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Impact of mobile phone-specific electromagnetic fields on DNA damage caused by occupationally relevant exposures: results of *ex vivo* experiments with peripheral blood mononuclear cells from different demographic groups

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Abstract

The aim of this study was to investigate if age and body mass of humans have an impact on the DNA-damaging properties of high-frequency mobile phone-specific electromagnetic fields (HF-EMF, 1950 MHz, universal mobile telecommunications system, UMTS signal) and if this form of radiation has an impact on the genotoxic effects of occupationally relevant exposures. Pooled peripheral blood mononuclear cells (PBMC) from three groups [young normal weight, young obese (YO), and older age normal weight individuals] were exposed to different doses of HF-EMF (0.25, 0.5, and 1.0 W/kg specific absorption rate—SAR) and simultaneously or sequentially to different chemicals which cause DNA damage (CrO_3, $NiCl_2$, benzo[a] pyrene diol epoxide—BPDE, and 4-nitroquinoline 1-oxide—4NQO) via different molecular mechanisms. We found no difference in regard to the background values in the three groups but a significant increase of DNA damage (81% without and 36% with serum) in cells from old participants after radiation with 1.0 W/kg SAR 16 h. In combined treatment experiments we found no impact of the UMTS signal on chemically induced DNA damage in the different groups in general. However, a moderate decrease of DNA damage was seen in simultaneous treatment experiments with BPDE and 1.0 W/kg SAR in the YO group (decline 18%). Taken together our findings indicate that HF-EMF cause DNA damage in PBMC from older subjects (69.1 years). Furthermore, they show that the radiation does not increase induction of DNA damage by occupationally relevant chemicals.

Keywords: mobile phone radiation; DNA damage; comet assay; chemical co-exposure

Introduction

Results of epidemiological and animal studies indicate that exposure to mobile phone-specific electromagnetic fields (HF-EMF) may increase the risk of cancer, in particular in the nervous system [1–3]. It is known that damage of the genetic material plays a key role in the aetiology of human cancer [4]. Therefore, human genotoxicity studies were realized with mobile phone-specific HF-EMF, for reviews see [5–8]. The results of these studies are contradictory and it was emphasized that it is difficult to draw firm conclusions due to methodological limitations of the experimental designs [9]. It was repeatedly postulated that relevant mechanisms by which HF-EMF cause damage of the genetic material are formation of reactive oxygen species [6,10] and interference with DNA-repair systems [11–13].

In most previous investigations human lymphocytes, peripheral blood mononuclear cells (PBMC), and stable cell lines were exposed to different radiation doses, only few studies concerned the impact of HF-EMF on chemically induced DNA damage [1,6]. The aim of the present investigation was to find out if the universal mobile telecommunications system - UMTS signal (1950 MHz), simulating third-generation mobile phone exposure causes DNA damage in lymphocytes from groups differing in regard to age and body mass indices (BMI) as these demographic parameters may have an impact on DNA-repair functions [14,15] and on the redox status [16,17]. All experiments were conducted under conditions that are relevant for human mobile phone users, that is with specific absorption rate (SAR) values ≤1.0 W/kg. Furthermore, we investigated also the impact of HF-EMF on the DNA damaging effects of occupationally relevant chemicals in PBMC from groups that differ in age and BMI. In most previous studies concerning the combination effects of chemicals, pharmaceutical drugs (in particular cytostatics) were studied [18].

Indicator cells from three groups (young obese, YO; young normal weight, YN; and old normal weight individuals, ON) were exposed to HF-EMF and simultaneously or sequentially to the UV radiomimetic compound [19] 4-nitroquinoline 1-oxide (4NQO), to benzo(a)pyrene-7,8-diol 9,10-epoxide (BPDE), the most relevant metabolite of benzo(a)pyrene,

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which belongs to the group of polycyclic aromatic hydrocarbons [20], and to two metal compounds (nickel(II) chloride, $NiCl_2$, and chromium trioxide, CrO_3). These chemicals cause damage of the genetic material via different molecular mechanisms, including formation of thymidine dimers (4NQO) [19,21], formation of bulky DNA adducts (BPDE) [22], release of ROS (metals and 4NQO) [23,24], formation of DNA-DNA and DNA-protein crosslinks and interactions with DNA-repair systems ($NiCl_2$ and CrO_3) [23–25]. Details concerning occupationally relevant exposures, induction of DNA damage, and carcinogenic properties of the individual compounds can be found in Supplementary Table 1.

DNA damage was measured in single-cell gel electrophoresis (SCGE) assays, which are based on the quantification of DNA migration in an electric field and enable the detection of single and double-strand DNA breaks and apurinic sites [26,27]. This method is increasingly used in genetic toxicology [26]. The experiments were conducted in agreement with international guidelines [26–30]. To avoid false positive results due to acute toxic effects, the viability of the cells was monitored in all experimental series [26]. Furthermore, it is notable that all experiments were performed in agreement with the quality criteria defined for studies with HF-EMF [9], they included (i) blind collection of the data, (ii) adequate description of the dosimetry and (iii) inclusion of positive controls, and (iv) inclusion of sham-exposed negative controls.

Methods

Participants and blood sampling

The realization of the study was approved by the Ethics Committee of the Medical University of Vienna (MUW, EK Nr: 2350/2019) and informed consent was obtained from all participants. Visitors of shopping centres were invited to participate in the study by the scientists/students that were involved in the realization. Blood was collected by vein puncture in heparinized tubes (27 ml/participant). All participants were healthy Caucasians and consumed a mixed diet. They did not consume dietary supplements and were asked not to perform exhausting physical activities up to 3 days before the blood collection. The participants did not consume pharmaceuticals (except contraceptives) over a longer time period. Furthermore, we excluded individuals with occupations that may be related to DNA instability (e.g. miners, hair dressers, medical staff, petrol station attendants, workers exposed to paints, farmers that are exposed to agrochemicals, workers in plastic and chemical factories, flight attendants, car and battery repair workers). Three groups were established, that is YN, n = 7♀ and 5♂, YO, n = 11♂, and ON, n = 6♀ and 6♂. The characteristics of the different groups are specified in Table 1.

