Bundesamt
für Strahlenschutz

Spotlight on EMF Research

Spotlight on “Transcriptional landscape of human keratinocyte models exposed to 60-GHz millimeter-waves” by Martin et al. in Toxicology in Vitro (2024)

Category [radiofrequency, in vitro study]

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Competence Centre Electromagnetic Fields (KEMF)

1 Putting the paper into context by the BfS

Millimetre waves (mmWs), i.e. electromagnetic fields (EMF) at frequencies between 30 and 300 GHz and wavelengths in the mm-range (hence the name), will become carriers of information in upcoming 5G/6G networks and enable higher data rates with reduced latency. MmWs exhibit penetration depths into human tissues below 1 mm. They are therefore absorbed in the superficial skin layers, where their energy is converted to heat [2]. Previous studies have not been able to show any significant non-thermal biological effects of mmWs [3]. More research in this area has been recommended [4].

2 Results and conclusions from the authors‘ perspective

In their previous work, the authors of the present study had exposed human keratinocytes – the cells of the most superficial skin layer – to mmWs. They used microarray analysis to examine gene expression in their exposed and control cell culture samples. There were no reproducible changes in RNA molecules present (“expressed” in the following) in the cells. The detected variations could be attributed to heating effects [5, 6].

Biological heterogeneity among individuals might contribute to difficulties in reproducing previously obtained results. To account for that in the current study, the authors used three different keratinocyte specimens [1]. Two of these – referred to as HEK and NHEK – were primary, i.e. freshly isolated and only short-term cultured, keratinocytes from pools of three human donors each, obtained from two different vendors. The third was the immortalized human HaCaT cell line that had already adapted to prolonged cell culture conditions. To investigate the impact of mmWs on their gene expression profiles, Martin et al. subjected the three cell types to either sham-exposure or exposure to reactive near-field EMF at 60.4 GHz [7] and 20mW/cm² incident power density (IPD) for three hours. They measured by how much the exposure increased the temperature of the cell culture medium and included corresponding heat shock controls by adjusting the temperature of the cell culture incubator to the same level. In addition, they treated HaCaT cells with sham, heat or mmW at an IPD of 10 mW/cm² for 14 hours. A dose-response

experiment with exposures of HaCaT cells to different IPDs of 5, 10 and 20 mW/cm² for three hours was also conducted. Heat shock controls matched the heat generated by these IPD levels. According to the authors, these IPD levels were chosen because they encompass the previous and recent exposure limits recommended by the International Commission on Non-Ionizing Radiation Protection (ICNIRP). The mRNA composition of the obtained cell material was analysed by Bulk RNA barcoding sequencing (BRB-seq) [8]. The widely used linear models for microarray data (LIMMA) R package was used to identify statistically significant hits.

In the first part of the analysis, the authors verified the sensitivity of the BRB-seq approach to detect biological differences between samples. They compared the three different sham-exposed keratinocyte specimens to each other. The two primary cultures were more similar to each other than to the HaCaT cell line, as reflected by the number of statistically significant genes obtained from the comparisons. The differentially expressed genes between primary and HaCaT cells were related to skin differentiation and cell division. This confirmed the expectation that immortalized cell lines, which had adapted to cell culture conditions, are biologically different from primary cell material which is more similar to the tissue of origin.

Next, within each individual cell type, gene expression profiles after three hours of sham-, heat- or mmW-exposure at 20 mW/cm² were compared with each other. Differentially expressed genes were only found in HaCaT (14 genes) and HEK cultures (four genes) with an overlap in two genes. The same genes that were changed after exposure to mmWs were also changed during exposure to heat. They were functionally annotated to the “Protein processing in endoplasmic reticulum” pathway, which includes genes encoding for the important heat shock proteins HSP70 and HSP27. When comparing mmW exposure with the heat control, only the gene for HSP70 was significantly differentially expressed and only in HEK cells. This result could not be validated by the quantitative polymerase chain reaction (QPCR) assay. In addition, QPCR showed HSP70 to be overexpressed after treatment in the other two keratinocyte cultures, but also without statistically significant differences between mmW- and heat-exposed samples. HSP27 overexpression after exposure of HaCaT cells to heat was confirmed by QPCR.

