Cytogenetic Investigations on Microwaves Emitted by a 455.7 MHz Car Phone

(RF radiation / 455.7 MHz / chromosome aberrations / SCE / synergy / lymphocytes)

A. MAES, M. COLLIER, L. VERSCHAEVE

Vito, Environmental Toxicology, Mol, Belgium

Abstract. The chromosome aberration or sister chromatid exchange frequency was determined in 455.7 MHz microwave-exposed human lymphocytes and in lymphocytes that were subsequently exposed to MMC or X-rays. The exposure was performed by placing the cells at 5 cm from the antenna of a car phone. In this way the specific absorption ratio was approximately 6.5 W/kg. The temperature and humidity was kept constant during the experiments. No statistically significant difference was found between microwave-exposed and unexposed control samples. When the microwave exposure was followed by exposure to MMC, some differences were found between the combined treatments and the MMC treatments alone. However, there was no consistency in the results. Combined treatments with X-rays did not provide any indication of a synergistic action between the RF fields and X-rays, either. Our data therefore do not support the hypothesis that RF fields act synergistically with chemical or physical mutagens.

Progressing modern life standards have consequences on the living environment, caused in part by an increasing electromagnetic pollution. Microwaves, especially those emitted by wireless communication devices, have an important contribution in our daily domestic exposure. Possible harmful effects are of great concern. The relationship between exposure to mobile telephone frequencies and an increased risk for brain cancer is often discussed in the scientific literature as well as in the media (Elmer-Dewit, 1993; Davidson, 1998; Moulder et al., 1999). As cancer is most often thought to involve a genetic event, investigations of microwave-induced genetic effects may be considered of particular importance in studies on this subject. That microwaves may have genetic effects is considered rather unlikely, as it is well known that their energy is far too low to break chemical bonds (Léonard et al., 1983). Yet, although originally proposed for electromagnetic field (ELF) effects and not for radiofrequency (RF) radiation, some mechanisms of interaction, e.g., with moving electrons within DNA (Blank and Goodman, 1997), have been postulated. Results from earlier studies were rather conflicting, but an overview of the literature shows that the general conclusion should be that microwaves indeed are not genotoxic (Verschaeve 1995; Brusick, 1998; Verschaeve and Maes, 1998). The majority of the investigations have shown that exposure of cells or animals to microwaves of different frequencies, at least under non-thermal conditions, does not lead to chromosome or DNA damage. Most "positive" results could indeed be ascribed to thermal exposure conditions or methodological shortcomings. However, the lack of a clearly accepted direct DNA damaging effect does not exclude the possibility of an indirect mechanism of action [e.g., DNA damage caused by oxidative stress (Scott, 1992)]. Furthermore, a number of reports show that low-power microwaves may be able to damage DNA (Sarkar et al., 1994; Lai and Singh, 1995, 1996) or be cancerogenic (Repacholi et al., 1997). These reports asked for a number of replication studies that are presently still ongoing or did not reach the same conclusion (Malyapa et al., 1998). Another aspect that should also be highlighted is the possibility of synergistic effects, for example by a "sensitization" of cells to the effects of other (chemical or physical) mutagens or carcinogens. Up to now, only a few studies were performed in this respect. There was no synergistic effect when the cells were simultaneously exposed to the microwaves and a chemical mutagen (Ciaravino et al., 1987, 1991; Meltz and Walker 1987; Melz et al., 1989, 1990; Kerbacher et al., 1990), but when the mutagen mitomycin C (MMC) was given after the cells were microwave-irradiated, the results were more controversial. We found no evidence for a synergistic action between MMC and 900 MHz electromagnetic fields (Maes et al., 2000), but previous investigations involving 954 MHz and 935.2 MHz fields did provide evidence for a synergistic action between the RF fields and MMC (Maes et al., 1996, 1997).

These microwave frequencies belong to the frequency band used by the new global system for mobile communication (GSM), a digital equipment which employs pulse transmissions of radiowaves. The use of this frequency band will certainly further increase in the years to come. Yet, other systems are still in use, e.g., one operating in the 450 MHz band, which employs a continuous

Received April 27, 2000. Accepted June 9, 2000.

Corresponding author: Luc Verschaeve, Vito, Environmental Toxicology, Boeretang 200, B-2400 Mol, Belgium. E-mail: luc.verschaeve@vito.be.

Abbreviations: MMC – mitomycin C, RF – radiofrequency, SCE – sister chromatid exchanges.

analogue transmission technology. It is anticipated that this frequency will be used for other applications later on.

