Cytogenetic investigation of subjects professionally exposed to radiofrequency radiation

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Nowadays, virtually everybody is exposed to radiofrequency radiation (RFR) from mobile phone base station antennas or other sources. At least according to some scientists, this exposure can have detrimental health effects. We investigated cytogenetic effects in peripheral blood lymphocytes from subjects who were professionally exposed to mobile phone electromagnetic fields in an attempt to demonstrate possible RFR-induced genetic effects. These subjects can be considered well suited for this purpose as their RFR exposure is ‘normal’ though rather high, and definitely higher than that of the ‘general population’. The alkaline comet assay, sister chromatid exchange (SCE) and chromosome aberration tests revealed no evidence of RFR-induced genetic effects. Blood cells were also exposed to the well known chemical mutagen mitomycin C in order to investigate possible combined effects of RFR and the chemical. No cooperative action was found between the electromagnetic field exposure and the mutagen using either the comet assay or SCE test.

Introduction

With the increasing utility and escalating popularity of radiofrequency radiation (RFR) emitting consumer devices, such as handheld mobile phones (especially GSM and UMTS), public attention has been drawn to possible adverse health effects of exposure to RFR. Exposures to fields emitted by a base transceiver station (BTS) as well as by the mobile phone itself are often regarded with suspicion. This is especially important as virtually everybody in the world is now exposed to this kind of ‘physical environmental pollutant’ that is, together with electromagnetic fields from overhead power lines, sometimes referred to as electrosmog. Worldwide, many investigations were already conducted in order to study possible adverse health effects. Among them, effects on the genetic material (DNA) are considered very important since damage to the DNA can, among other effects, lead to the development of cancer. Overall it is assumed that RFR radiation does not induce genetic damage in cells after \textit{in vitro} or \textit{in vivo} exposure, but suspicion remains, especially because a number of studies indeed show RFR-induced genetic damage in a number of cell types and for particular conditions of exposure [for a review see (1–5)]. There were also a number of reports indicating that RFR, while being not genotoxic itself, can enhance the effect of a chemical mutagen (6,7).

Studies on humans being exposed to RFR-radiation from mobile phone devices are rather scarce. Exposure of the population to radiation from BTSs is generally at very low field intensities as they are at several tens of meters or more from the emitting antennas. Mobile phone users may be exposed to higher but localized fields and usually only for a limited period of time. Personnel from the mobile phone operators, e.g. maintenance workers, may be exposed to much higher levels and for a more prolonged period of time and may therefore constitute the best possible population to investigate \textit{in vivo} human exposure to eventually high, yet ‘realistic’ exposure levels of RFR. We here present the results of a cytogenetic investigation on radio field engineers and other subjects working for two of the Belgian mobile phone operators. The chromosome aberration, sister chromatid exchange (SCE) and alkaline comet assays were used to assess RFR-induced genetic effects. We furthermore also exposed the subject’s blood to the well-known chemical mutagen mitomycin C (MMC) in order to investigate possible higher (or lower) sensitivity of the blood cells from the RFR-exposed subjects to the chemical (cooperative or antagonistic effects). Here, the alkaline comet assay and SCE test were used. Results were compared to those obtained in unexposed control subjects.

Materials and methods

\textit{Test population and RFR exposure}

Blood samples were obtained from 49 individuals who were working for a Belgian mobile phone company: 27 worked in one company (operator 1, all radio field engineers) and 22 worked in another company (operator 2, 11 were radio field engineers, 11 were administrative workers). Questionnaires were used to obtain data on, e.g. their age, sex, professional activity, estimated exposure to RFR, duration of exposure and involved electromagnetic frequencies, events such as smoking, therapeutic radiographies, recent illnesses, etc.

The radio field engineers were in charge of the maintenance and repair of transmission antennas linked to the mobile phone network. Although antennas are normally switched off when the work requires close proximity or contact with the antennas these subjects considered themselves as moderately exposed to fields from different frequencies (450, 900 MHz and other frequencies in the MHz or GHz frequency range). ‘Real’ exposure was estimated to be more than 1 h a day during at least 1 year (2.3 years on the average). The distance to emitting antennas was very often judged to be <1 m. Unfortunately, these workers do not wear a dosimeter so that quantification of the exposure level was not possible.

