

Non-thermal DNA breakage by mobile-phone radiation (1800 MHz) in human fibroblasts and in transformed GFSH-R17 rat granulosa cells in vitro

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Abstract

Cultured human diploid fibroblasts and cultured rat granulosa cells were exposed to intermittent and continuous radiofrequency electromagnetic fields (RF-EMF) used in mobile phones, with different specific absorption rates (SAR) and different mobile-phone modulations. DNA strand breaks were determined by means of the alkaline and neutral comet assay.

RF-EMF exposure (1800 MHz; SAR 1.2 or 2 W/kg; different modulations; during 4, 16 and 24 h; intermittent 5 min on/10 min off or continuous wave) induced DNA single- and double-strand breaks. Effects occurred after 16 h exposure in both cell types and after different mobile-phone modulations. The intermittent exposure showed a stronger effect in the comet assay than continuous exposure. Therefore we conclude that the induced DNA damage cannot be based on thermal effects.

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1. Introduction

The increase in the use of mobile phones has brought about an urgent need to determine whether their microwave radiation (radio-frequency-modulated electromagnetic fields, RF-EMF at 1800 MHz) may con-

stitute a risk to human health. The importance of this issue was considerably increased when in vivo findings obtained in the mid-1990s [1,2] were recently supported by results of in vitro studies indicating that electromagnetic fields are capable of causing DNA damage [3]. It is, therefore, the aim of this study to confirm or disprove the genotoxic potential of RF-EMF by investigating possible DNA-damaging effects of RF-EMF in different cell types using a newly developed highly sophisticated exposure system [8]. In vitro studies with defined exposure conditions and with genotoxic effect markers as endpoints are suitable to provide evidence in favour or

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against the occurrence of genotoxic effects due to RF-EMF exposure.

2. Materials and methods

2.1. RF-EMF exposure conditions and cell culture

Human diploid fibroblasts initiated from skin biopsies from a healthy donor (ES1, male, 6 years old) and maintained in culture as previously described [4,5] and transformed rat granulosa cells GFSH-R17 [6] were seeded into 35 mm Petri dishes at a density of 2×10^5 cells/3 ml, 24 h prior to RF-EMF exposure.

The exposure system was built and provided by the IT'IS-Foundation (Foundation for Information Technologies in Society, Zürich, Switzerland). For this setup R18 hollow rectangular waveguides operating at a frequency of 1800 MHz are used. On one side of the waveguide is a coupler with tuner, on the other side is a shortcut. For loading the setup, the shortcut can easily be removed and the whole cell dish holder can be placed inside. Six Petri dishes can be exposed simultaneously. The dishes were placed in the H-field maxima. Two waveguides (exposed, sham-exposed) were placed inside a commercial incubator to ensure constant environmental conditions (37 °C, 5% CO₂, 95% humidity). In addition, the temperature and the SAR were monitored at the location of the dishes during exposure [7,8]. The temperature difference between the chambers did not exceed 0.1 °C. To enable blind exposures, a computer randomly determined which of the two waveguides was exposed. This information was provided to the investigator by the IT'IS Foundation in Zurich via e-mail in exchange with the transmission of comet assay results. The fields as well as all sensors were continuously monitored. All experiments were performed at a carrier frequency of 1800 MHz at continuous or intermittent exposure (5 min field on/10 min field off). The field was either applied without modulation of the signal (=continuous wave) or with two different types of modulation. (1) *GSM basic*: GSM signals were amplitude-modulated by rectangular pulses with a repetition frequency of 217 Hz and a duty cycle of 1:8, yielding frames of a length of 4.61 ms each including a 576 μ s burst. Since every 26th frame is idle, an 8 Hz modulation component is integrated into the signal. In order to save battery power during active periods without speaking into the phone the number of active frames is reduced (=discontinuous transmission mode, DTX). (2) *GSM-talk* alternates between GSM basic and DTX at an average rate of 97 and 50 s and simulates hereby a mobile-phone conversation. An average

specific absorption rate (SAR) of 2 W/kg was selected, because it is the safety limit for the mobile-phone microwave-radiation emission as defined by ICNIRP [9,10]. SAR defines the tissue-absorbed energy per unit mass (expressed in W/kg) and is dependent on the geometry of the exposure chamber. Measurements of our exposure system by the provider (IT'IS Foundation, Zürich, Switzerland) revealed a non-uniformity of SAR with exposed monolayer cell cultures of less than 30%, and a temperature load of less than 0.03 °C per W/kg SAR. For further technical details see [10a,b]. The exposure time varied between 4 and 24 h. After exposure the cells were detached with trypsin and suspended in fresh culture medium. Each exposure level was tested in duplicate.