Chemicals and media

Organic and inorganic chemicals, including Roswell Park Memorial Institute (RPMI) medium, foetal calf serum (FCS), 4NQO, $NiCl_2$, CrO_3, propidium iodide, Triton X-100, Trizma base, and NaOH were provided by Sigma–Aldrich (Steinheim, Germany). Low melting point agarose (LMPA) was obtained from Gibco (Paisley, UK) and normal melting point agarose (NMPA) from Serva (Heidelberg, Germany). BPDE was provided by A. Seidl (Biochemisches Institut für Umwelt-Karzinogene, Gernot Grimmer Stiftung, Germany). 4NQO and BPDE were first dissolved in pure dimethyl sulfoxide (DMSO); subsequently, the stock solutions were diluted with fresh RPMI. The metal compounds were dissolved in aqueous medium (RPMI). All solutions were freshly prepared before each experiment.

Table 1. Demographic data of the participants.[1]

Group	Young normal weight (YN, n = 7♀ and 5♂)	Young obese (YO, n = 11♂)	Old normal weight (ON, n = 6♀ and 6♂)
Age	24.8 ± 3.1[a]	27.5 ± 2.7[a]	69.1 ± 7.6[b]
Weight	69.2 ± 10.9[a]	95.4 ± 7.7[b]	77.8 ± 13.8[a]
BMI	23.6 ± 2.9[a]	34.4 ± 4.9[b]	24.6 ± 1.9[a]
Smoking	0	3	2

1Mean ± S.D. Statistical comparison was performed by one–way ANOVA followed by Bonferroni's Multiple Comparison Test and data marked in different letters (a, b) are statistically significant ($p \leq 0.05$) if they do not share identical letters.

Design of the exposure system

The waveguide-based and computer-controlled exposure system was built and provided by the IT'IS Foundation (Foundation for Research on Information Technologies Society, Zurich, Switzerland, www.itis.ethz.ch). A detailed description of the experimental set-up and of the dosimetry can be found in Schuderer *et al.* [31]. The exposure unit consists of two rectangular waveguides operated at a frequency of 1950 MHz. Both waveguides were placed in a commercial incubator (HeraCell 240 CO_2 incubator, Kendro Laboratory, Germany), which provided the environmental conditions for the cell cultures (37°C ± 0.1°C, 5% CO_2, 95% humidity). A commercial broadband coax-to-waveguide coupler was used to excite the waveguides, each one containing up to six 35 mm ø Nunc Petri dishes (Nunc, Roskilde, Denmark). The detailed electrothermal analysis of the waveguide exposures included both, numerical estimation and experimental validation. The field level was controlled via monopole antennas in the waveguides and linked to the corresponding SAR and temperature values from the dosimetric assessment. The Petri dishes were placed in the H-field maxima of the standing wave within the waveguide, such that the highest SAR level occurred at the bottom layer of the dishes, where the monolayer cells are located. The dishes were filled with 3.0 ml medium. Exposure to the different intensities followed the same UMTS protocol at 5 min on/10 min off schedule. The cells were exposed to three different doses for 16 h, namely 0.25, 0.5, and 1.0 W/kg. The SAR were chosen on the basis of the exposure standards for localized exposure of the head (2 W/kg in 10 g contiguous tissue in Europe [32] and 1.6 W/kg in 1 g of tissue in the USA [33]).

A computer program was used for randomizing exposure of the cells to the UMTS signal (in order to ensure blinded exposure and evaluation) in all individual experimental series. Information concerning the exposure of the cultures was only available to an operator (located at the IT'IS Foundation in Zurich) and disclosed after completion of the experiments and evaluation of the cells.

Separation of PBMC and storage

PBMC were isolated by gradient centrifugation (800 *g*, 15 min, 16°C) with Histopaque (Sigma–Aldrich, Steinheim, Germany).

The pellets were suspended in 100 µl RPMI and aliquoted in Biofreeze Medium (Biochrom AG, Berlin, Germany), frozen overnight at –80°C, and stored in liquid nitrogen.

Dose-response experiments with individual chemicals

Before the main experiments, dose-response studies were conducted with the individual test compounds to define the optimal experimental conditions for the combined treatment experiments. Cells from YN individuals were treated with different concentrations of the chemicals in Petri dishes (35 mm ø Nunc, Roskilde, Denmark), 75 × 10^3 cells per ml in incubator (37°C, relative humidity 95%, 5% CO_2) in the dark for 16 h. Subsequently, they were collected, washed twice (250 *g*, 5 min) with medium, and re-suspended in 1.0 ml RPMI. Per experimental point, three cultures were made. The viability was determined with a CASY counter (TT2QA2589, OMNI Life Science GmbH, Bremen, Germany), three measurements per culture. Only cultures with a viability ≥70% were analysed for comet formation.

Experimental design of combination experiments

Deep frozen PBMC suspensions were thawed, washed with RPMI containing 10% FCS, and seeded in 3 ml medium (RPMI) in Petri dishes (35 mm ø Nunc, Roskilde, Denmark). Six dishes were exposed simultaneously in a waveguide chamber; an identical number of cultures was sham exposed (37°C, relative humidity 95%, CO_2 5%). Two study designs (Fig. 1) were used: in simultaneous exposure experiments, the cells were exposed in solutions of the respective chemicals to different HF-EMF doses (SAR 0.25, 0.5, and 1.0 W/kg) for 16 h. The exposure time was chosen on the basis of the results of earlier experiments with human-derived cells. Detailed results are described in a thesis of Al Serori [34], part of the findings was published [11,35], and it was shown that an exposure of 16 h caused alterations of biologicals effect that are causally related to DNA stability (apoptosis, DNA-repair functions) therefore we used this treatment period in the present study. In experiments with sequential design, the cells were first exposed to HF-EMF for 16 h, subsequently they were treated with the test chemicals (16 h). After exposure,