The administrative personnel worked in buildings that were surrounded by RF-dipole antennas (147.25, 164.35 and 169.625 MHz) and parabolic antennas with frequencies ranging between 6 and 40 GHz. According to the questionnaire and discussions held at the company’s medical service their ‘electromagnetic field exposure’ was considered quite important. No systematic dosimetric evaluation was performed but a measuring campaign carried out at the time of the present investigation revealed that the investigated subjects could be exposed to fields that were usually below, but sometimes very close to the ICNIRP guidelines (8). These guidelines were exceeded in a few locations (at near field for 147.25, 164.35 and 169.625 MHz frequencies) where maintenance workers (but not the administrative personnel) could be present.

Blood samples from 25 subjects who were unrelated to the operators and had occupations that excluded exposure to RFR sources other than the ‘normal’ domestic sources (eventually use of a microwave oven or cordless telephone)

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were used as a ‘non-exposed’ control group. The blood was taken during the compulsory yearly medical examination at their companies’ medical service. The subjects did not at the time of the study possess a mobile phone and declared not using one, unless on one or a few exceptional occasions. All RFR-exposed subjects did report that they used cell phones on a regular basis (at least half an hour per day). However, they did not feel able to estimate their mobile phone use adequately, as this seemed to be very variable from one day to the other.

Except for smoking, no excessive professional or leisure exposure to potential genotoxic compounds was reported. No one consumed alcohol more than occasionally. One of the RFR-exposed subject did report exposure to welding fumes, one underwent a X-ray examination about 6 months before blood sampling and a few persons self-reported traffic pollution as a possible confounder (all were radio field engineers). None of the subjects reported any recent or recurrent illness. All but one subject (operator 2, radio field engineer) were men. Some information on the age distribution and smoking behaviour of the subjects is given in Table I. All subjects (test groups and controls) were informed about the purpose of the study and gave their consent to participate as a blood donor. All filled in the requested questionnaires.

**Cytogenetic investigations**

The following cytogenetic endpoints were investigated:

(i) the frequency of chromosomal aberrations
(ii) DNA damage as revealed by the alkaline comet assay
(iii) the SCE frequency

All tests were performed according to routine procedures. For the chromosome aberration test (9), 0.5 ml of fresh blood was added to 5 ml RPMI-1640 culture medium supplemented with 15% of fetal calf serum, antibiotics and phytohaemagglutinin (8 μg/ml). The cultures were incubated at 37°C for 48 h. To block the cells in metaphase, colcemid (0.2 μg/ml) was added 2 h before harvest of the cells. The cells were harvested by centrifugation, suspended in KCl hypotonic solution (0.075 M) and then fixed in three changes of methanol/acetic acid (3:1, v/v). Cell suspensions were dropped onto pre-chilled slides, air dried and stained with Giemsa. At least 200 metaphase figures were analysed per subject for the presence of structural chromosome aberrations.

For analysis of SCE lymphocyte cultures were set up and treated as above, except that cultivation was for 72 h in the presence of BrDU (5-bromo-2′-deoxyuridine). Following slide preparation we used the standard fluorescence plus Giemsa (FPG) method of Perry and Wolff (10); cells were treated with a Hoechst 33258 solution, placed under white light for 24 h and then treated with sodium saline citrate (SSC) solution at 65°C for 1.5 h. Finally, the cells were stained with a 5% Giemsa solution in SSC. The SCE frequency was determined in 50 second division metaphases per subject.

