# EFFECT OF GSM-900 RADIOFREQUENCY ON APOPTOSIS OF IMMUNE AND NERVOUS CELLS[1].

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## INTRODUCTION

Apoptosis, or programmed cell death, is a major mechanism of protection against genotoxic agents since potential cancer cells are removed by apoptosis. Dysregulation in the apoptotic pathways is also involved in different pathologies. Hence, excessive apoptosis can contribute to diseases such as AIDS or neurodegenerative diseases [1], whereas default in apoptosis is involved in cancer or autoimmune diseases.

The exponential development of mobile telephony has raised the concern of possible health hazards related to exposure to such environmental electromagnetic fields. Therefore, we focused our research on the investigation of the potential role of GSM-900 radiofrequency (RF) fields on apoptosis in immune and nervous cells, namely in the U937 human lymphoma cell line, as well as in C6 rat glioma cells and primary neurones and astrocytes.

## EXPERIMENTAL STRATEGY

### Exposure to GSM-900

GSM-900 was generated in a wire-patch antenna (Fig. 1, [2]). GSM-900 was amplitude modulated by rectangular pulses with a repetition frequency of 217 Hz and a duty cycle of 1:8 (pulse width 0.576 ms). Temperature measurement in the culture medium was performed throughout the exposure duration using optic-fibre Luxtron probes that are immune to the microwaves.

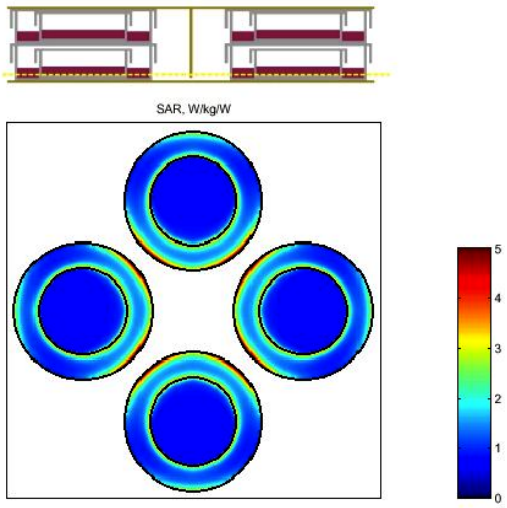


Fig. 1 : Upper panel shows a transversal scheme of the wire-patch antenna. Lower panel gives an example of numerical FDTD modelling for SAR in the wire-patch antenna

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Two protocols were performed: (i) U937 cells (ATCC n° CRL-1593.2) were grown in DMEM-HamF12 medium (supplemented with 10% fetal calf serum (FCS), antibiotics and non-essential amino acids) and exposed to GSM-900 microwaves for 48 hours at a specific absorption rate (SAR) of 0.7 W/kg. In these cultures, camptothecin (4 µg/ml, 4 hours) was used as positive control for apoptosis (ii) Primary neuronal cells were prepared from postnatal day 4-5 rat cerebella and grown in HMEM medium supplemented with 10% horse serum and antibiotics. Primary glial cells (astrocytes) were obtained from postnatal day 9 rat cerebella and cultured in DMEM medium supplemented with 10% fetal calf serum and antibiotics.
Primary nervous cells were submitted to a 1-hour exposure to GSM-900 at a SAR of 2 W/kg. C6 rat glioma cells (ECACC N° 85040101) were grown in DMEM / Ham's F12 medium (with 10% FCS, antibiotics and 2 mM sodium pyruvate).

