Exposure of Two Cell Lines
to Intermittent 1.8 GHz Mobile Phone Signals:
Evaluation of Different Biological Endpoints

Paola Valbonesi*, Silvia Franzellitti*, Ferdinando Bersani*, Andrea Contin*,+ and Elena Fabbri*
*Interdept. Centre for Research in Environmental Sciences, University of Bologna, 48123, Ravenna, Italy
+Department of Physics, University of Bologna, 44100 Bologna, Italy

Summary— We evaluated different biological endpoints in cells exposed to 1.8 GHz mobile phone signals, looking for a possible marker to infer their biological effects. Among the signals applied, the 217-GSM signal induced some positive responses. Interestingly, it caused HSP70 gene overexpression in two cell lines with different physiological functions, reproductive and neural.

I. INTRODUCTION

The potential health risks of exposure to high frequency electromagnetic fields (HF-EMF) associated with mobile telephony have become a great public concern. One area of particular interest relates to the ability of HF-EMF to induce a cellular stress response, which could conceivably cause alterations in cell physiology. Our research group evaluated different biological endpoints in cells exposed to intermittent 1.8 GHz mobile phone signals, looking for a possible candidate to infer biological effects of HF-EMF.

II. BIOLOGICAL MODELS

As a biological model we used two different cell lines:

1- HTR-8/SVneo cells, derived from human trophoblast, represent a good model for the in vitro study of molecular mechanisms at the basis of placentation. In particular HTR-8/SVneo cells sensitivity to external stimuli make them an attractive model for investigating possible detrimental effects of HF-EMF on cell physiology and more specifically gestation processes.

2- PC12 cells, tumoral cells derived from rat pheochromocytoma, are widely used for studying nervous system functions and effects of neurotoxic agents and are therefore employed by several research groups as a neuronal model to study the molecular mechanism that mediate HF-EMF biological effects.

III. HF-EMF EXPOSURE SYSTEM

Cells were exposed to HF-EMF in an exposure system (Fig 1) developed and built by IT’IS Foundation (Zurich, Switzerland), following the specifications outlined by Schönborn et al. [1]. All the experiments were performed at a carrier frequency of 1.8 GHz, with intermittent exposure (On/Off cycles of 5/10 min), at a time-averaged SAR of 2 W/kg. The following signals were applied:

1- continuous-wave (CW) signal (1817 MHz carrier frequency only).

2- GSM-217Hz (speaking only): signals were amplitude modulated by rectangular pulses with a repetition frequency of 217 Hz and a duty cycle of 1:8, following the Time Division Multiple Access (TDMA) GSM scheme. Every 26th frame is idle, adding an 8 Hz modulation to the signal.

3- GSM-Talk (33% of speaking and 67% of hearing): GSM-Talk generates temporal changes between GSM-217 Hz and GSM-DTX and simulates a typical conversation with average periods of speaking and of hearing. The DTX mode (discontinuous transmission) is active during periods of non-speaking into the phone. The transmission is reduced to 12 active frames per 104 frames. The frame structure of the DTX signal results in 2, 8 and 217 Hz modulation components.

Fig. 1 The waveguide resonators constituting the HF-EMF exposure system inside the incubator.

IV. RESULTS

1- HSP70 expression: 70 kDa Heat Shock Proteins (HSP70) expression is a sensitive and rapid response indicator of cell stress, therefore HSP70 overexpression was suggested as a possible marker of HF-EMF exposure. In HTR-8/SVneo cells we reported effects on HSP70 transcript after prolonged (4 to 24 h) exposure to amplitude-modulated GSM signals. In particular we observed that levels of the inducible HSP70 transcript were significantly enhanced after 24 h of exposure to GSM-217Hz and reduced after 4 and 16 h of exposure to GSM-Talk signals [2]. Conversely, these effects were not detected after 1 h of exposure [3]. HF-EMF exposure did not affect HSP70 protein expression. Raising the possibility that HF-EMF impacts specific cell subpopulations or cell types, the World Health Organization encourages the
employement of rigorous quantitative methods on different cells to confirm positive results [4]. In fact, cells of different types, species, genetic background, metabolic activity, might respond differently to mobile phone radiation or might have different sensitivity to this weak stimulus. Therefore we applied the same 1.8 GHz signals for 4-24 h, to PC12 cells. Ongoing experiments indicated that also in PC12 cells the response to HF-EMF takes place only at the mRNA level and after prolonged exposure to the GSM-217 Hz signal.

