Membrane receptor-mediated ROS generation upon ELF exposure in neuroblastoma cells: an experimental and computational study.

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In this work, the role of NADPH oxidase (Nox1) in extremely low frequency- magnetic field (ELF-MF) mediated ROS generation is investigated. To this aim, we treated SH-SY5Y human neuroblastoma cells with the DPI a molecule able to inhibit ROS production, DPI was administered prior to the exposure to 50 Hz, 1 mT MF for 24 hours. When cells were pre-treated with the DPI, the pro-oxidant function elicited by ELF exposure was partially reverted. The protective (anti-oxidant) effect was observable at all DPI doses in terms of the expression of a fluorescence markers (H2-DCFDA, and DHE) evaluated by flow cytometry.

To locally quantify electric quantities and verify their biological relevance in the different cell compartments, microscopic electric fields and current densities in the surroundings of the plasma membrane of single cells or aggregates have been computed. In setting up such type of models, we took into account realistic SH-SY5Y cell topologies.

Five different cell configurations were extracted from images of cultured SH-SY5Y taken during our experiments. Simulations were carried out using the AC-DC module of Comsol Multiphysics v. 5.0. To quantitatively describe the phenomenon, microdosimetric analysis of the current densities, evaluated at the membrane interface within the first 1 μm shell of the extra-cellular medium, were computed. The results indicates an increase of the microscopic induced current densities for SH-SY5Y topologies where a certain distance among cells is present. On the other end, high confluent cell topologies present the lowest levels of induced current densities as the internal cells are shadowed by the external ones. The induced current density values, in this case, are around 0.45 mA/m2. While, lower confluent cells present current densities more shifted towards higher values up to 1 mA/m2. This result is due to the reduction of the internal cell shielding. These latter levels of reached current densities can be considered able to interact with biological functions and specifically to activate Nox1 as evidenced by our bio-experiments.

Globally, our results demonstrate, that NADPH oxidase enzyme is involved in the ELF-induced ROS generation in SH-SY5Y human neuroblastoma cells in vitro [1]. Our microdosimetric findings support the hypothesis that ELF-MF mainly acts at plasma-membrane level and stimulates down-stream intracellular events, such as oxidative stress. The methodological approach followed in this studies can be considered as a rigorous way to proceed towards the comprehension of complex ELF-mediated bio-effects.

References

[1] C. Merla, M. Liberti, C. Consales, A. Denzi, F. Apollonio, C. Marino, B. Benassi “Evidence of Plasma Mmebrane-Mediated ROS Generation upon ELF Exposure in Neuroblastoma Cells Supported by a Computational Multiscale Approach” *BBA-Biomembranes*, **1861**, June 2019, pp. 1446-1457, doi: 10.1016/j.bbamem.2019.06.005..