Biological Effects of Radiofrequency Fields

In Vitro Studies

Since laboratory conditions are easier to set up for *in vitro* studies, and because apparently simpler and more stable biological systems can be studied with this approach, there is an exceedingly large experimental literature on RF interactions on these aspects, which we do not intend the review in detail here. Recent exhaustive reviews by other authors have been published elsewhere and the reader is referred to them (see particularly D'Inzeo, 2009; Marino, 2008a and 2008b; ICNIRP, 2009).

In vitro studies try to answer the fundamental question which is at the roots of all putative RF bioeffects: if these effects cannot be demonstrated clearly and without doubt at the level of molecules or isolated cell preparations, there should be no reason to continue the scientific search for these effects at other levels of complexity in living organisms.

D'Inzeo (2009) proposed a layered or hierarchical model for interpreting results according to these levels of complexity, whereas

> *"Interaction models aiming at the evaluation of possible health consequences have to take into account the complex organization typical of living systems. All biological systems must be considered, from a logical point of view, as a stratification of complexity levels, from the microscopic one of atoms and molecules, up to the macroscopic one of the whole organism, going through sub-cellular structures, cells, tissues, organs, and systems. (…) Due to the complex structure of biological systems, for electric or magnetic fields to initiate or promote adverse health effects in an organism, they must trigger a series of steps, through different levels of biological complexity, from molecular level up to cell, organ and organisms"*

These levels are:

- Small inorganic molecules and ions at the atomic, physico-chemical level
- Large organic molecules, such as proteins and nucleic acids
- Cell membrane, including receptors, ionic gates and active transport mechanisms
- Metabolic function and biochemical pathways in the cellular cytoplasm and organellesl
- Aggregates of cells, such as tissues, networks of excitable cells, immune networks, etc

Furthermore, even if they are demonstrated to exist at a certain level, this doesn't mean automatically that they are significant or influence other levels of complexity above them. Also, according to D'Inzeo, *"(Although) the functionality of each level is related to those of all lower levels it is not completely determined by them, i.e. each upper level shows the so-called emergent proprieties."*

From a didactic point of view, possible bioeffects of RF fields at the molecular level have been classified into two main types:

1) **thermal effects** due to the dielectric heating phenomenon that is typical of non-ionizing radiation (NIR), such as microwaves. NIR has not sufficient quantized energy to interact with the outer orbitals of atoms and break intra- or extra-molecular bonds, so radiation-induced agitation of polar molecules accounts for temperature enhancement as its only plausible effect. This has been well established to occur, even for very small temperature changes, since cells have a complex mechanism to respond to them, including molecular cascades, heat shock proteins, etc. With exposure to large temperature changes for a sufficient time, denaturation of some molecules, such as proteins, may ensue. This explains the cooking effect on foods by a microwave oven. For small thermal heating, which might occur when cells are exposed to low-level RF, only indirect effects such as these are plausible and they have been sufficiently well documented;

2) **non-thermal effects** have been theoretically proposed as other interaction mechanisms not due to direct or indirect increases in local temperature. A large number of these models have been proposed and experimentally studied in *in vitro* preparations. A number of authors have claimed that they could be demonstrated, but this is still an open debate in the scientific community, since many studies have also been unable to demonstrate that they exist. In many cases, it has been argued that these effects are actually due to normal responses of living cells to heating. As shown below, of all non-thermal effects that have been reported to occur in cells, such as changes in enzyme levels, none have been shown to have any health consequence, since the body easily compensates for them.

Thus, in relation to low level RF, the debate centers on

1) the existence of non-thermal effects at a given level of organization,

2) whether they are of sufficient magnitude and

3) whether they interact with other levels of complexity above it in order to play a role in pathophysiology and causation of disease in intact (in vivo) organisms.

As we will examine later on, the scientific answers to all these three questions have been overwhelmingly negative.