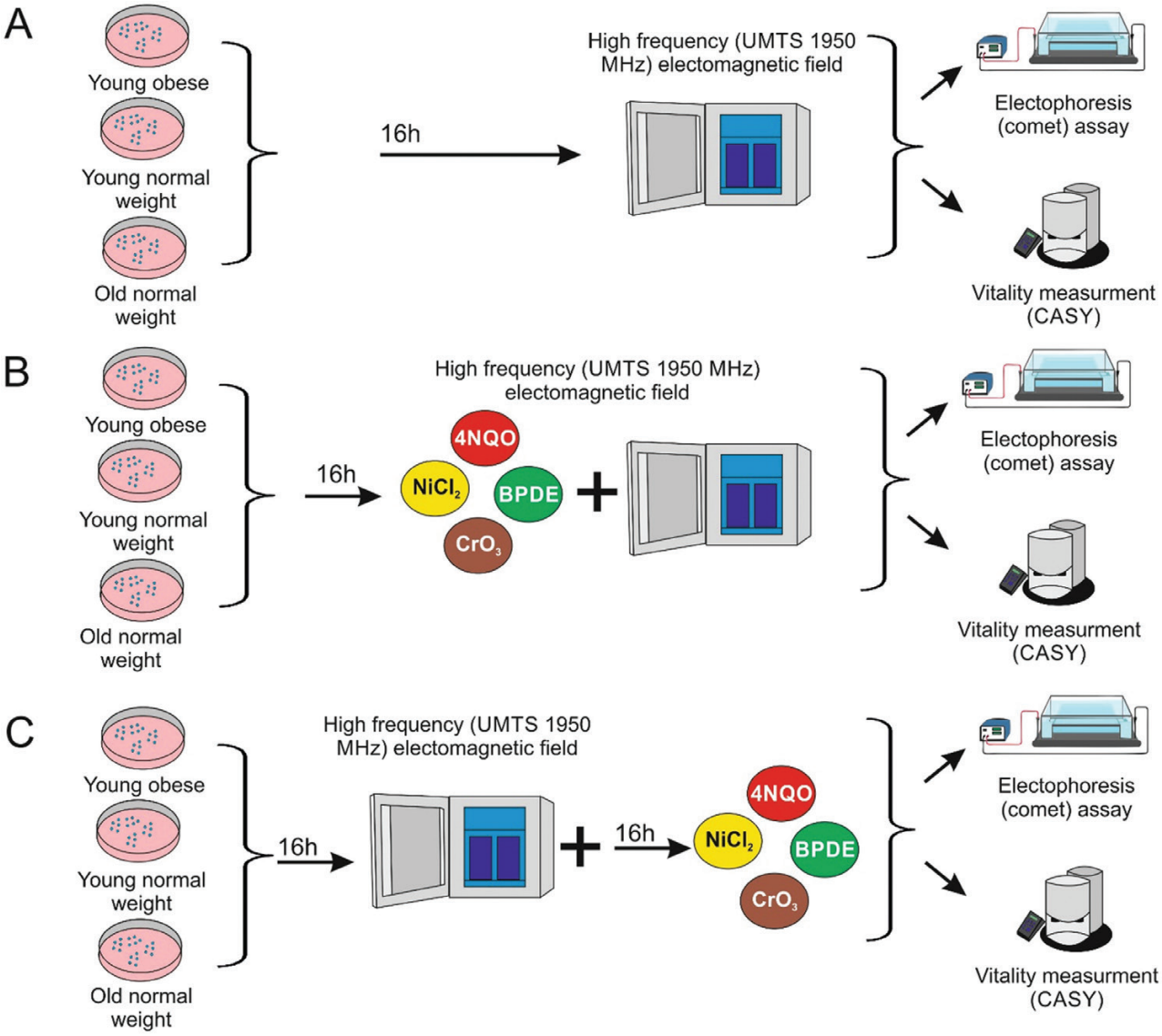

Figure 1. Experimental design of the study. (A) Exposure to HF-EMF only; (B) simultaneous exposure to HF-EMD and different chemicals (4NQO, BPDE, CrO_3, $NiCl_2$ for 16 h); and (C) sequential treatment to HF-EMF (16 h) and compounds exposure (4NQO, BPDE, CrO_3, $NiCl_2$ for 16 h).

the cells were collected, washed twice (with RPMI, 250 g, 5 min), re-suspended in 1.0 ml RPMI, and stored in a refrigerator (4°C) for up to 1 h. The temperature in each chamber of the sXc1950 exposure system was continuously monitored and recorded; to avoid fluctuations a ventilation system was integrated in the exposure apparatus. The fluctuations of the temperature were in all experiments <0.5°C. We know from earlier experiments these alterations of the temperature in the medium have no detectable impact on DNA migration on human lymphocytes and other cell types. For each experimental point at least two experiments were performed. In each experiment two cultures (sham and exposed conditions) were treated. The viability of the cells was monitored in each experiment with a CASY counter (TT2QA2589, OMNI Life Science GmbH, Bremen, Germany). Only cultures with a viability ≥70% were analysed for comet formation.

Single-cell gel electrophoresis assay

The SCGE experiments were conducted under standard alkaline conditions [26,27] which allow the detection of single and double-strand breaks and apurinic sites [27]. The cells were re-suspended in LMPA and spread on pre-coated agarose slides (1.5% NMPA). Electrophoresis was carried out under alkaline conditions (30 min, 300 mA, 0.8 V/cm, at 4°C, pH > 13) after lysis for ≥ 1 h (pH 10.0) and 30 min unwinding. Neutralization was performed twice with ddH_2O for 8 min, then the air-dried slides were stained with propidium iodide (20.0 µg/ml). The percentage of DNA in tail was measured by use of a computer-aided image analysis system (Comet IV, Perceptive Instruments Ltd., Haverhill, UK). From each culture, two slides were prepared and 50 randomly distributed cells were evaluated per slide. Positive controls were included and described in the legends for Fig. 4.

Statistics

The distribution of %age DNA in tail for each slide was analysed to determine possible multimodality that may occur due to the heterogeneity of the cell population. In all cases, either a single mode occurred and the median of the measurements virtually coincided with the mode, or a slight bi-modality was detected with a second mode at higher values with up to 15% of the cells measured. In the latter case the median was computed after leaving out this second group of cells. The medians of percent DNA in tail were arcsine transformed to remove correlations between mean and standard deviations and to make variances homogeneous. For all experiments the code number of the experiment was included as a random factor in the model. A general linear model was applied with group (ON, YO, and YN), SAR (0.25, 0.5, and 1 W/kg), and exposure (sham/exposed) as fixed factors. Comparisons between sham and exposed within group and SAR level were done by linear contrasts with sequential Sidak correction. For all experiments, normality of residuals was checked by Shapiro–Wilk tests and homogeneity of variances by Brown–Forsythe tests. Dose-response experiments were similarly evaluated, however, with dose as the only experimental factor and comparisons of each dose against the unexposed controls. Effect sizes have been computed: difference w/o serum Δ% = 81%, Cohen's d = 3.9, difference with serum: Δ% = 36%, Cohen's d = 1.3.

