Genetic effects of radiofrequency radiation (RFR)

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Abstract

The possible effects of radiofrequency (RF) exposure on the genetic material of cells are considered very important since damage to the DNA of somatic cells can be linked to cancer development or cell death whereas damage to germ cells can lead to genetic damage in next and subsequent generations. This is why the scientific literature reports many investigations on the subject. According to a number of review papers, the conclusion so far is that there is little evidence that RFR is directly mutagenic and that adverse effects that were reported in some of the papers are predominantly the result of hyperthermia. Yet, some subtle indirect effects on DNA replication and/or transcription of genes under relatively restricted exposure conditions cannot be ruled out. Furthermore, the possibility of combined effects of RFR with environmental carcinogens/mutagens merits further attention.

The present paper takes into account more recent investigations but the conclusion remains the same. A majority of studies report no increased (cyto)genetic damage but yet, a considerable number of investigations do. However, many studies were not sufficiently characterized, are therefore difficult to replicate and cannot be compared to others. Experimental protocols were very different from one study to another and investigations from a single laboratory were very often limited in the sample size or number of cells investigated, preventing a robust statistical analysis. Subtle, but significant differences between RFR-exposed and sham-exposed cells cannot be found in such conditions. For the above reasons, it was concluded at a workshop in Löwenstein (November 2002) that further investigations by individual laboratories most probably will not add much to the discussion of radiofrequency radiation (RFR) genotoxicity. Large, well coordinated, international collaborative studies involving participation of several experienced scientists are considered an alternative of uttermost importance. One such study is now being planned.

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Introduction

Users of mobile telephones are exposed to radiofrequency electromagnetic fields. Although the average exposure levels are low compared to exposure limits, the rapid increase of personal telecommunication devices has activated discussion on the possible health risks of radiofrequency radiation (RFR). Possible increase of cancer risk is one of the main concerns. Some studies have reported cancer-enhancing effects of RF exposure in transgenic mice (Repacholi et al., 1997) but these findings are so far inconclusive, especially since replication studies were not able to confirm these results (Utteridge et al., 2002; La Regina et al., 2003). Additional animal experiments and well-sound epidemiological investigations are necessary. In this context, also the possible effects of RFR exposure on the genetic material of cells are considered very important as damage to the DNA of somatic cells cannot only be linked to cell death but also to cancer development. Furthermore, genetic effects in germ cells can lead to genetic damage in next and subsequent generations. This is why the scientific literature reports many investigations on the subject.

Genetic studies of RFR were conducted in vitro as well as in vivo and were devoted to RFR alone, as well as to the combined action of RFR with known chemical mutagens. A majority of the published reports suggested that exposure of mammalian cells and animals to radiofrequency radiation do not result in increased (cyto)genetic damage, assessed from DNA strand breaks, incidence of chromosomal aberrations, micronuclei and gene mutations. Most experimental studies therefore suggested lack of direct genotoxic effects from RFR exposure. Because of the very low quantum energy of RF radiation, this is not unexpected. Yet, some of the ‘positive’ data are intriguing and in need of clarification.

Furthermore, also non-genotoxic carcinogens are known and many agents are cocarcinogenic when delivered together with a genotoxic agent. Therefore, also investigations on combined effects of RFR and (chemical or physical) mutagens/carcinogens merit our attention.

Extensive reviews of the literature have been published (Brusick et al., 1998; Verschaeye, 2001; Verschaeye and Maes, 1998; Vijayalaxmi and Obe, 2004). Therefore, this paper will restrict itself to a short overview of the main investigations and conclusions on RFR-induced genetic effects alone or in combination with a known mutagen/carcinogen. Some considerations on further developments and research are provided.

Review of the literature

Cytogenetic effects in cells following in vitro RFR exposure

As for other studies in genetic toxicology, many investigations were performed on human blood lymphocytes. The main reasons are that they (1) are from human origin facilitating extrapolation to the human situation, (2) are readily available, (3) come everywhere in the body and may therefore reflect damage at different organs, and (4) that they repeatedly proved efficient in biomonitoring studies and investigations of genetic damage induced by chemical mutagens and (ionizing) radiation. It was for example demonstrated that an increased chromosome aberration frequency in human blood lymphocytes correlates well with an increased cancer risk in the considered human populations (Hagmar et al., 1994; Bonassi et al., 1995).

