Bundesamt
für Strahlenschutz

Spotlight on EMF Research

Spotlight on “Evaluation of mitochondrial stress following ultraviolet radiation and 5G radiofrequency field exposure in human skin cells” by Patrignoni et al. in Bioelectromagnetics (2023)

Category [radiofrequency, in vitro study]

Spotlight - Mai/2024 no.3 (Eng)

Competence Centre Electromagnetic Fields (KEMF)

1 Putting the paper into context by the BfS

Technology-generated radiofrequency electromagnetic fields (RF-EMF) have been present in the environment since the last century. The recent rollout of the fifth generation of mobile communications technology (5G) has introduced a widespread use of the frequency band around 3.5 GHz to enhance the speed of data transmission. RF-EMF with this frequency exhibit small penetration depths into biological tissues and the field energy is mostly absorbed in human skin. While RF-EMF effects at other frequencies have been extensively studied, there is a lack of research on potential health effects of RF-EMF at these frequencies on human skin. One previously implicated mediator of health effects from RF-EMF exposure, for which the evidence is not yet conclusive, is oxidative stress [2]. It occurs when reactive oxygen species (ROS), such as the superoxide radical anion $O_2^{\bullet -}$ (molecular oxygen with one additional unpaired electron), are in excess, leading to damage to macromolecules and organelles [3]. ROS form naturally during biochemical processes and are controlled by antioxidant enzyme systems. In contrast to ionizing radiation and ultraviolet light (UV) [4], no plausible physicochemical mechanism for putative ROS formation induced by RF-EMF exposure has been proposed so far.

2 Results and conclusions from the authors‘ perspective

Patrignoni et al. [1] used an *in vitro* cell culture exposure system to determine experimentally if RF-EMF alone or after exposure to UV-B radiation impacts health parameters of two cell types found in human skin. They used human immortalized KHAT keratinocytes and Xp6be skin fibroblasts. The former represent the cell type forming the epidermis, i.e. the outermost layer of the skin, while the latter represent a cell type found in the dermis, the connective tissue layer directly beneath the epidermis.

Exposure to RF-EMF was generated inside a self-developed reverberation chamber that ensures a homogenous and isotropic field within the cell culture plates and is suitable for use in combination with a cell culture incubator [5]. The setup was tuned to generate specific absorption rates (SAR) of 0.25, 1.0 or 4.0 W/kg at 3.5 GHz within the exposed cell cultures. These exposure levels were validated by temperature measurements of the medium in the cell culture vessels and using finite difference time domain (FDTD)-based numerical simulations. Additionally, cell cultures were exposed to UV-B radiation at a wavelength of 312 nm, which lasted no more than a minute, and were then exposed to RF-EMF or sham-exposed.

After 24 hours of exposure to RF-EMF or sham-exposure, three different fluorescence-based assays were performed to determine biological effects of RF-EMF on skin cells: a) mitochondrial ROS, and especially superoxide, detection by the MitoSox™ Red probe; b) mitochondrial membrane depolarization using the Mitostatus®TMRE dye; c) cellular viability and apoptosis measurement using Annexin V and Sytox Blue Dead Cell Stain. Measurements were performed in blinded conditions after coding of the samples.

Using appropriate positive controls, for each assay and cell type, the authors determined a UV-B dose that resulted in statistically significant effects and achieved about 50% of the maximum response. This ensured that assays were carried out within a dynamic range where any additional effects of RF-EMF would be detectable. The authors state that the UV-B doses were within the range of the standard erythemal dose of 30 mJ/cm², suggesting an environmentally relevant exposure level.

Comparing RF-EMF-exposed to sham-exposed cells, they found a statistically significant decrease in mitochondrial ROS production by about 15% in fibroblasts at 1.0 W/kg SAR. No other changes were observed, neither at the two other SAR levels, nor in any other assay, nor in keratinocytes. When the authors investigated the impact of RF-EMF exposure following UV-B irradiation, they found statistically significantly elevated mitochondrial ROS production in keratinocytes at 0.25 W/kg by 28% and at 1.0 W/kg by 20%. At 4.0 W/kg, the signal of the probe was increased by 16%, without reaching statistical significance. In fibroblasts, RF-EMF exposure did not alter UV-B-induced ROS production. RF-EMF exposure alone or after UV-B irradiation had no effect on mitochondrial membrane potential and cellular apoptosis in either cell type.