Recently, more than 100 papers in 15 different journals on mechanisms of molecular and cellular action have been reviewed by D'Inzeo (2009), allowing for the classification of putative non-thermal effects into four groups of models:

- resonance mechanisms;
- coupling with non linear systems;
- effects due to the direct action of electric and magnetic fields;
- cooperative mechanisms due to interactions among several membrane components

All have been documented experimentally, sometimes at exceedingly low power density levels of exposure, but authors and reviewers diverge widely as to the most plausible mechanism for non-thermal interaction of EMF with matter. D'Inzeo concludes that "*however, such results are hardly extendable to higher levels of biological complexity and thus to possible hazardous effects on human health."*

Other recent reviews, by Swicord & Balzano (2009) and by ICNIRP (2009), examined in detail the current evidence in published literature which supports mechanisms of interaction of RF with living matter both at cellular and organism level. The reader is referred to these two comprehensive reviews for more detailed coverage and analysis. In the following pages we will review some of the more significant findings.

Oncogenesis Studies at Molecular and Cellular Level

Since cancer-related effects of EMF are considered particularly important, the potential impact of cellular and sub-cellular effects for oncogenesis have been given a high importance in international research, and, accordingly a large literature body has been produced. Marino (2009) has reviewed the literature on these aspects before 2000 and from 2000 to 2007. The review has classified the papers according to a four-point scale (comprising Sufficient Evidence, Limited Evidence, Inadequate Evidence, and Evidence Suggesting Lack of Effect) in order to describe the degree of uncertainty for the effects reviewed. The scale has been adapted from one developed by the International Agency for Research on Cancer (Repacholi and Cardis 1997)

Oncogenesis at subcellular and cellular levels is exceedingly complex and still under study. Cancer is in fact a generic denomination to probably hundreds of different diseases, with different causes and different natural histories. However, a common denominator is genetic instability, caused by a cumulative chain of changes in intracellular DNA-repair mechanisms, activation of tumor inhibition genes or expression of oncogenes, the apoptosis of defective cells, the reproduction, growth and survival mechanisms of cells, etc. Eventually, such accumulated changes in the genomic machinery of cells lead to a cell line that inherits the changes and gains reproductive advantage over normal cells and do not die (**tumorigenesis**).

In vitro studies can be classified into six areas of inquiry and experimentation.

Genotoxicity: is the name given to the property of external agents, such as EMF, to damage directly DNA. DNA damage can be evaluated experimentally by the so-called comet assay, which identifies whether base damage and single-strand breaks in the DNA molecule inside the cell nucleus have occurred (neutral comet test) or double-strand breaks have occurred (alkaline comet test). The first indicates repairable damage, while the latter is non-repairable, and thus more dangerous damage to DNA.

Another common class of experiments is to test whether low-level RF is able to potentiate genotoxicity induced by a second known genotoxic agent, or in already abnormal (cancerous) cell lines. Another way of testing genotoxicity is investigating for the appearance of micronuclei and aneuploidy (abnormal number of chromosomes), which are related to DNA alterations. A number of experiments on genotoxicity of low-level RF published before 2000 have demonstrated effects (usually very weak or difficult to interpret due to technical inadequacies, improper controls, etc), but there are also many experiments that didn't show any genotoxic effect, including replication or confirmation studies or previously positive ones (in some cases by the same authors under exactly the same conditions (Marino, 2008a). Her recent review of 83 published papers on genotoxicity have revealed that 69% of them reported absence of effects, 20% reported presence of effects, and the rest were inconclusive. A general evaluation following the classification of evidence used by IARC (International Agency for Research on Cancer) arrived at the conclusion that there is so far inadequate evidence for a low-level RF

interaction causing genotoxicity as well as potentiation of other mutagens. Therefore, since oncogenesis depends strictly on its occurrence at cellular level, there is no plausible mechanism for cancer causation at or below international safety levels.