All analyses were performed with Stata 17 (StataCorp, College Station, TX, USA).

Results

Comet formation in sham-treated cells

Figure 2 depicts the extent of DNA damage in the three groups. No significant differences were seen between the ON,

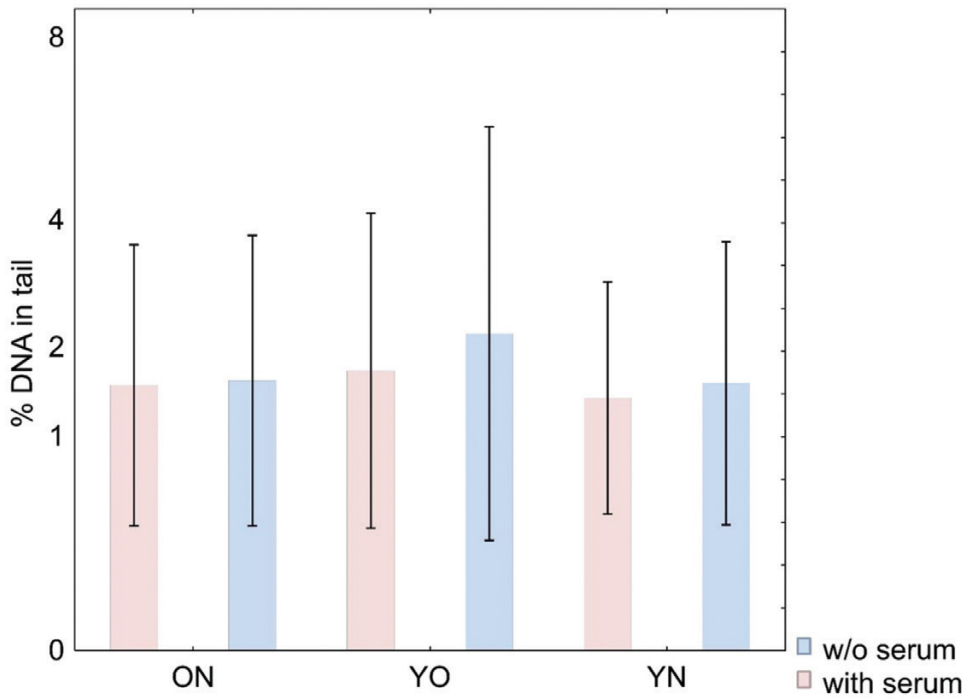

Figure 2. Comet formation in PBMC from young participants with normal body BMI (YN), young participants with high BMI (YO) and older participants with normal BMI (ON) without exposure to chemicals, in presence and absence of serum (exposure 16 h). Bars show means ± SD of results from eight individual experiments without and with serum, respectively. In each experimental series two cultures were treated, and 50 cells were evaluated per culture.

YO, and the YN groups, regardless when the cells were cultivated in presence or absence of serum.

Dose-response effect of model compounds

To define the optimal experimental conditions for the combined treatment experiments, PBMC from the YN group were treated with different concentrations of the model compounds. The results are shown in Fig. 3. The most pronounced effects were caused by 4NQO and BPDE followed by $NiCl_2$ and CrO_3. Experiments with higher Cr concentrations (≥90 µM) were not evaluated due to acute toxic effects (i.e. the viability of the cells was below 70%).

Impact of high-frequency radiation on DNA stability

Figure 4 shows the results of experiments in which PBMC from the different groups were exposed to different radiation doses. We detected a significant increase in experimental series with blood cells from ON donors after treatment of the cells with 1.0 W/kg. To find out if the effect is also seen when the cells are grown in presence of serum, additional experiments were conducted. Also under this condition a clear increase of DNA migration was observed after exposure of the cells to the highest dose (1.0 W/kg).

Impact of age and BMI on chemically induced DNA damage

Table 2 shows the results of experiments in which the cells were treated with different model compounds. We did not detect differences of the sensitivity towards 4NQO and metal compounds. In experiments with BPDE, we found a slight increase (non-significant) of the comet tail intensity in the ON group compared to the other groups (difference compared to YN 15% and 11% compared to the YO group, respectively).

Results of combined treatment experiments

Cells from the three groups were either treated simultaneously or sequentially with HF-EMF and the model chemicals (for details see Fig. 1). Simultaneous exposure did not cause an increase of chemically induced DNA damage in general; however, with BPDE and 1.0 W/kg a significant but moderate decrease (by 18%) was seen in the YO group (see Table 3).