The investigation that we report now focuses on the possible cytogenetic effects caused by exposure of cells to this frequency (more precisely 455.7 MHz). We studied the effects of the microwaves alone, as well as of the microwaves followed by exposure to the well-known mutagens MMC and X-rays.

Material and Methods

Cytogenetic endpoints

Three kinds of exposure were investigated on blood samples from healthy male and female donors that were exposed for 2 h to 455.7 MHz microwaves:

(1) chromosome aberrations in white blood cells from 28 donors following the microwave exposure alone (2 h whole blood exposure before 48 h cell cultivation started),

(2) sister chromatid exchanges (SCE) in cells from 4 donors after a combined treatment by microwaves (as above) followed by MMC. MMC was administered at the onset of cell cultivation and remained present in the culture medium during the complete cultivation time (72 h) (synergy experiment).

(3) chromosome aberrations in cells from 6 donors after a combined treatment by microwaves followed by X-rays (synergy experiment). X-ray exposure was also performed before the onset of cell cultivation.

All microwave exposures were thus performed before cell cultivation. This was because the particular exposure facility did not allow exposure inside an incubator. We furthermore deliberately did not expose the samples at body temperature in order to avoid possible heating phenomena resulting in non-physiological temperature conditions. At the applied exposure conditions at 17°C (see below), a preliminary experiment showed that the 2 h exposure time did not change the temperature of the samples, but no intracellular measurements were performed. Exposure of cells at the G0 stage of the cell cycle furthermore had the advantage that a synchronized cell population was exposed, preventing any interference resulting from possible cell-cycle dependencies.

In the combined treatment with MMC, SCE were chosen as the cytogenetic endpoint because MMC is known to be a good SCE inducer (Evans, 1977). On the other hand, X-rays induce chromosome aberrations more readily than SCE (Evans, 1977). Therefore, we used these aberrations as the cytogenetic endpoint in our synergy studies with X-rays.

Modes of exposure

The microwave exposure was performed by placing whole blood samples at 5 cm from the antenna of a Siemens Carvox S 24859-C 1003-A5 unit (ATF2 net). Samples were thereby placed in a cooled box at 17°C ± 1°C. The electric field was measured as 155 dBμV/m (approx. 50 V/m; calculated specific absorption rate (SAR) = approx. 6.5 W/kg), but it should be noted that the exposure was in the near field and that the field distribution is not uniform, and any evaluation is therefore very approximated. Control samples were kept in the same conditions of temperature and humidity as the exposed ones.

For the study of synergism between 455.7 MHz waves and X-rays, blood samples were irradiated at room temperature with different X-ray doses (5, 10, 20, 30 or 100 cGy). This was performed with a Philips RT 200/250 exposure unit (250 kV, 15 mA, 1 mm Cu-filter, dose-rate of 1 Gy per min).

As indicated above, the microwave and X-ray exposure always immediately preceded cell cultivation.

Cell cultivation procedures

Cells were cultivated in different ways depending on the genetic endpoint that was tested. Two parallel cultures were made for each of the exposures. All analyses were performed on coded slides and the code was broken only after all slides had been scored.

(a) For the chromosome aberration analysis of 455.7 MHz-exposed blood cells alone, phytohemagglutinin (PHA; 8 μg/ml) stimulated 48 h cell cultures were set up according to standard procedures (International Atomic Energy Agency (IAEA), 1996). Usually, two hundred Giemsa-stained metaphase figures were analysed per sample.

(b) For the combined exposure of 455.7 MHz waves and MMC, phytohemagglutinin-stimulated cultures were set up in the presence of 10 μg/ml bromodeoxyuridine (BrDU). The cultures were initiated immediately after the microwave exposure and the cultivation time was 72 h. MMC was added in concentrations of 0.5 or 0.1 μg/ml for the whole cultivation period. The SCE analysis was performed on 50 second-division cells (M2) after staining with a standard fluorescence-plus-Giemsa (FPG) method (Perry and Wolff, 1984).

(c) In the experiment on 455.7 MHz field and X-ray exposure, PHA-stimulated lymphocyte cultures were performed as in (a).

Statistical analysis

Analyses of chromosome aberrations were performed using a percentage test and Kastenbaum and Bowman tables (1970), whereas SCE were analysed with the Student's t-test and χ^2 test.

Results

Table 1 gives the results of the chromosome aberration analysis performed in lymphocytes from microwave-exposed whole blood samples from 28 different donors. The number of analysed metaphase figures and chromosome aberrations that were found for each donor (identified by