Even so, REFLEX, a high-profile European research on genotoxicity, which alleged finding evidence of double strand DNA breakage in cultured human fibroblasts by EMF in the cell phone range, made the news worldwide in 2008 (Schwarz *et al.*, 2008), but was later found to have unblinded and possible fabricated data. This demonstrated the perils of flawed research techniques when biased expectations are in play.

Another parameter for carcinogenicity in cell lines is the transformation potential of external agents (*i.e.*, transforming healthy cells into neoplastic ones). This can be investigated either by assessing direct effects (initiation) or whether RF increases transformation under other known agents (promotion or co-promotion). The absolute majority of experiments reported so far were unable to detect neoplastic transformation from microwave signals used in mobile communication; thus reviewers have concluded that there is lack of evidence for these effects.

Cancer-related gene and protein expression: a more modern and technically superior testing of carcinogenicity potential of exposure to low level RF, including the use of high-throughput techniques for investigating simultaneously the expression of thousands of genes and several proteins related to cancers (genomics and proteomics), have been used recently (i.e., after the year 2000). Up to 10,000 genes can be tested simultaneously using so-called micro-array probes. Among the genes of importance for cancer are the proto-oncogenes inside the cell genome, such as *c-fos*, *c-jun* and *c-myc*, and proteins such as P53, related to cancer suppression. When a gene is found to be up-regulated, it means that it is expressing at a higher rate than normal (i.e. synthesising more of its related protein); when it is down-regulated it is the contrary. A detrimental effect can be evidenced by either increased or decreased expression, but generally the results are not easy to interpret.

The results so far have been highly heterogeneous: some experiments using large scale testing of gene expression have found in some cases complete absence of effect of low-level RF, while others have found up-regulation and down-regulation in a significant proportion of genes. For example, Zhao R. et al. (2007) investigated the effects of intermittent exposure of cultured rat neurons to RF at a SAR of 2 W/kg on gene expression. Among 1,200 candidate genes, 24 up-regulated genes and 10 down-regulated genes were identified. Several other papers have identified mostly the up-regulation of apoptosis-related genes (expressing caspase proteins), while down-regulation was related to cell cycle functionalities. Validation data was lacking in most of these positive studies, so their significance is hard to interpret. In contrast to these studies others have failed to provide evidence for significant changes in gene expression using microarray technologies. For example, Gurisik et al (2006) found changes in only 6 of 8,400 genes tested, and even then they were only slightly down-regulated.

The finding of a large number of altered genes or proteins, without a consistent pattern, also points to nonspecific effects, most probably due to heat shock, although experimenters have claimed to control for temperature changes. The alteration of proteins of the heat shock proteins (hsp) family in many of the positive studies provides evidence that such might be the case, particularly because they are altered when no RF irradiation is present, but that the temperature of the culture medium is raised. In addition, the

pathological significance of such gene expression is unknown and difficult to interpret. In general, reviewers have concluded that the evidence is limited or there is a lack of consistent evidence for cancer-related low level gene and protein expression from RF exposure.

Cell proliferation and differentiation: These are two important characteristics of neoplastic cell lines: an increase in cell proliferation, leading to tumour growth, in general, and the decrease in differentiation (*i.e.,* de-differentiation of neoplastic cells, increasing its resistance to chemo- and radiotherapy, its metastasis potential and its overall malignance). The most common cell lines investigated are normal human or murine fibroblasts (cells from the conjunctive tissue) and some neoplastic cell lines, such as lymphoblasts and neuroblastomas. Again, there is wide variability of results among different papers, which are difficult to interpret, since the methods and conditions used could not be adequately identified or compared, including the most important controls for exposure; level of power density and controls for temperature variation. The reviewers concluded that "there is globally inadequate evidence for positive effects of low-level RF on these parameters.