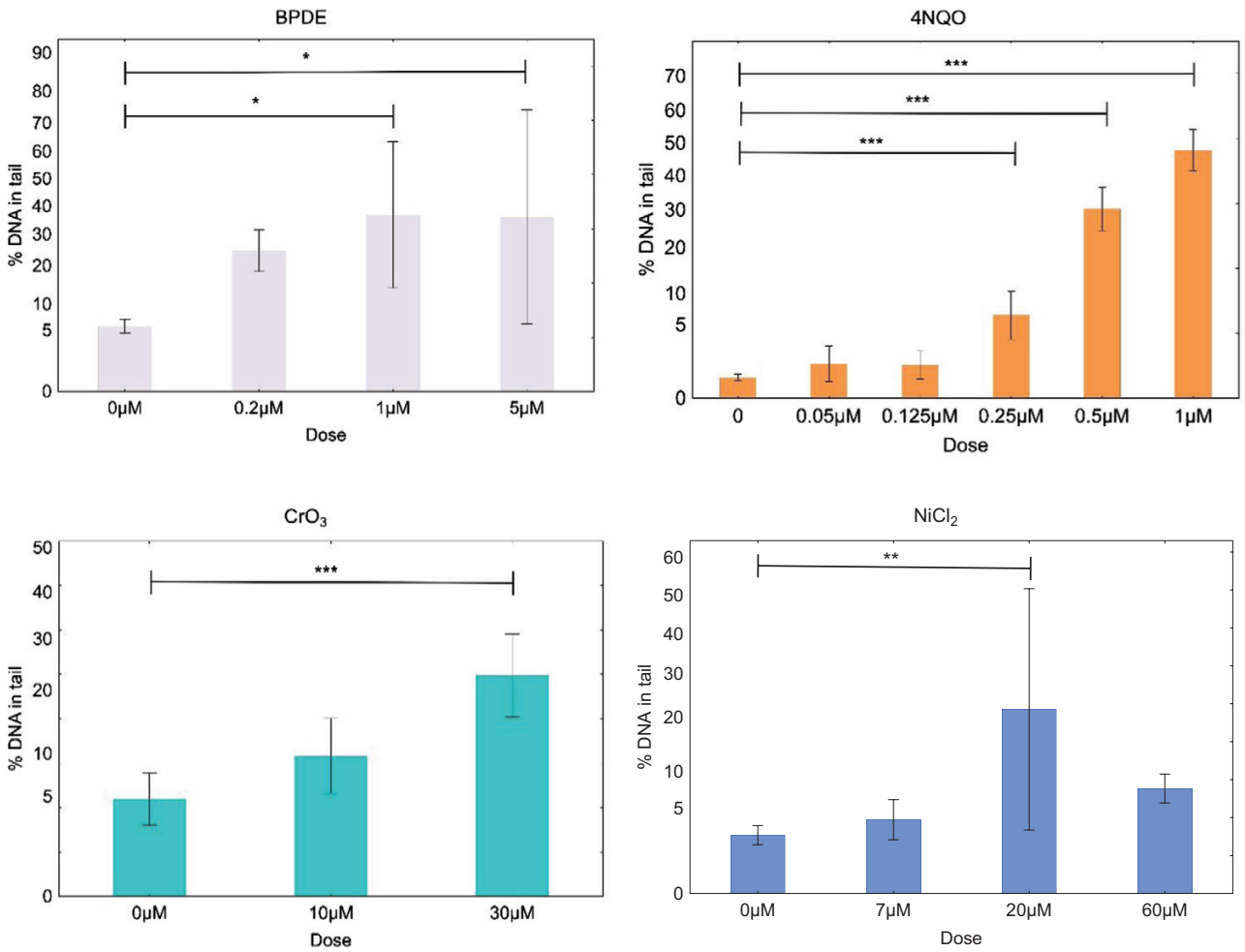

Figure 3. Induction of DNA damage by the different model compounds. PBMC from young participants with normal BMI (YN) were treated for 16 h with different concentrations of the chemicals. Per experimental point three cultures were made. From each culture two slides were prepared and 50 cells were evaluated per slide. Bars show means ± SD. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$.