2.2. Comet assay analysis

We followed the technique described by Östling and Johanson [11] with minor modifications by Singh et al. [12–14]. RF-exposed and sham-exposed cells (10,000–30,000) were mixed with 100 μ l low-melting agarose (0.5%, 37 °C) and this cell suspension was pipetted onto slides pre-coated with 1.5% normal melting agarose, spread with a cover slip, and maintained on a cold flat tray for about 10 min to solidify. After removal of the cover slip the third layer of 0.5% low-melting agarose was added and solidified. The slides were then immersed in freshly prepared cold lysis solution (2.5 M NaCl, 100 mM Na₂EDTA, 10 mM Tris, pH 10, 1% sodium sarcosinate, 1% Triton X-100, 10% DMSO, pH 10) and lysed for 90 min at 4 °C. Subsequently, the slides were drained and placed in a horizontal gel electrophoresis tank side by side, nearest the anode. The tank was filled with fresh electrophoresis buffer (1 mM Na₂EDTA, 300 mM NaOH, pH > 13 in case of the alkaline comet assay, and 100 mM Tris, 300 mM sodium acetate, 500 mM sodium chloride, pH 8.5 in case of the neutral comet assay) to a level of approximately 0.4 cm above the slides. For both alkaline and neutral comet assay, slides were left in the solution for 40 min to allow equilibration and unwinding of the DNA before electrophoresis. Electrophoresis conditions (25 V, 300 mA, 4 °C, 20 min, field strength: 0.8 V/cm) were the same for neutral and alkaline comet assay. All steps were performed under dimmed light to prevent the occurrence of additional DNA damage. After electrophoresis the slides were washed three times with Tris buffer (0.4 M Tris, pH 7.5) to neutralize, then air-dried and stored until analysis. Comets were visualized by ethidium bromide staining (20 μ g/ml, 30 s) and examined at 400 $\times$ magnification using a fluorescence microscope (Axiophot,

Zeiss, Germany). One thousand DNA spots from each sample were classified into five categories corresponding to the amount of DNA in the tail according to Anderson et al. [15], with modifications as previously described [4,5,16,17].

2.3. Statistical analysis

Statistical analysis was performed with STATISTICA V. 5.0 package (Statsoft, Inc., Tulsa, USA). All data are presented as mean $\pm$ standard deviation (S.D.). The differences between exposed and sham-exposed, as well as between different exposure conditions were tested for significance with the independent Student's *t*-test. A difference at $p < 0.01$ was considered statistically significant.

3. Results

The influence of RF-EMF exposure on the generation of DNA strand breaks in cells derived from two different tissues (human fibroblasts, rat granulosa cells) was evaluated using the alkaline and the neutral comet assay. Four different sets of exposure conditions were tested: continuous (1800 MHz, SAR 2 W/kg), intermittent (5 min on/10 min off, 1800 MHz, SAR 2 W/kg), pulse modulation (5 min on/10 min off, 1800 MHz, SAR 2 W/kg, amplitude 217 Hz) and talk modulation (continuous 1800 MHz, SAR 1.2 W/kg, DTX 66%, GSM basic 34%). All these modulations are characteristic for the

normal use of mobile phones. Different exposure durations were applied: 4, 16 and 24 h.

An increased response in the alkaline comet assay in exposed human fibroblasts compared with sham-exposed controls could be detected in each of these experiments, also at continuous exposure (Fig. 1). This elevation became significant at 16 h of exposure ($p < 0.01$), but no significant differences between 16 and 24 h could be detected. At intermittent pulse modulation and talk modulation, effects in the comet assay were significantly higher than at continuous exposure. Human fibroblasts and rat granulosa cells responded equally to RF-EMF, but the latter exhibited higher basal and higher end levels (Fig. 2). The comet factors with the neutral comet assay were similar, albeit lower (Figs. 3 and 4).