**Detection of apoptosis**

The occurrence of apoptosis was assessed immediately after exposure to GSM-900 using Annexin V/PI and $DiOC_6(3)$/IP staining.
During apoptosis, phosphatidylserine is exposed on the outer leaflet of the plasma membrane that causes a loss of membrane asymmetry. Annexin V binds to phosphatidylserine and can be detected by flow cytometry using the Annexin-V-Fluos® kit (Roche Diagnostics, France). Immediately after exposure, cells were washed with PBS and centrifuged at 200 g for 5 minutes. Cell pellet was resuspended and $10^6$ cells were incubated for 10-15 minutes in 100 µl of labelling solution (2 µl of Annexin-V-Fluos® in 100 µl of a Hepes buffer [10 mM Hepes/NaOH, pH 7.4, 140 mM NaCl, 5 mM $CaCl_2$]. 400 µl of Hepes buffer containing 2.5 µl of Propidium Iodide (PI, 50 µg/ml) were then added and samples were analysed on a FacsCan® flow cytometer.
Mitochondrial physiology is disrupted in cells undergoing apoptosis. Mitochondrial permeability is altered resulting in a decrease of the mitochondrial membrane potential ($\Delta\Psi_m$) that can be measured using the carbocyanine dye $DiOC_6(3)$. Briefly, immediately after exposure, cells were washed and centrifuged as indicated above. Then $2x10^5$ to $10^6$ cells were incubated for 10-15 minutes in 500 µl of PBS containing 40 nM of $DiOC_6(3)$ and PI (50 µg/ml) was added before the sample was analysed. Data analysis was performed in a blind manner (analysis without the knowledge of the exposure conditions).

**RESULTS**

**Effects of GSM-900 on apoptosis in U937 cells**

Data from three blind experiments show that no significant induction of apoptosis was detected in U937 cells immediately after a 48 hour-exposure to GSM-900 compared to sham-exposed samples whatever the marker used (Fig. 1). By contrast, about 15% of U937 cells underwent apoptosis when treated for 4 hours with the apoptogenic agent camptothecin.

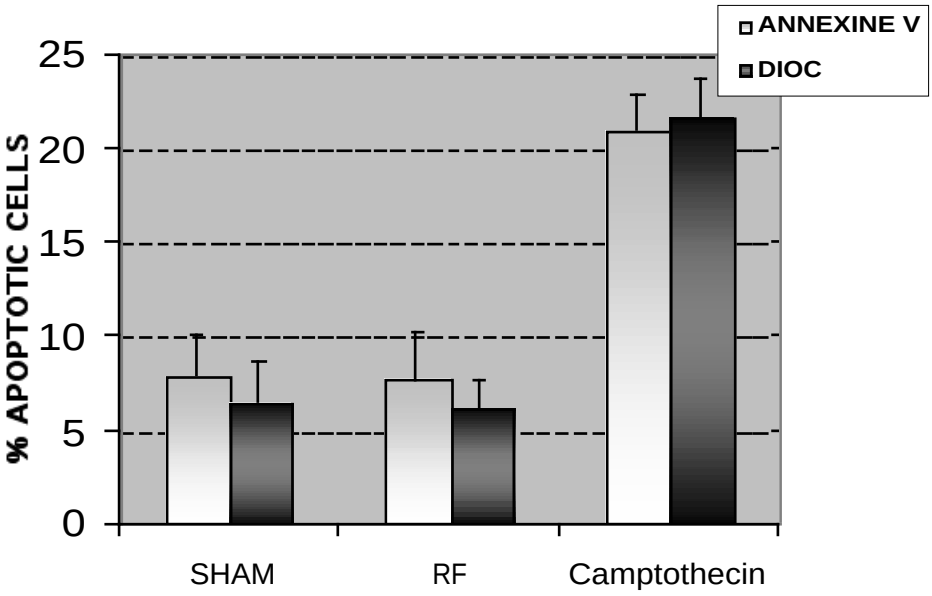


Fig. 1: Detection of apoptosis in U937 lymphoblastoma cells immediately after a 48-hour exposure to GSM-900 at 2 W/kg. Negative (sham) and positive (camptothecin) controls are shown. Annexine V and DiOC6 are used as markers of apoptosis.

**Effects of GSM-900 on apoptosis in nervous cells**

When C6 glioma cells were exposed to GSM-900 for 48 hours at 2 W/kg, no increase in the percentage of cells with depolarised mitochondrial membrane potential was detected (Fig. 2). The cell viability was not affected by exposure to radiofrequency (data not shown).