The comet test was performed as described by Singh et al. (11) with minor modifications. Cells were mixed with agarose gel, which was spread onto a microscope slide. The cells were then lysed with high salt concentrations and detergents for at least 1 h enabling the release of DNA from individual cells. The remaining nuclear DNA was then denaturated in an alkali buffer and modifications. Cells were mixed with agarose gel, which was spread onto a microscope slide. The cells were then lysed with high salt concentrations and detergents for at least 1 h enabling the release of DNA from individual cells. The remaining nuclear DNA was then denaturated in an alkali buffer and micrographs of the comet tail were analyzed. The comet test was performed as described by Singh et al. (11) with minor modifications. Cells were mixed with agarose gel, which was spread onto a microscope slide. The cells were then lysed with high salt concentrations and detergents for at least 1 h enabling the release of DNA from individual cells. The remaining nuclear DNA was then denaturated in an alkali buffer and micrographs of the comet tail were analyzed.

**Results**

In this study peripheral blood lymphocytes from three groups of RFR-exposed subjects (two populations of radio field engineers and one group of administrative subjects) were cytogenetically investigated along with a control group. As indicated in Table I their age and smoking habits were very well comparable. There was also no indication of any difference in other exposures or lifestyle factors (see Materials and methods section). Except for the RFR exposure, the subjects appeared statistically not significantly different from each other (P > 0.05).

The results of the chromosome aberration test are summarized in Table II. All types of aberrations were within normal limits found in our laboratory. There was no statistically significant increase in the aberration frequency in RFR-exposed subjects (radio field engineers and administrative personnel, considered separately or taken together) compared with the control subjects (P > 0.05).

Results on the SCE frequency in professionally RFR-exposed subjects and the sensitivity towards (0.1 μg/ml) MMC are summarized in Table III. Here, 16 subjects (operator 1) were compared with the 25 subjects from the control population. The effect of MMC is obvious in both populations but there was no statistically significant difference between RFR-exposed and RFR-unexposed subjects.

Data on the alkaline comet assay are briefly summarized in Table IV. The Mann–Whitney U-test revealed no difference between RFR-exposed and unexposed subjects. It should be noted that, while MMC showed a significant increase in DNA damage, the comet test did not reveal any significant differences between RFR-exposed and unexposed subjects. The effect of MMC is obvious in both populations but there was no statistically significant difference between RFR-exposed and RFR-unexposed subjects.

**Table I. Age distribution and smoking behaviour of the investigated subjects**

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Smoking behaviour (number of subjects)</th>
<th>Age distribution (years)</th>
<th>Non-smoking</th>
<th>1–5 cigarettes/day</th>
<th>5–10 cigarettes/day</th>
<th>10–20 cigarettes/day</th>
<th>&gt;20 cigarettes/day</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>Operator 1: radio field engineers</td>
<td>(27 male subjects)</td>
<td>34.5 ± 6.9 (26–46)</td>
<td>16</td>
<td>0</td>
<td>5</td>
<td>5</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>Operator 2: administrative personnel (11 male subjects)</td>
<td></td>
<td>35.1 ± 9.0 (25–59)</td>
<td>6</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1 pipe (2–3 per day)</td>
</tr>
<tr>
<td>Operator 2: radio field engineers</td>
<td>(10 males, 1 female)</td>
<td>37.7 ± 9.1 (25–67)</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>1</td>
<td>–</td>
</tr>
<tr>
<td>Unrelated control subjects</td>
<td>(25 male subjects)</td>
<td>35.3 ± 8.4 (25–60)</td>
<td>15</td>
<td>0</td>
<td>5</td>
<td>4</td>
<td>1</td>
<td>–</td>
</tr>
</tbody>
</table>
Twenty-five external control subjects