2- connexins expression and localization: connexins (Cx) are membrane proteins able to influence trophoblast functions. Cx expression is affected by chemical pollutants, ionizing radiations and other environmental stresses, therefore we exposed HTR-8/SVneo cells to the GSM-217 Hz signal for 1 h and reported that this irradiation can selectively modify Cx mRNA expression pattern and protein localization, but not Cx protein expression [5].

3- DNA fragmentation: one of the most controversial issues regarding EMF exposure is their ability in altering DNA molecule integrity. We reported a transient and reversible increase of comet protein products (by Alkaline Comet assay) after prolonged irradiation of HTR-8/SVneo cells with 1.8 GHz signals [6]. In particular, the un-modulated CW was ineffective, whilst the amplitude-modulated signals GSM-217 Hz and GSM-Talk induced a significant increase in comet parameters in HTR-8/SVneo cells after 16 and 24 h of exposure. Conversely, these effects were not detected after 1 h of exposure [3]. Alterations were rapidly recovered and the DNA integrity of irradiated cells was similar to that of sham-exposed cells within 2 h of recovery in the absence of irradiation. We hypothesised that the EMF-induced DNA fragmentation do not lead to chromosomal aberrations or reduction of cell viability. Differently, is related to DNA repair phenomena and induced in parallel with expression of genes and related repair proteins.

4- AChE enzymatic activity: acetylcholinesterase (AChE) activity was assessed as possible target of EMF in numerous studies. After EMF exposure, AChE activity is reduced in some cellular models and increased in others. It may be hypothesized that the electrostatic features of AChE have some role in this phenomenon. PC12 cells are a good model for studying AChE activity, because they synthesize and secrete acetylcholine and the degradative enzyme AChE. A few evidence obtained in our laboratory point to a stimulation of the enzyme activity in PC12 cells after exposure to 1.8 GHz signals.

5- MAPK phosphorylation: stress induced pathways mediated by HSP70 and Myogen Activated Protein Kinases (MAPK) are strictly correlated between each other. For this reason, after assessing the effects of HF-EMF on HSP70 gene expression, also the MAPK-dependent signaling pathway was examined in irradiated PC12 cells. Ongoing experiments suggest that HF-EMF exposure do not affect the phosphorylation status of MAPK in PC12 cells.

V. CONCLUSIONS

Some of the biological endpoints assessed by our research group gave a positive response after cell exposure to HF-EMF (those obtained after application of the GSM-217 Hz signal are summarised in Table I), but these responses are probably too weak to have a significant impact on cell physiology.

Indeed, the HSP70 gene overexpression induced both in HTR-8/SVneo cells and in PC12 cells, represents a weak effect, considering the lack of a corrspondent overexpression of the protein product; the same it’s true for the up-regulation of Cx transcripts. Moreover, alterations induced by HF-EMF on DNA integrity were rapidly recovered by HTR-8/SVneo cells within a 30- and 120-min incubation in the absence of irradiation.

It is worth noting, however, that the same modulation scheme, namely the 217-GSM signal, induced the same effect, HSP70 overexpression, in two cell lines with different physiological functions (reproductive and neural), which were exposed and analyzed in different times, using the same apparatus. Cells exposed to 217-GSM showed also further positive effects on Cx gene expression and protein localization, DNA molecule integrity and AChE activity. Such weak but repeatable effects deserve further investigations, mainly concerning the identification of the targets for EMF interaction, and a possible role of signal modulation.

REFERENCES


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<th>Biological endpoint</th>
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$^{a}$ experiments performed on HTR-8/SVneo cells; $^{b}$ experiments performed on PC12 cells; o.e.: ongoing experiments

Table 1: Biological Effects of Cell Exposure to GSM-217 Hz Signal.