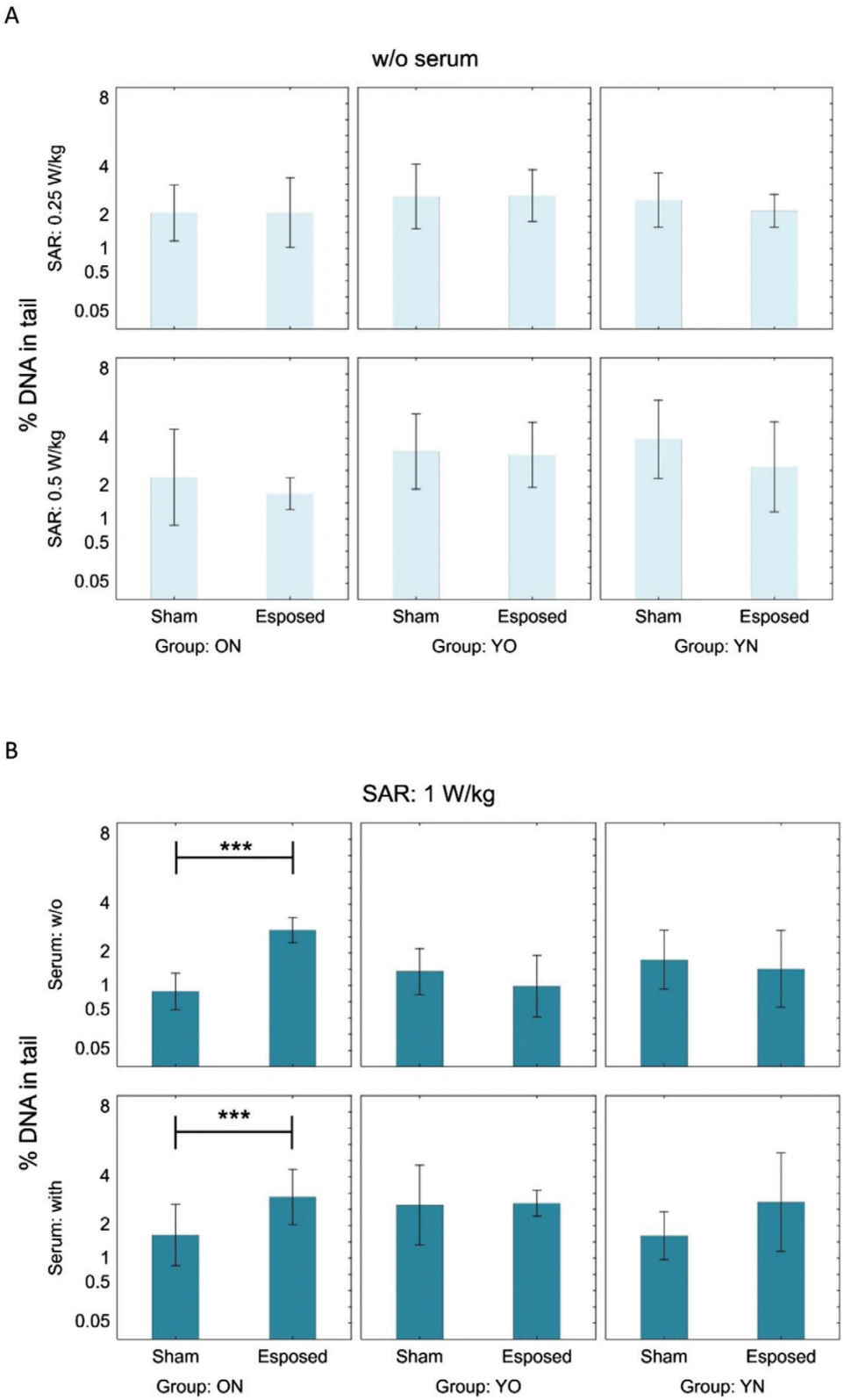

Figure 4. Impact of exposure to HF-EMF on DNA stability in peripheral PBMC from young participants with normal BMI (YN), young participants with high BMI (YO) and older participants with normal BMI (ON). The cells were treated with different HF-EMF doses (SAR 0.25 and 0.5 W/kg) in absence (A) and absence and in presence of serum (1 W/kg) for 16 h (B). Bars show means ± SD of results obtained in two independent experiments. Per experiment two cultures were made, three slides were prepared per culture and 50 cells were evaluated per slide. ***$p < 0.001$. H_2O_2 was used in all experiments as a positive control. Lymphocytes were exposed for 5 min to 50 µM, subsequently they were washed and the cells were processed as described in the Materials and Methods section. In all individual experiments clear positive results were obtained with a positive control (i.e. H_2O_2 induced comets with a size between 18.0 and 27.3% DNA in tail). These results are in agreement with findings of earlier studies [57].

Table 2. Induction of DNA damage by selected chemicals in the three demographic groups.[1]

	Group[2]		
Substance	**ON**	**YO**	**YN**
BPDE (2 µM)	64.0 (60.4–67.6)	57.1 (53.4–60.8)	53.6 (49.8–57.3)
4NQO (0.5 µM)	54.7 (50.9–58.4)	58.1 (54.3–61.8)	47.5 (43.8–51.3)
$NiCl_2$ (60 µM)	7.7 (5.8–9.8)	7.3 (5.5–9.4)	7.8 (5.9–9.9)
CrO_3 (30 µM)	14.2 (11.7–16.9)	16.5 (13.8–19.4)	16.5 (13.8–19.4)

1Numbers indicate means % DNA in tail (95% confidence intervals) found in PBMC after treatment for 16 h. They indicate results obtained in six independent experiments. From each experiment, three slides were prepared and 50 cells evaluated.
2Young normal weight participants (YN), young participants with high body weight (YO), and older normal weight participants (ON).

Table 3. Impact of chemicals and HF-EMF exposure (simultaneous treatment) on DNA damage in the three demographic groups.[1]

Exposure sequence	Substance	Exposure	Group[2]		
			ON	**YO**	**YN**
Simultaneous	BPDE (2 µM)	Control	3.5 (0.6–8.6)	11.6 (5.5–19.5)	6.8 (2.4–13.4)
		Sham	68.1 (62.0–73.9)	63.0 (56.7–69.0)	37.9 (31.8–44.1)
		0.25 W/kg	64.9 (58.7–70.9)	46.3 (40.0–52.6)	53.2 (46.8–59.5)
		Control	1.3 (0.0–5.0)	4.6 (1.1–10.3)	1.3 (0.0–5.0)
		Sham	59.7 (53.4–65.9)	59.7 (53.4–65.9)	62.2 (56.0–68.3)
		0.5 W/kg	54.3 (47.9–60.6)	63.9 (57.7–69.9)	62.7 (56.4–68.7)
		Control	2.4 (0.4–5.9)	0.8 (0.0–3.2)	1.5 (0.1–4.5)
		Sham	64.1 (57.9–70.1)	53.0 (46.6–59.3)	41.1 (34.9–47.4)
		1 W/kg	56.6 (50.2–62.8)	38.4 (32.3–44.7)*	47.9 (41.6–54.3)
	4NQO (0.5 µM)	Control	2.0 (0.1–6.2)	0.4 (0.2–2.9)	0.5 (0.2–3.2)
		Sham	60.2 (53.9–66.4)	61.3 (55.0–67.4)	62.6 (56.3–68.6)
		0.25 W/kg	57.1 (50.7–63.3)	59.1 (52.7–65.2)	56.8 (50.5–63.1)
		Control	4.9 (1.3–10.7)	7.8 (3.0–14.8)	3.4 (0.5–8.4)
		Sham	51.4 (45.1–57.8)	54.9 (48.6–61.2)	59.6 (53.3–65.8)
		0.5 W/kg	59.0 (52.6–65.1)	50.9 (44.5–57.2)	59.4 (53.1–65.6)
		Control	0.2 (0.2–1.8)	0.4 (0.1–2.4)	1.2 (0.0–4.8)
		Sham	52.4 (46.0–58.7)	58.9 (52.5–65.1)	63.3 (57.1–69.3)
		1 W/kg	48.8 (42.4–55.1)	57.0 (50.6–63.2)	66.5 (60.4–72.4)
	$NiCl_2$ (60 µM)	Control	0.5 (0.0–2.6)	0.5 (0.0–2.7)	0.5 (0.0–2.5)
		Sham	10.0 (6.5–14.1)	10.1 (6.6–14.3)	10.8 (7.1–15.0)
		0.25 W/kg	10.1 (6.6–14.3)	7.3 (4.4–11.0)	7.7 (4.6–11.4)
		Control	2.9 (0.6–6.6)	3.1 (0.8–7.0)	1.1 (0.0–3.7)
		Sham	7.3 (4.3–10.9)	7.4 (4.4–11.0)	8.8 (5.5–12.7)
		0.5 W/kg	9.1 (5.8–13.1)	8.4 (5.2–12.3)	10.7 (7.1–14.9)
		Control	0.6 (0.0–2.7)	0.5 (0.0–2.6)	1.4 (0.1–4.2)
		Sham	5.9 (3.3–9.3)	4.8 (2.5–7.9)	4.5 (2.2–7.5)
		1 W/kg	5.0 (2.6–8.1)	4.0 (1.9–6.8)	5.5 (2.9–8.7)
	CrO_3 (30 µM)	Control	1.9 (0.3–4.5)	1.9 (0.4–4.7)	1.5 (0.2–4.0)
		Sham	10.5 (6.9–14.7)	9.6 (6.2–13.7)	11.8 (8.0–16.3)
		0.25 W/kg	9.0 (5.7–13.0)	9.6 (6.2–13.7)	9.5 (6.1–13.5)
		Control	0.2 (0.2–1.8)	1.0 (0.0–3.5)	0.7 (0.0–3.0)
		Sham	13.9 (9.8–18.6)	18.9 (14.2–24.1)	20.6 (15.7–26.0)
		0.5 W/kg	14.9 (10.6–19.7)	18.0 (13.4–23.1)	19.4 (14.6–24.7)
		Control	3.4 (1.1–6.8)	3.5 (1.2–6.9)	3.2 (1.1–6.6)
		Sham	18.7 (14.0–23.9)	22.1 (17.0–27.6)	17.6 (13.1–22.8)
		1 W/kg	19.8 (15.0–25.1)	19.4 (14.6–24.7)	15.5 (11.1–20.3)