Non-exposed control subjects

Eleven administrative workers, operator 2

Eleven radio field engineers, operator 2

Without MMC 3.43

With MMC 34.98

and their sensitivity towards MMC

Without MMC 1.20

With MMC 2.18

There are many papers on the presence or absence of RFR-induced genetic effects [for a review see (1–5)]. The majority of them were \textit{in vitro} investigations reporting negative results. Few studies were devoted to human (cyto)genetic biomonitoring. Furthermore, as is almost always the case, the results from these few studies were conflicting. For example, Garaj-Vrhovac \textit{et al}. (12) found increased chromosomal aberration and micronucleus frequencies in lymphocytes of radar station workers who had experienced occupational exposure to 30–300 GHz at 1000–5000 W/m². The same research group reported an increased micronucleus frequency in other occupationally exposed subjects (13). However, Maes \textit{et al}. (14) found no chromosomal aberrations in antenna maintenance workers who had been exposed to various RF fields (including 450 and 950 MHz) at least 1 h each day for more than a year. This was a very preliminary study involving only a few subjects but the results were in accordance with those of Garson \textit{et al}. (15) who also found no increased chromosome aberration frequency in the blood of radio lineman exposed to 0.4 MHz up to 20 GHz fields. On the other hand, Balode (16) found significant effects in the blood of cattle on farms close to a major radar installation in Latvia.

An ‘Expert Group on Health Risks of Radiofrequency Fields from Wireless Telecommunication Devices’ (http://www.rsc.ca/english/RFreport.pdf) (17) stated that: ‘Perhaps some of the most important observations are those obtained from \textit{in vivo} studies with occupationally exposed individuals who were followed for over a year without any indication of an induction of chromosomal aberrations’. Reference was made to our preliminary investigation (14) that was not really informative as only a few subjects were involved. The present study was performed as an extension of this preliminary investigation.

We used the chromosome aberration, SCE and alkaline comet tests for assessing genetic effects in lymphocytes from RFR-exposed subjects, whereas the comet assay and SCE test were used for the investigation of possible RFR-induced modulatory effects after exposure to a chemical mutagen. The choice of the tests was based on previous results (6,14,18–20). Although doses received were not systematically recorded it may be considered that the exposed subjects (27 + 11 + 11) belong to a category of subject exposed to ‘more than average exposure levels’. Apart from the exposure the subjects could be considered very well comparable with the controls in terms of age distribution, smoking habits and other possible confounders. The results did not suggest any increased genetic damage in the lymphocytes due to RFR exposure. There was also no evidence of RFR-mediated increased sensitivity towards the action of the chemical mutagen MMC as was

### Table II. Results of the chromosome aberration study in RFR-exposed subjects and a control population

<table>
<thead>
<tr>
<th></th>
<th>Gap/Isogap</th>
<th>Chromatid breaks</th>
<th>Interchromatid exchange</th>
<th>Chromosome breaks</th>
<th>Dicentric chromosomes without acentric fragment</th>
<th>Dicentric chromosomes with acentric fragment</th>
<th>Other aberrations (translocations)</th>
<th>Chromatid type aberrations</th>
<th>Chromosome type aberrations</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Exposed subjects</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Twenty seven radio field engineers, operator 1</td>
<td>0.41 ± 0.50</td>
<td>1.93 ± 1.17</td>
<td>0.04 ± 0.19</td>
<td>1.19 ± 1.57</td>
<td>0.07 ± 0.27</td>
<td>0</td>
<td>0.04 ± 0.19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eleven radio field engineers, operator 2</td>
<td>0.91 ± 0.14</td>
<td>1.82 ± 1.33</td>
<td>0</td>
<td>0.91 ± 0.83</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eleven administrative workers, operator 2</td>
<td>1.09 ± 1.76</td>
<td>1 ± 0.89</td>
<td>0.36 ± 0.92</td>
<td>0.91 ± 1.14</td>
<td>0.18 ± 0.40</td>
<td>0.27 ± 0.47</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Non-exposed control subjects</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Twenty-five external control subjects</td>
<td>0.52 ± 0.87</td>
<td>1.16 ± 1.18</td>
<td>0.04 ± 0.2</td>
<td>1.28 ± 1.4</td>
<td>0.2 ± 0.41</td>
<td>0.04 ± 0.2</td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table III. SCE frequencies (±SD) in professionally exposed subjects and their sensitivity towards MMC

<table>
<thead>
<tr>
<th></th>
<th>RF-exposed subjects</th>
<th>RF-unexposed subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without MMC</td>
<td>3.43 ± 0.70</td>
<td>5.1 ± 0.81</td>
</tr>
<tr>
<td>With MMC</td>
<td>34.98 ± 10.61</td>
<td>43.42 ± 10.52</td>
</tr>
</tbody>
</table>