4. Discussion

In view of the results of our previous ELF-EMF research [4] we used an 'on' duration time of 5 min and an 'off' duration time of 10 min for the intermittent RF exposure. This study showed that the effects are much stronger at intermittent than at continuous exposure. It is highly unlikely that the observed genotoxic damage is caused non-specifically by spots of increased temperature within the cell layer, as a secondary effect of the electromagnetic field. If so, the damage would be largest at continuous exposure due to the much higher energy impact under this condition. Therefore, we conclude, that at least the major part of the observed induction

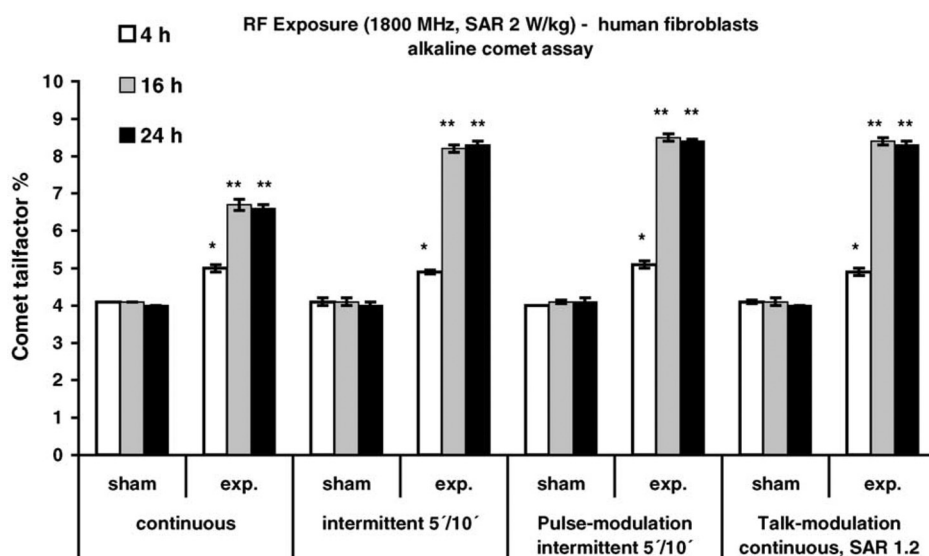


Fig. 1. Influence of exposure time and different exposure conditions on the formation of DNA single- and double-strand breaks in human fibroblasts, determined with the comet assay under alkaline conditions (* statistically not significant, ** $p < 0.01$).

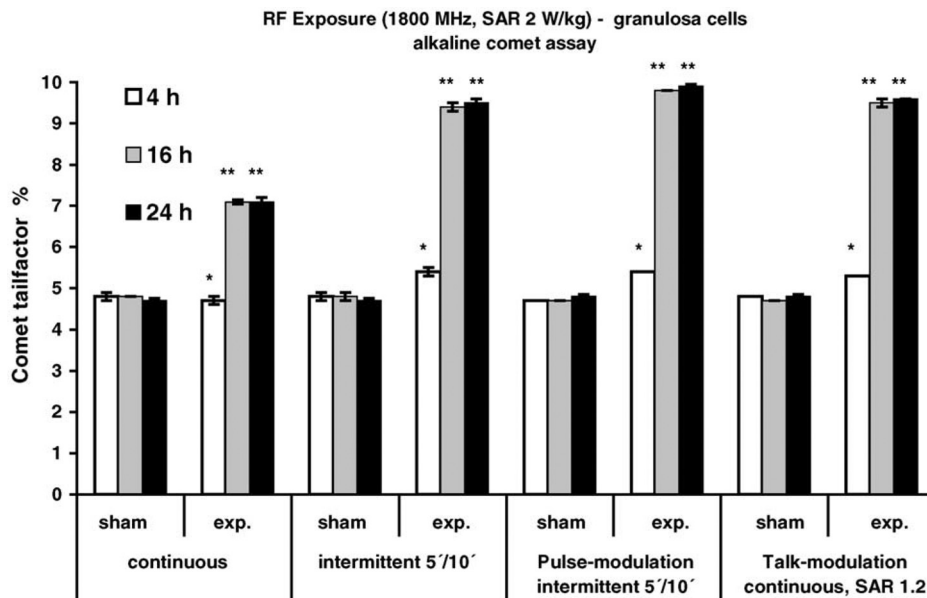


Fig. 2. Influence of exposure time and different exposure conditions on the formation of DNA single- and double-strand breaks in transformed rat granulosa cells, determined with the comet assay under alkaline conditions (* statistically not significant, ** $p < 0.01$).

of DNA single- and double-strand breaks is a direct consequence of intermittent exposure to RF-EMF and not the result of a temperature increase. The identification of the processes that lead to this DNA breakage will finally help to determine the extent of biological effects directly induced by RF-EMF exposure. Importantly, cellular effects observed in this study were induced with

SAR values set at the highest level allowed by the European safety limits. This suggests that the allowed radiation emission levels for mobile phones might be sufficient to induce biological effects also in vivo. We know from our previous studies that the effect of the ELF-EMF [18] depends on the cell type and the duration of the 'on'/'off' cycles used in the experiments. It