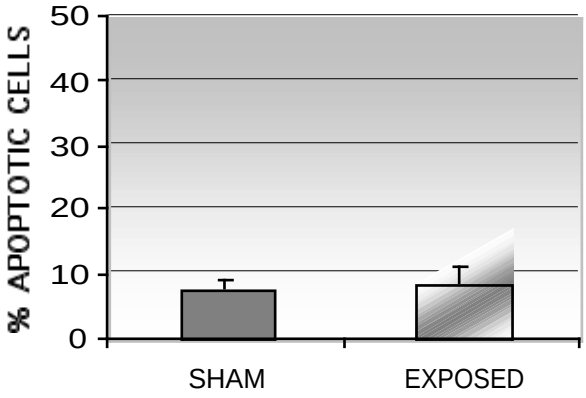


Fig. 2: Detection of cells with depolarised mitochondrial membrane potential in C6 glioma cells immediately after a 48-hour exposure to GSM-900 at 2 W/kg. Negative (sham) control is shown.

Exposure of primary astrocytes cells at 1 W/kg for one hour did not induce apoptosis as determined by the time-kinetics shown in Fig. 3. However, inter-experimental variations were much higher in primary cultures compared to cell lines.

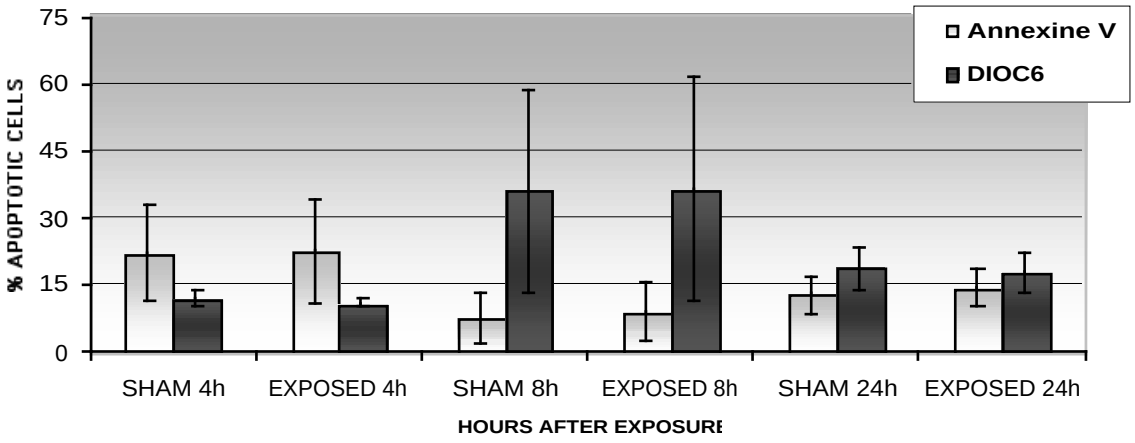


Fig. 3: Detection of apoptosis in primary astrocytes 4, 8 and 24 hours after a one-hour exposure to GSM-900 at 2 W/kg. Negative (sham) control is shown. Annexine V and DiOC6 are used as markers of apoptosis.

## Conclusions

The SAR of 2 W/kg that was used in our study corresponds to the guidelines for the public local exposure to radiofrequency. In the exposure conditions tested, GSM-900 did not induce apoptosis in the U937 immune cell line. Results on glial cells (rat C6 astrocytes and primary astrocytes) do not show evidence that mobile phone–related radiofrequency could induce apoptosis in these cells. Whether primary neuronal cells are sensitive to the GSM-900 radiofrequency is still under evaluation.
Very few data are available of potential pro- or anti- apoptotic effects of radiofrequency at non-thermal level. The data shown here suggest that GSM-900 frame signal could not influence by themselves the outcome of apoptosis in the cell types studied so far. The investigation of primary neurons will be of high interest since it is known that apoptosis in these cells can lead to neurodegenerative diseases. Further work will aim at the study of interaction of such radiofrequencies with known apoptogenic agents.

## References

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