In the dose-response experiment, the gene expression profiles of HaCaT cells showed a higher number of differentially expressed genes the higher the exposure level. Altered genes were related to the response to heat and to protein folding. Two, seven or two genes were differentially expressed at IPD levels of 5, 10 or 20 mW/cm², respectively, when compared to the matched heat control. Most of these genes also changed in response to heat exposure when compared to sham exposure. No differentially expressed genes were found after an overnight mmW- or heat-exposure.

The authors conclude that the effects of millimetre waves on gene expression in cells of the most superficial skin layer are due to heating. The small differences detected were likely due to differences in how heat is delivered by millimetre waves or the air of a warm cell culture incubator.

3 Comments by the BfS

The present study investigated the effects of short-term, near-field exposures to millimetre waves (e.g. to be used for 5G/6G) on cells of the most superficial skin layer, simulating the exposure scenario of a body-worn device emitting mmWs. All observed alterations in gene expression could be attributed to thermal effects of mmWs, consistent with current mechanistic understanding and already considered by limit values. The results provided no indication of non-thermal effects.

The study was carefully controlled and allows for generalisation of the findings to a certain extent, because different and independent keratinocyte sources, exposure levels and durations were used. The detected differences between primary keratinocyte cultures and the established cell line, as well as the observed dose-response effect, are plausible results indicating that the approach was technically valid. The finding that differentially expressed genes after heat or mmW exposure were associated with the pathway

“Protein processing in endoplasmic reticulum” is expected, since such a response to heat helps the cell manage proteins that misfold due to overheating.

Four replicates per condition and cell type were analysed by BRB-seq which is a common compromise between cost, feasibility and statistical power in comparable gene expression experiments. To call a gene differentially expressed between sham and mmW- or heat-exposures, the current paper used a standard rule: a gene is considered differentially expressed if its level changes by at least 1.5 times. However, to detect even smaller changes in gene expression, it is recommended to include at least six and up to twelve biological replicates [9].

Two differentially expressed genes were validated by QPCR and results of BRB-seq were only partly confirmed. It is therefore unclear if the other differentially expressed genes would have been validated if QPCR had been performed on all of them.

There is no information on investigator blinding during the experiments. However, observer bias is unlikely to have influenced the current results, as no major differences in gene expression were found. Additionally, large-scale genomic analyses, as conducted in this study, are less likely to be systematically affected by observer bias, because there is a long delay between the collection of the biological material by the biologist or physician and the processed sequencing results, which are obtained by reproducible computational methods that are usually performed by a bioinformatician.

In this study, the IPD has been used to describe exposure levels. However, the “reference levels” for the IPD recommended by the International Commission on Non-Ionizing Radiation Protection (ICNIRP) in the 2020 guidelines are not considered appropriate for assessing local reactive near-field exposures at frequencies above 6 GHz. For such exposures, as applied in this study, ICNIRP advises compliance with “basic restrictions” to prevent excessive heating or thermal effects in human tissues [2]. Specifically, for frequencies above 6 GHz, the occupational exposure limit should be based on the local absorbed power density (APD), which should not exceed 10 or 20 mW/cm², depending on the averaged area [2]. Numerical field simulations are usually required to accurately assess the APD. Nevertheless, the study’s results remain meaningful as concludingly explained.

The exposure to mmWs with an IPD of 5 mW/cm² resulted in a heating of the cell culture medium by approximately 1.2 °C. Exposure with an IPD of 20 mW/cm² heated the medium by 4.3 °C to 5.7 °C. According to the ICNIRP guidelines, which set specific thresholds to prevent harmful health effects due to heating, such as to keep skin temperature increases below 5 °C for local exposures, these levels are close to the operational adverse health effects threshold. No effects on gene expression could be detected at these levels that were not also detected in appropriate heat control samples. This is a relevant and methodologically robust result for radiation protection. However, it cannot be ruled out that exposure to mmWs may induce minor alterations in gene expression that the study lacked sufficient statistical power to detect.

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