### Table IV. Mean DNA content (and standard error) in the ‘DNA comet tail’ from white blood cells of RFR-exposed subjects and their sensitivity towards MMC

<table>
<thead>
<tr>
<th></th>
<th>RF-exposed subjects</th>
<th>RF-unexposed subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without MMC</td>
<td>1.20 ± 0.19</td>
<td>1.74 ± 0.23</td>
</tr>
<tr>
<td>With MMC</td>
<td>2.18 ± 0.40</td>
<td>2.25 ± 0.31</td>
</tr>
</tbody>
</table>

damage ($P < 0.05$), this was not as obvious as for the sister chromatid exchange test ($P < 0.0001$).

### Discussion

There are many papers on the presence or absence of RFR-induced genetic effects [for a review see (1–5)]. The majority of them were \textit{in vitro} investigations reporting negative results. Few studies were devoted to human (cyto)genetic biomonitoring. Furthermore, as is almost always the case, the results from these few studies were conflicting. For example, Garaj-Vrhovac \textit{et al}. (12) found increased chromosomal aberration and micronucleus frequencies in lymphocytes of radar station workers who had experienced occupational exposure to 30–300 GHz at 1000–5000 W/m². The same research group reported an increased micronucleus frequency in other occupationally exposed subjects (13). However, Maes \textit{et al}. (14) found no chromosomal aberrations in antenna
suggested in some (6,7) but not in all (20–23) previous reports. That MMC showed a much less significant increase in DNA damage as assessed by the comet tail DNA content compared with the SCE test can probably be ascribed to DNA–DNA cross linking induced by MMC treatment that produce a retardation in the rate of DNA migration (24).

It is also interesting to note that, apart from chromatid type aberrations (Table II) all other cytogenetic endpoints indicated a low, although statistically non-significant, decrease in genetic damage compared with non-exposed subjects. As such this may be anecdotic and not worth being mentioned if other recent investigations did not show the same tendency (e.g. the EC’s 5th framework research programs CEMFEC and PERFORM B in which genetic effects were investigated in the blood of, respectively, in vivo exposed rats or in vitro exposed human lymphocyte cultures; to be published soon).

Overall, our results are in agreement with the majority of published papers on RFR-induced genetic effects. Literature data indicate that most scientists do agree that solid scientific evidence in favour of RFR-induced genetic effects in cells or animals is lacking, at least when fields are too low to result in a measurable increase in temperature (1,3,4). Yet, controversial results were occasionally reported. A reason for the controversy could be the differences in the use of test material (cell types, animals, humans), methods (DNA comets, micronuclei, SCE, chromosomal aberrations and others that measure different genetic events), conditions (frequency, modulation, specific absorption rate) and/or duration of RF exposure. Additionally, in some investigations the RF fields did not guarantee homogeneous RF-exposure and therefore ‘hot spots’ (namely points of higher field intensities, as well as temperature gradients in the exposed material) might have been created leading to convection heating of the test material. Also, some of the measured effects could probably be just reactions of the activated system of thermoregulation even without any measurable alteration in core or surface temperature of animals and human volunteers (http://www.cost281.org/newsletter.php) (25). Furthermore, in some of the reports the above mentioned experimental details which are critical for independent verification were either inadequately- or non-described (RFR exposure conditions, dosimetry, specific absorption rate and temperature measurements). There is also a concern that the number of cells that were examined by the investigators were often not sufficient enough to bring out subtle, but significant differences between RFR-exposed and sham-exposed cells.

Hence Vijayalaxmi and Obe (5) stated that ‘the data from well coordinated, multicenter collaborative studies with adequate statistical power will be needed to identify the factors contributing to these controversial observations…’. Such an international coordinated research on genotoxic effects of electromagnetic radiation from mobile communication systems has also been recommended by the European COST281 framework (http://www.cost281.org/activities/Gentox-recomm-090304AW.doc) so as to accommodate the above formulated critics and obtain a solid basis for a final answer on the question whether radiofrequency radiation is or is not genotoxic.

References