Apoptosis: is programmed cell death, *i.e.,* a very specific and complex chain of intrinsic and extrinsic cellular events that induce defective cells to "suicide". The contents of ruptured cells are digested by cells of the immune system, such as macrophages. A line of enzymes (caspases) are involved in the process, so they can be evaluated in experimental assays, thus providing indirect evidence of the level of apoptosis activity in a given tissue or cell culture preparation. Apoptosis in normal cells as well as in cancerous (neoplastic) cells of tumour lines have been investigated following exposure to low- and high-level RF. A potential induction of apoptosis in normal cells is considered deleterious, while it is considered beneficial in tumor cell lines (this could be the explanation for the efficacy of some kinds of instrumented irradiation such as X-rays and gamma rays, in decreasing tumor growth, since one of the main defects observed in cancer is the production of cells that are incapable of apoptosis, i.e., "eternal" cell lines).

Again, the overall results of the assessment of published papers on this subject are that there is no evidence that low-level RF exposure could induce apoptosis in normal cells limited evidence that RF acts as a pro-apoptotic agent in tumoral cells, and inadequate evidence that low-level RF exposure may interact with known pro-apoptotic agents and/or on the genetic background in vitro (Marino, 2008a).

Conclusions of *In Vitro* Studies

The current scientific evidence on molecular and cellular mechanisms of RF has been evaluated by several international specialized bodies. In all of them so far, conclusions hav been the same, such as:

Sweden SSI (2008) Recent Research on EMF and Health Risks- Fifth Annual Report from SSI: Independent Expert Group on Electromagnetic fields, 2007(Revised edition 15 April, 2008
http://www.stralsakerhetsmyndigheten.se/Global/Publikationer/Rapport/Stralskydd/2008/ssi-rapp-2008-12.pdf

Most of these studies have not demonstrated effects of RF exposure on the studied outcomes, including also attempts to replicate the genotoxic effects observed in the REFLEX European program.

Six recent studies on carcinogenicity, some with higher exposure levels than previously used, consistently report lack of carcinogenic effects, and two studies on genotoxicity report no increase in micronuclei or DNA strand breaks after RF exposure.

ICNIRP (2009): "Exposure to high frequency electromagnetic fields, biological effects and health consequences (100 kHz-300 GHz)"
http://www.icnirp.de/documents/RFReview.pdf

The mechanisms by which RF exposure heats biological tissue are well understood and the most marked and consistent effect of RF exposure is that of heating, resulting in a number of heat-related physiological and pathological responses in human subjects and laboratory animals. Heating also remains a potential confounder in in vitro studies and may account for some of the positive effects reported

Research in Latin America

Very few experimental in vitro studies of bioeffects of RF have been carried out in Latin America. Working at the State University of Campinas in Brazil, Heinrich and collaborators (2006, preliminary communication WHO-EMF database) have studied the effect of microwave radiation emitted by cell phones on the chromosomes of human lymphocytes in vitro. Spectral karyotyping was used for this purpose. The researchers concluded that no chromosomal damage could be observed at levels compatible with or below the ICNIRP standards, at least for 800 MHz AMPS CDMA devices. Levels exceeding 10 W/kg were observed to cause some damage indicating a dose dependent effect on increasing acrocentric chromosomes and altering satellite length

Conclusions

As judged from the available literature on oncogenesis-related cell function and exposure to low-level RF, the general conclusion is that there is, so far, inadequate evidence or lack of consistent and validated evidence, that such a cause-effect relationship can be established. There is some confusion and controversy in this area of research, because many times experiments that had rendered positive effects could not be validated or replicated.

Particularly, short-term effects on cell cycle and regulation, gene and protein expression, damage to genetic material and transformation/dedifferentiation cannot be automatically translated to causation of cancer. For example, most of the cell cultures used in these experiments are highly susceptible to any external agent, such as cells from the hematopoietic system. Even small temperature changes affect them, but this probably happens when they are irradiated when cultured externally, thus removing the strong protection from inside the body, due to the homeostatic mechanisms. Thus the relevance of these results for human health can be contested.

In the following section we will discuss cancer-related studies in intact *(in vivo*) organisms.