Investigations on (cyto)genetic effects of RFR-exposed human lymphocytes (chromosomal aberrations, sister chromatid exchanges and micronucleus induction) yield contradictory and often intriguing results. Many studies failed to find any indication of a RFR-induced genetic effect but some did. Among the ‘positive’ findings, studies from Garaj-Vrhovac et al. (1992), Maes et al. (1993, 2000), Zotti-Martelli et al. (2000) and Tice et al. (2002) are often cited. The results may, to a certain extend, all be attributed to hyperthermia rather than to the radiofrequency radiation. Yet, in recent years, especially the observation of RFR-induced micronucleus frequencies in resting lymphocytes following a 24h exposure (Tice et al., 2002) attracted a lot of attention, as this was corroborated by other investigations where non-thermal exposure conditions could be assumed (e.g., in cattle living near a radar station; Balode, 1996), and as no effects were found in the same (or related) investigation(s) with regard to other genetic endpoints (e.g., single strand breaks). As micronuclei may not only contain chromosome fragments, but also whole chromosomes, this could be indicative of a possible aneugenic action of RFR. In other words, RFR could be capable of inducing aneuploidy (e.g., due to unequal segregation of chromosomes during cell division resulting in daughter cells with an abnormal chromosome count). Aneuploidy was not extensively investigated so far with regard to RFR. However, recently, Mashevich et al. (2003) reported a linear and SAR (Specific Absorption Rate)-dependent increase in aneuploidy in RFR-exposed cells compared with sham-exposed cells and concluded that the RFR exposure induced aneuploidy via a non-thermal pathway. It should be noted that they used fluorescence in situ hybridization techniques applied to chromosome 17 only and that they found a 2.5-fold increase in aneuploidy for this single chromosome. Extrapolation to all human chromosomes will thus give an enormous yield of aneuploid cells in the cell population which can hardly be conceived. This investigation should therefore at least be replicated in another laboratory before any conclusion can be drawn. It should furthermore be noted that other investigations devoted to RFR-induced micronuclei (and hence, eventually to aneuploidy) failed to find any significant increase in the micronucleus frequency in...
RFR-exposed cells from human origin (McNamee et al., 2002a, 2002b, 2003, Bisht et al., 2002; Vijayalaxmi et al., 2001a, 2001b). Investigations in cultured rodent cells again gave positive (Garaj-Vrhovac et al., 1990a, 1991) and negative (Bisht et al., 2002) results.

It is also interesting to note that d’Ambrosio et al. (2002) found that the micronucleus frequency was not affected by continuous wave exposure but that cells exposed to pulsed waves did show a statistical increase in micronuclei. This suggests a differential response of the cells according to the RFR-modulation. Similar results were found in other studies related to, for example, extreme low frequency electromagnetic fields (e.g., Ivancsits et al., 2002).

Finally, very recent (and so far unpublished) results from EC’s 5th framework program were again contradictory. According to the PERFORM B program, no (cyto)genetic effects could be attributed to RFR, but in the REFLEX program, RFR-(as well as ELF-magnetic field) exposures showed genotoxic potential provided exposures are intermittent according to a particular “on/off” protocol and according to the investigated cell type. Blood lymphocytes were apparently not responding contrary to, e.g., fibroblasts. This was observed in two different laboratories and was interpreted as a very important finding and an explanation why most of the lymphocyte studies were negative. Unfortunately, a repeat study was already partially performed in a third laboratory where these findings could not be replicated (Scarfi, personal communication).

It may also be important to mention that some of the studies do show a reduced rather than increased cytogenetic effect in RFR-exposed cells compared to sham-exposed cells. Although this reduction was not necessarily statistically significant, it is observed quite often at ‘low dose exposures’ and may be indicative of some protective effect, e.g., as a result of the activation of DNA repair processes. This was for example found in experiments related to the PERFORM B project but also in other studies. An example is given by the work of Phillips et al. (1998) who used the alkaline comet assay to examine single strand breaks in the DNA of a variety of cells exposed to RFR. These authors found a decrease in single strand breaks at low SARs but an increase, at least in some experiments, at high SARs compared to sham-control cells. They interpreted these findings as a ‘protective’ effect of RFR. The comet assay was also applied by others in rodent as well as human cells and this immediately after exposure as well as at 4 h post-exposure (based on in vivo studies, see below). All studies failed to find RFR-induced DNA damage (e.g., Maes et al., 1997; Malyapa et al., 1997a, 1997b; Vijayalaxmi et al., 2000; Li et al., 2001; Tice et al., 2002; McNamee et al., 2003).