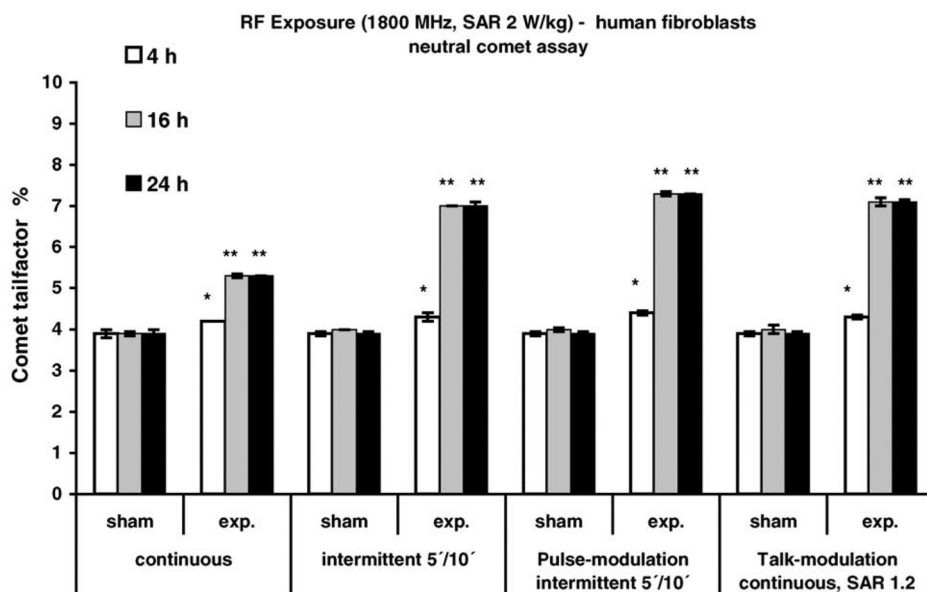


Fig. 3. Influence of exposure time and different exposure conditions on the formation of DNA single- and double-strand breaks in human fibroblasts determined with the comet assay under neutral conditions (* statistically not significant, ** $p < 0.01$).

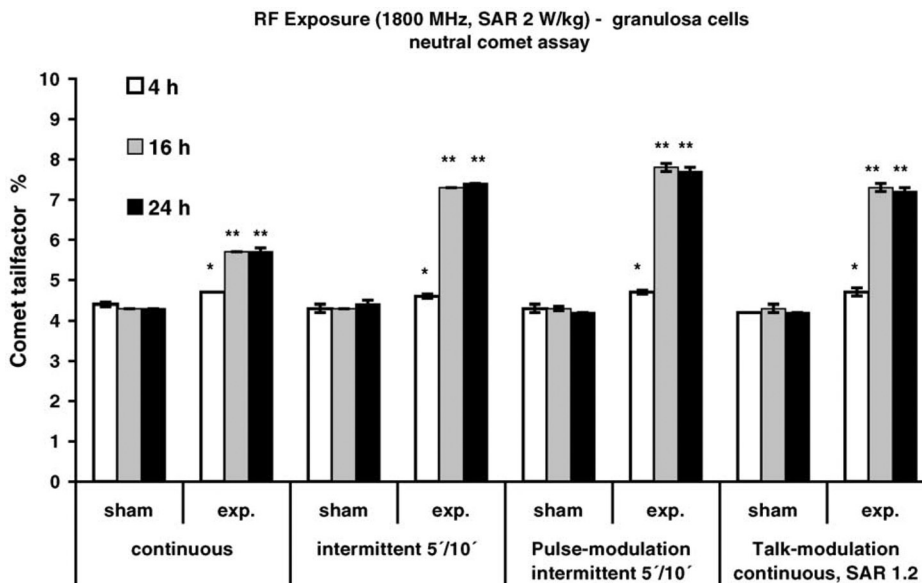


Fig. 4. Influence of exposure time and different exposure conditions on the formation of DNA single- and double-strand breaks in transformed rat granulosa cells determined with the comet assay under neutral conditions (*statistically not significant, ** $p < 0.01$).

is, therefore, not surprising that no genotoxic effects of RF-EMF have been reported from experiments with lymphocytes under continuous exposure conditions [19–21]. Of course, further investigations on cell-type dependence and dose-dependence are required.

In conclusion, under conditions of several different mobile-phone modulations RF-EMF induces DNA single-strand and double-strand breaks in human diploid fibroblasts and in rat granulosa cells in culture.

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