According to the authors' conclusion, the present study is the first to show an increase in ROS production by RF-EMF after exposure to UV-B in keratinocytes, but not in fibroblasts. The authors conclude that 3.5 GHz RF-EMF exposure was not sufficient to further disrupt mitochondrial membrane potential, nor to cause additional apoptosis or necrosis of cells. They suggest further studies on skin organoids and *in vivo* experimental studies to follow up on their findings and take into account the complexity of the whole skin tissue.

3 Comments by the BfS

That a previously stressed biological system is exposed to RF-EMF is a realistic scenario. Incident UV radiation from sunlight or tanning beds may overwhelm buffering safeguards and might sensitize the affected tissue to additional damage by otherwise harmless stressors. Patrignoni et al. investigated the impact of 3.5 GHz RF-EMF exposure with and without preceding UV-B irradiation on mitochondria, which are a known target organelle for UV-induced damage [6]. They found that keratinocytes irradiated with UV-B showed a further increase in mitochondrial ROS when additionally exposed to low or medium intensity RF-EMF. The applied SAR values were within the current limits for general public exposure for the limbs recommended by the International Commission on Non-Ionizing Radiation Protection (ICNIRP) [7].

The chosen cellular models have some limitations which prevent a further generalization of the findings. The Hp6be fibroblasts are derived from a patient with the monogenic disease Xeroderma pigmentosum and are inherently sensitive to UV-induced DNA damage [8]. In addition, it is not mentioned whether the KHAT cells were derived from different individual donors or from a single donor, what the age of the donors was, and if biologically individual or the same pool of KHAT cells were used for independent experiments. It

remains unclear whether the sham-exposed cells were simultaneously cultured within a second, but switched off, reverberation chamber, both for RF-EMF-only exposure and for sequential UV-B and RF-EMF exposures.

The presented distribution of local SAR values shows heterogeneity within the tissue culture plate wells and between positions of the wells and positions of the plates. The differences appear much greater than the "maximum variation between different locations of the wells <30%" as stated in the paper. The maximum temperature increase measured in the medium at the bottom of the cell culture wells was 2.03 ± 0.08 °C at a local SAR value of 4 W/kg. The authors did not mention which exact incubator temperature settings they used to offset the RF-EMF-induced warming at each SAR level. This impairs the replicability of the study and leaves open the possibility that localized heating effects might have contributed to the observed differences.

To assess the impact of RF-EMF, six independent experiments in duplicates were performed. The authors pooled the results to generate whisker-box-plots. It is unclear whether the duplicates were averaged to correctly obtain six independent values, or if technical and biological replicates were incorrectly conflated. Furthermore, experimental cultures were prepared explicitly from the same batch of cells. This allows using the paired Wilcoxon signed-rank test. Instead, without explanation, they used the unpaired Wilcoxon rank-sum test for independent samples that has a lower sensitivity.

The authors demonstrate that the MitoSox probe is clearly sensitive to strong inducers of ROS. Since UV-B induced much lower levels of ROS and the dihydroethidium-based MitoSox probe is prone to artefacts [9], the specificity of the probe signal for mitochondrial superoxide at the chosen concentration could have been, at least, corroborated by fluorescence microscopy. The increased mitochondrial ROS production by RF-EMF exposure after UV-B irradiation at small and moderate SAR is an interesting finding. To better understand this process, the authors could have attempted to rescue the additional rise in ROS by providing available superoxide scavengers during different phases of the protocol or by overexpressing the mitochondrial superoxide dismutase enzyme (SOD2). This could, for example, clarify whether RF-EMF exposure boosts ROS by exploiting some capacity overload after UV-B irradiation.

Addressing the lack of an incremental dose-response relationship, the authors discuss their findings in the light of a U-shaped hormetic response, i.e. an ability of the organism to adapt to low or moderate doses of a stressor in a beneficial manner. Hormesis does not seem to be a plausible model to explain the findings since upon exclusive RF-EMF exposure a decreased ROS production was observed, but the opposite happened when the samples were pretreated with UV-B irradiation. Reversing the sequence of exposures, i.e. UV-B irradiation after RF-EMF exposure, would have been a helpful approach to resolve this issue.