1Numbers indicate means % DNA in tail (95% confidence intervals) found in PBMC after simultaneous exposure to radiation and chemicals (for 16 h). For each experiment control cultures (neither exposed to HF-EMF not to substances), sham and real exposure conditions were evaluated. For each condition, two independent experiments were performed. From each experiment, three slides were prepared and 50 cells evaluated. *Statistically significant difference ($p \leq 0.05$) compared to corresponding sham values.
2Young normal weight participants (YN), young participants with high body weight (YO), and older normal weight participants (ON).

Table 4. Impact of chemicals and HF-EMF exposure (sequential treatment) on DNA damage in the three demographic groups.[1]

Exposure sequence	Substance	Exposure	Group[2]		
			ON	YO	YN
Sequential	BPDE (2 µM)	Control	1.8 (0.2–5.0)	1.8 (0.2–5.0)	1.5 (0.1–4.4)
		Sham	60.3 (54.0–66.4)	67.7 (61.6–73.5)	62.5 (56.3–68.6)
		0.25 W/kg	62.5 (56.3–68.6)	64.8 (58.6–70.8)	62.8 (56.5–68.8)
		Control	1.9 (0.2–5.2)	0.6 (0.0–2.9)	1.6 (0.1–4.7)
		Sham	68.3 (62.2–74.0)	64.6 (58.4–70.6)	65.0 (58.8–70.9)
		0.5 W/kg	68.0 (61.9–73.8)	67.7 (61.6–73.5)	69.6 (63.5–75.2)
		Control	5.0 (1.8–9.6)	7.8 (3.7–13.3)	1.6 (0.1–4.6)
		Sham	61.8 (55.6–67.9)	54.3 (47.9–60.6)	50.4 (44.1–56.8)
		1 W/kg	64.3 (58.1–70.3)	55.9 (49.5–62.1)	53.6 (47.3–59.9)
	4NQO (0.5 µM)	Control	1.8 (0.2–5.0)	1.8 (0.2–5.0)	1.5 (0.1–4.4)
		Sham	53.0 (46.0–59.9)	61.7 (55.9–67.4)	62.8 (56.6–68.9)
		0.25 W/kg	60.1 (53.7–66.2)	56.1 (49.8–62.4)	59.9 (53.6–66.1)
		Control	1.9 (0.2–5.2)	0.6 (0.0–2.9)	1.6 (0.1–4.7)
		Sham	67.8 (61.7–73.6)	65.2 (59.1–71.2)	65.1 (58.9–71.0)
		0.5 W/kg	64.0 (57.8–70.0)	63.5 (57.3–69.5)	67.2 (61.1–73.0)
		Control	5.0 (1.8–9.6)	7.8 (3.7–13.3)	1.6 (0.1–4.6)
		Sham	62.0 (55.7–68.0)	70.3 (64.4–76.0)	57.4 (51.0–63.6)
		1 W/kg	62.0 (55.7–68.1)	65.6 (59.5–71.5)	61.8 (55.5–67.9)
	$NiCl_2$ (60 µM)	Control	1.5 (0.1–4.5)	0.6 (0.0–2.9)	0.1 (0.3–1.6)
		Sham	12.7 (8.7–17.2)	21.2 (16.2–26.6)	20.3 (15.4–25.7)
		0.25 W/kg	14.7 (10.5–19.5)	22.3 (17.3–27.8)	12.8 (8.8–17.3)
		Control	1.9 (0.2–5.2)	0.6 (0.0–2.9)	1.6 (0.1–4.7)
		Sham	24.3 (19.1–30.0)	24.1 (18.9–29.8)	21.7 (16.7–27.1)
		0.5 W/kg	24.0 (18.8–29.6)	29.2 (23.6–35.2)	18.6 (13.9–23.8)
		Control	5.0 (1.8–9.6)	7.8 (3.7–13.3)	1.6 (0.1–4.6)
		Sham	29.2 (23.6–35.2)	26.1 (20.7–31.8)	21.9 (16.9–27.4)
		1 W/kg	37.8 (31.7–44.1)	24.2 (19.0–29.9)	21.7 (16.7–27.2)
	CrO_3 (30 µM)	Control	2.2 (0.3–5.6)	0.1 (0.4–1.4)	0.4 (0.1–2.4)
		Sham	16.6 (12.1–21.6)	22.6 (17.5–28.2)	26.9 (21.5–32.7)
		0.25 W/kg	18.0 (13.4–23.1)	14.2 (10.0–18.9)	31.6 (25.9–37.7)
		Control	1.9 (0.2–5.2)	0.6 (0.0–2.9)	1.6 (0.1–4.7)
		Sham	24.9 (19.6–30.6)	26.2 (20.8–32.0)	20.4 (15.5–25.7)
		0.5 W/kg	25.2 (19.9–30.9)	28.0 (22.5–33.9)	23.2 (18.1–28.8)
		Control	5.0 (1.8–9.6)	7.8 (3.7–13.3)	1.6 (0.1–4.6)
		Sham	25.2 (19.9–30.9)	25.5 (20.1–31.2)	31.6 (25.8–37.6)
		1 W/kg	21.3 (16.3–26.7)	26.4 (21.0–32.2)	30.1 (24.4–36.1)

1Numbers indicate means % DNA in tail (95% confidence intervals) found in PBMC after sequential treatment (16 h HF-EMF radiation followed by 16 h chemical treatment). For each experiment control cells (neither exposed to HF-EMF not to substances), sham and real exposure conditions were evaluated. For each condition, two independent experiments were performed. From each experiment, three slides were prepared and 50 cells evaluated. *Statistically significant difference ($p \leq 0.05$) compared to corresponding sham values.