As mentioned above, also investigations on combined effects of RFR and (chemical or physical) mutagens/carcinogens merit our attention. Theoretically, it may indeed be well that RFR exposure is not genotoxic but may enhance the cytogenetic damage induced by other chemical or physical agents. This was investigated on several occasions. In a series of in vitro studies, the group of Meltz at St. Louis found no indication of a collaborative (or synergistic) effect of RFR and the chemical mutagens adriamycin, mitomycin C (MMC) and profalin, or UV-radiation in Chinese hamster ovary cells, human diploid fibroblasts or L5178Y mouse leukemia cells (see references in Verschaeve, 2001). In these studies, the RFR and chemical exposures were simultaneous. On the other hand, Maes et al. (1996, 1997, 2000) have investigated human blood lymphocytes that were RFR-exposed before cells were cultivated in the presence of MMC. In a first investigation, a clear enhanced SCE frequency was found in the cells that were exposed to both agents compared to cells that were exposed to MMC alone (Maes et al., 1996). This was found in blood from 8 different donors and was therefore highly reproducible. These results were confirmed by Zhang et al. (2002) in a later investigation. However, other investigations by Maes et al. gave less clear results that actually varied between a ‘weak collaborative effect’ up to absence of any collaborative effect (Maes et al., 1997, 2000). The reasons for differences in response as observed in ‘similar’ experiments performed by the same investigators are unknown. Differences in experimental protocol, RFR-frequencies or exposure regimes may certainly be among the possible explanations.

Cytogenetic effects in animals following in vivo RFR exposure

Cytogenetic damage was assessed in short term and chronic exposure experiments using ‘normal’ and transgenic animals. Again, contradictory data were obtained. No increase in micronucleus frequency was found in bone marrow cells from rats that were exposed to RFR for a continuous period of 1 day (Vijayalaxmi et al., 2001a, 2001b). The same authors also failed to observe an increase in micronuclei in bone marrow cells of rats in a chronic near field exposure (Vijayalaxmi et al., 2003) but Trosic et al. (2002) reported a significantly increased micronucleus frequency in the peripheral blood of rats exposed to RFR for 2 h/day during an 8 days period. After 15 and 30 days, no induced micronucleus frequency was found. This was explained as an adaptive or recovery mechanism in the rats. On the other hand, an investigation on chronic RFR-exposed C3H/HeJ mice showed a small but statistically significant increase of micronuclei in polychromatic erythrocytes (Vijayalaxmi et al., 1998). These mice were chosen because of their predisposition to develop mammary tumors and their possible ‘hypersensitivity’ to RFR. Because all micronucleus indices were within the spontaneous range of historical controls and because of the lack of any correlation with the carcinogenicity data in the same animals, the biological relevance of the results should be questioned. Sykes et al. (2001) also found some indication of RFR-induced genetic effect in pKZ1 mice. In this study, a
reduction below the spontaneous frequency of intra-chromosomal recombination inversion was found in sections of the spleen. The biological significance of this finding also remains speculative.

The rationale behind the 5th framework CEMFEC project was that classical carcinogenicity studies are not likely to produce much new information given the evidence against direct genotoxicity of RFR. Animal models assessing cocarcinogenesis will more likely reveal the effects if there are any. Such studies – if negative – would also be much more convincing evidence against the existence of carcinogenesis-related effects. The 5th framework CEMFEC program included investigations of cytogenetic effects in the blood, liver and brain of RFR + MX\(^1\) exposed rats. Here, micronuclei were investigated in the blood of rats that were exposed to RFR and the chemical carcinogen MX for 2 h/day during 3, 6 and 24 months. There was no increased incidence of micronuclei compared to animals that were exposed to MX alone or to cage control animals. The alkaline comet assay also did not show any increased incidence of DNA damage in the rat blood, neither in liver nor brain cells (after 24 months exposure). On the contrary, this study also provided some indication of a DNA protective effect in the rat blood (essentially after 3 and 6 months exposure). There was also no indication of a RFR cocarcinogenic activity (results to be published).