Importantly, none of the observed statistically significant changes in mitochondrial ROS were reflected in membrane potential and cellular viability, even at the much higher UV-B doses than the ones applied to the mitochondrial ROS assay. Likewise, no increase in mitochondrial ROS was detected when only RF-EMF was applied.

Because of the mentioned shortcomings, the results of this study provide a limited contribution to the state of knowledge whether RF-EMF exposure of prestressed human skin might result in potentially adverse effects. More conclusive research is needed to make definitive statements. To help improve the evidence base, the BfS has initiated studies on human skin cells investigating the impact of exposures on gene expression and DNA methylation, at frequencies typically used in 5G networks. Further studies to assess health effects of RF-EMF on the eye are planned.

References

[1] Patrignoni L, Hurtier A, Orlacchio R, et al. Evaluation of mitochondrial stress following ultraviolet radiation and 5G radiofrequency field exposure in human skin cells. *Bioelectromagnetics*. Apr 2024;45(3):110-129 doi:10.1002/bem.22495 [published Online First: 20231219].

[2] Henschenmacher B, Bitsch A, de Las Heras Gala T, et al. The effect of radiofrequency electromagnetic fields (RF-EMF) on biomarkers of oxidative stress in vivo and in vitro: A protocol for a systematic review. Environ Int 2022;**158**:106932 doi: 10.1016/j.envint.2021.106932 [published Online First: 20211015].

[3] Redza-Dutordoir M, Averill-Bates DA. Activation of apoptosis signalling pathways by reactive oxygen species. Biochim Biophys Acta 2016;**1863**(12):2977-92 doi: 10.1016/j.bbamcr.2016.09.012 [published Online First: 20160917].

[4] Dunaway S, Odin R, Zhou L, Ji L, Zhang Y, Kadekaro AL. Natural Antioxidants: Multiple Mechanisms to Protect Skin From Solar Radiation. Front Pharmacol 2018;**9**:392 doi: 10.3389/fphar.2018.00392 [published Online First: 20180424].

[5] Orlacchio R, Andrieu G, Joushomme A, et al. A Novel Reverberation Chamber for In Vitro Bioelectromagnetic Experiments at 3.5 GHz. Ieee T Electromagn C 2023;**65**(1):39-50 doi: 10.1109/Temc.2022.3216045.

[6] He H, Xiong L, Jian L, Li L, Wu Y, Qiao S. Role of mitochondria on UV-induced skin damage and molecular mechanisms of active chemical compounds targeting mitochondria. J Photochem Photobiol B 2022;**232**:112464 doi: 10.1016/j.jphotobiol.2022.112464 [published Online First: 20220513].

[7] International Commission on Non-Ionizing Radiation P. Guidelines for Limiting Exposure to Electromagnetic Fields (100 kHz to 300 GHz). Health Phys 2020;**118**(5):483-524 doi: 10.1097/HP.0000000000001210.

[8] Takayama K, Salazar EP, Lehmann A, Stefanini M, Thompson LH, Weber CA. Defects in the DNA repair and transcription gene ERCC2 in the cancer-prone disorder xeroderma pigmentosum group D. Cancer Res 1995;**55**(23):5656-63.

[9] Murphy MP, Bayir H, Belousov V, et al. Guidelines for measuring reactive oxygen species and oxidative damage in cells and in vivo. Nat Metab 2022;**4**(6):651-62 doi: 10.1038/s42255-022-00591-z [published Online First: 20220627].

Bundesamt
für Strahlenschutz

Impressum

Bundesamt für Strahlenschutz
Postfach 10 01 49
38201 Salzgitter

Tel.: +49 30 18333-0
Fax: +49 30 18333-1885
E-Mail: spotlight@bfs.de
De-Mail: epost@bfs.de-mail.de

www.bfs.de

Please always use the following URN when citing this document:
urn:nbn:de:0221-2024053044055

Spotlight - Mai/2024 no.3 (Eng)