration, Investigation, Funding acquisition, Data curation, Conceptualization. **Florian Eibensteiner:** Writing – review & editing, Writing – original draft. **Miroslav Mišík:** Writing – review & editing, Writing – original draft, Visualization. **Siegfried Knasmüller:** Writing – review & editing, Writing – original draft, Validation, Supervision, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: S. Knasmueller reports financial support was provided by Allgemeine Unfallversicherungsanstalt (AUVA) Austria. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The authors do not have permission to share data.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2024.118634.

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