Above mentioned investigations on DNA single and double strand breaks using the comet assay were conducted following the initial reports of Lai and Singh who examined the brain cells of rats exposed to RFR. These studies certainly need to be mentioned in this short review. Lai and Singh found a significant increase in DNA strand breaks immediately (in one experiment) and at 4 h post-exposure (Lai and Singh, 1995, 1996a) and suggested that this could be due to either a direct effect on the DNA and/or an effect of the radiation on DNA repair mechanisms (Lai and Singh, 1996a). Furthermore, they provided data suggesting that free radicals may play a role in the observed DNA single and double strand breaks as the addition of free radical scavengers reduced the effect (Lai et al., 1997). The fact that effects were observed at 4 h post exposure was especially criticized (Williams, 1996) although arguments in favor of the findings were subsequently presented by Lai and Singh (1996b). In a replication study, Malyapa et al. (1998) failed to confirm the earlier data. This was attributed to differences in procedure (especially differences in the way the animals were killed and in the time lag between the death of the rats and dissection of the brain and slide preparation for the comet assay). Other similar experiments were conducted since (e.g., CEMFEC project) but also failed to find RFR-induced DNA damage.

\(^1\) MX = 3-chloro-4-(dichlormethyl)-5-hydroxy-2(5H)-furanone. It is a by-product of water chlorination. It is strongly mutagenic in vitro and has been shown to be carcinogenic in rats.

Cytogenetic effects in humans

An increased incidence of chromosomal aberrations and micronuclei were found in peripheral blood lymphocytes from individuals who were occupationally exposed to RFR (Garaj-Vrhovac et al., 1990b; Fucic et al., 1992) but in other investigations, this was not found (Garson et al., 1999; Maes et al., 1995). It is generally assumed that these kinds of investigations are of uttermost importance for assessing RFR-genotoxic effects in humans (e.g., Royal Society of Canada, 1999), but abovementioned studies should be considered inconclusive, not only because of the different outcome, but also due to insufficient dosimetry, omission of potential confounders and inadequate sample size for statistical power analysis. In a recent investigation (manuscript submitted for publication), Maes et al. have extended the sample size of the exposed individuals. Here, also no cytogenetic damage was found above background level but further investigations are certainly necessary to come to a more definite conclusion.

Evaluation of the literature data

So far, a rather great number of cytogenetic investigations were already devoted to RFR radiations, including those from “mobile phone frequencies”. Most studies are negative suggesting that RFR is not directly mutagenic and that adverse RFR effects are predominantly the result of hyperthermia. However, there is too much controversy yet to allow a ‘definite’ conclusion. Reasons for the existence of controversial data may be that in some of the reports important experimental details which are critical for the independent verification were either inadequately or non-described, such as, RFR exposure conditions, dosimetry, specific absorption rate and temperature measurements (Vijayalaxmi and Obe, 2004). Hence, it is not always possible to estimate the exposure conditions adequately, and, e.g., discriminate between thermal or non-thermal exposures which may certainly account for differences in response of cells or organisms. Furthermore, it is clear that variables exist in experimental protocols in terms of the frequency applied, the modulation, investigated genetic endpoints, cell type used, etc. At least some papers tend to attribute the controversial results to these differences. Also, there is a concern that the numbers of cells that were examined by the investigators were not sufficient enough to bring out subtle but significant differences between RFR-exposed and sham-exposed cells. Therefore, the idea of an international collaborative study among different independent investigators with expertise in cytogenetics was launched at a workshop on Genetic and cytogenetic aspects of RF-field interaction in November 2002 (see http://www.cost281.org/documents.php?node=39&dir_session). It was assumed that more ‘individual’ experiments will add little to the scientific discussion as each will only bring one more negative or positive result that does not make a big
difference with regard to the overall picture. With such studies, the controversy will most probably remain. Only a large scale collaborative research can avoid most of the drawbacks or disadvantages of these studies and will be able to reach a generally accepted consensus, at least for the experimental protocol and cell systems used. The International COST 281 framework for international research and development cooperation on “Potential Health Implications from Mobile Communication Systems” formulated a recommendation for such a “coordinated research on genotoxic effects of electromagnetic radiation from Mobile Communication Systems” (see http://www.cost281.org/activities/Gentox-recomm-090304AW.doc). Such a collaborative study is foreseen for the near future provided sufficient funding can be obtained.

Conclusion

Although most of the investigations on genetic and cytogenetic effects of RFR do indicate that these electromagnetic fields are not capable of inducing any kind of genetic effect and also do not enhance the effect of chemical or physical mutagens/carcinogens, the scientific data remain sufficiently controversial to exclude any potential RFR-genetic hazard. It is assumed that only large-scale research under well controlled conditions and allowing the generation of results with sufficient statistical power may lead to a better risk estimate.

References