2Young normal weight participants (YN), young participants with high body weight (YO), and older normal weight participants (ON).

The findings which were obtained after sequential treatment of the cells (HF-EMF exposure followed by chemical treatment) are summarized in Table 4. We found consistently no impact of the radiation on the extent of chemically induced DNA damage.

Discussion

The main aim of the present study was to determine if HF-EMF cause DNA damage in blood cells from groups (YN, YO, and ON) which differ in regard to age and BMI. As mentioned in the introduction, numerous studies have been published concerning the genotoxic properties of mobile phone-specific radiation; for review see [5]. In slightly more than half of the studies with mammalian cells, evidence for induction of genetic instability was found [5]. However, the impact of age and increased BMI has not been studied so far according to our knowledge. Furthermore, we investigated in the present study also the impact of HF-EMF on chemically induced DNA damage in the three groups. The chemicals we used cause damage of the genetic material via different molecular mechanisms and reflect exposures which occur at workplaces (for details see Supplementary Table 1). As described in Fig. 2, we did not detect significant differences of the extent of basal DNA damage in the different groups. In this context it is notable that earlier investigations concerning

the impact of age on comet formation yielded controversial results. Some reported an increase with age [36–38], while others found no effect [39–41] or even an inverse association [42]. Also the results concerning the impact of obesity are inconsistent; however, a number of studies reported a moderate increase (up to 2-fold) in highly overweight individuals [17].

One of the most interesting results of the present study is the observation of increased DNA damage after HF-EMF exposure in ON subjects (≥69.1 ± 7.6 years). After radiation with the highest dose (1.0 W/kg) a significant increase of the tail intensity was observed. This effect was seen in presence and also in absence of serum. We performed experiments with and without serum; these cultivation conditions lead to different physiological states of the cells. When cells are grown in absence of serum they stop to divide almost completely, this situation resembles the condition in the human body where PBMC have a very low mitotic activity. In presence of serum the cells divide much faster; we included this experimental condition as most mutagenicity experiments with PBMC and other cell types are routinely conducted with serum-supplemented media. No influence of radiation was seen in the same group with lower doses (0.25 and 0.5 SAR) and no effects were detected in the other groups (YN and YO) under all experimental conditions. It was postulated earlier that the extent of oxidative stress increases with age [43]; furthermore, there is evidence that DNA-repair functions decline with the length of the lifespan [44]. Numerous studies have been published which concern the effects of age on oxidative DNA damage and on DNA-repair processes. A cumulative review of Moller *et al*. analysed the results of experiments with rodents in which oxidatively damaged nuclear DNA was quantified in inner organs. The authors state that there is convincing evidence (from 21 out of 29 studies) for ageing-associated accumulation of oxidatively damaged DNA [45]. Notably, also in experiments with human cells evidence for an increase of oxidative DNA damage was observed (for review see [46]). Also DNA-repair process change during ageing and a substantial decrease of the efficiency of several important DNA-repair pathways was found in a number of studies [47]. These factors may lead to increased sensitivity towards HF-EMF. It was repeatedly postulated that formation of ROS and alterations of DNA-repair functions may play a role in regard to the DNA-damaging effects of mobile phone-specific fields [1]. It is notable that the results of earlier studies with HF-EMF (frequencies between 800 and 1950 MHz) in lymphocytes are ambiguous; for review see [5]; approximately that is, in 36% of the studies no evidence for induction of DNA damage was found. These investigations were conducted with cells from a few donors and the effects of age and BMI were not studied.

All model chemicals which we used, caused DNA damage in the blood cells. The ranking order of genotoxic activities declined in the order BPDE > 4NQO > CrO_3> $NiCl_2$ (Fig. 3). Similar results were obtained in experiments with PBMC, however, the exposure time was in general shorter (1–6 h) [48–51]. As described in the results section, we did not find evidence for differences of the sensitivity of the cells from the three groups towards HF-EMF after treatment with the different chemicals in general. However, we observed a significant decrease of BPDE-induced comet formation (by 18%) when cells from the YO were exposed to the highest intensity (1.0 W/kg). In this context it is notable that we observed in an earlier radiation study an increase of the activity of base excision repair and nucleotide excision repair after radiation of a glioblastoma cell line under similar experimental conditions with the UMTS signal [11]. It is known that these repair systems are involved in the elimination of BPDE-caused lesions [52].

The results of a computer-aided literature search shows that 10 combined treatment studies have been published concerning the impact of HF-EMF on chemically induced DNA damage; for reviews see [5,6]. Most of them were performed with cytostatic drugs. The effects which were obtained with 4NQO are controversial, that is, an increase was detected after simultaneous treatment for 2 h by Baohong *et al*. [53] in human lymphocytes, while no impact was seen in a human leucocyte cell line (THP1) with this agent [54]. No effects were found with the alkylating agent MMS [53] while results with mitomycin C are controversial [53,55]. As mentioned above, 4NQO is an UV mimetic agent and no alteration of 4NQO-induced DNA damage was seen in the present experiments after radiation. In this context it is notable that reduction of comet formation was observed in lymphocytes 1–5 h after HF-EMF radiation (1800 MHz) [56]; on the contrary, the extent of DNA damage increased when a longer treatment period was used. The authors hypothesized that this phenomenon could be caused by alterations of repair processes.

Taken together, the results of the present study indicate that HF-EMF causes damage of the genetic material in cells from older subjects. However, we found no evidence for a negative impact of mobile phone-specific fields on induction of DNA damage by occupationally relevant chemical exposures.

Supplementary data

Supplementary data is available at *Mutagenesis* Online.

Conflict of interest statement: The authors declare that there is no conflict of interest.

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Data availability

The data underlying this article will be shared on reasonable request to the corresponding author. Information on individual participants will not be made available.

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