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PUBTEXTO

Compensation of Mobile Phone Radiation by the Medic Amber: *In Vitro* Investigations Using a Novel Test System

Dartsch PC*

Dartsch Scientific GmbH, Institut für zellbiologische Testsysteme, Auf der Voßhardt 25, D-49419 Wagenfeld, Germany

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Corresponding author: Dartsch PC, Dartsch Scientific GmbH, Institut für zellbiologische Testsysteme, Auf der Voßhardt 25, D-49419 Wagenfeld, Germany; E-mail: pc.dartsch@dartsch-scientific.com

Abstract

Background

Mobile phones are high-performance devices that consume a lot of energy and, thus, also generate heat in form of microwaves. This heat is mainly created by the high-frequency electromagnetic fields that transmit voice or data in mobile communications. Moreover, mobile phones also possess non-thermal radiation which has been shown to induce oxidative stress and cell damage. A novel test system was used which allows to evaluate the influence of the radiation of a commercially available current mobile phone on cellular processes such as cell regeneration/wound healing of connective tissue fibroblasts, and the formation of superoxide anion radical of functional neutrophils in the course of an oxidative burst . Moreover, also the question was studied whether a newly constructed device named Medic Amber might be able to reduce the cellular effects of mobile phone radiation.

Principal Findings

The use of the Medic Amber with an active mobile phone including WLAN resulted in: (1) A significant improvement of cell migration and proliferation in comparison to the exposed and unprotected cell cultures. Thus, an increased closure of the cell-free space was examined. (2) A significant improvement in both basal cell metabolism of functional neutrophils and generation of ROS when compared to the experimental situation without any protection.

Conclusions

As shown here, cell regeneration/wound healing of connective tissue fibroblasts and the activity of functional neutrophils as the first defense of the innate immune system against invading microbial pathogens, is significantly decreased by mobile phone radiation. The use of the Medic Amber was able to attenuate these unwanted effects. Based on the results of both tests, the use of the Medic Amber can be recommended to reduce the effects of mobile phone radiation.

Keywords: Mobile phone radiation; Cell regeneration; Wound healing; Oxidative burst; Connective tissue fibroblasts; Functional neutrophils; Cell culture

Abbreviations: ROS – Reactive oxygen species; SAR – Specific absorption rate; WLAN – Wireless local area network

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Introduction

Mobile phones are high-performance devices that consume a lot of energy and, thus, also generate heat in form of microwaves [1]. This heat is mainly created by the high-frequency electromagnetic fields that transmit voice or data in mobile communications [2,3]. Moreover, mobile phones also possess non-thermal radiation which has been shown to induce oxidative stress and cell damage [4-8]. However, current standards of the IEEE and the ICNIRP [9,10] are still based only on the thermal compatibility of biological organisms.

The main problem of studies on whole multi-cellular organisms such as experimental animals is the complexity of the test systems. There are numerous unknown variables which are difficult to be established. In contrast, cultivation of eukaryotic cells can be standardized and provides the opportunity to vary different factors depending on the experimental needs.

For this aim we used a novel test system which allows to evaluate the influence of the radiation of a commercially available current mobile phone on the cellular level. Moreover, we also studied the question whether a newly constructed device named Medic Amber might be able to reduce the cellular effects of mobile phone radiation. We investigated the effects of mobile phone radiation and its compensation by the Medic Amber on two different cellular processes: (1) cell regeneration/wound healing of connective tissue fibroblasts, and (2) superoxide anion radical formation of functional neutrophils in the course of an oxidative burst.

In vivo, the wound healing process can be divided into three distinct phases [11-13]. In this *in vitro* study the granulation

phase, characterized by the occurrence of cell migration and cell proliferation of fibroblasts for defect filling and wound closure, was simulated. We investigated whether the Medic Amber would be able to compensate mobile phone radiation and, thus, to reduce any effects which prolongate or even inhibit the migration and proliferation of the cells into a cell-free space.

Polymorphonuclear neutrophils (PMNs) belong to the group of phagocytes and are cells that possess the important properties of the innate immune defense in the blood, namely the production of reactive oxygen species (= ROS) by an oxidative burst [14-16]. *In vivo*, these cells recognize invading microbial pathogens in the blood, migrate to them, enclose them and kill them by forming ROS. The residues of the killed pathogens are then ingested by the cells. In the case that the metabolism and the formation of ROS is reduced by the influence of mobile phone radiation, the innate immune defense is no longer as effective as before.

Materials and Methods

Medic Amber

For the investigations conducted in this study, a Medic Amber was kindly made available to us from Somavedic Technologies s.r.o., CZ-41002 Lovosice**,** Czech Republic, for a period of several months.

The Medic Amber as stated by Somavedic Technologies, "is the strongest brand new model and protects the human body from the negative effects of electromagnetic fields. It creates a more coherent, natural environment and activates self-healing process in the body and mind. Somavedic creates a low-energy high-frequency electromagnetic field (wave characteristic), excited by several kinds of geometrically appropriately arranged silicium minerals (SiO_2) with a varying level of pollution around the centrally placed quartz in the core. This field leads to a partial or total elimination of the negative free radicals".

Mobile Phone

A current and commercially available mobile phone from a leading brand manufacturer with a SAR value of 0.76 W/kg was used for the study. However, one must consider that a poor signal strength causes a significant rise in radiation energy. Moreover, in this study no distinction was made between thermal and non-thermal radiation, because both are also present in reality when making a call and using WLAN and have an effect on the human body.

Cell Culture

The cell regeneration/wound healing experiments were conducted with connective tissue fibroblasts (L-929 cell line, ACC-2, Leibniz Institute DSMZ, Braunschweig, Germany). Cells were routinely cultured in RPMI 1640 with 10 % growth mixture and 0.5 % gentamycin and cultivated in an incubator at 37 °C with an atmosphere of 5 % CO_2 and 95 % air and a humidity of approximately 100 %.

The investigations on the oxidative burst were conducted with human promyelocytes (HL-60 cell line; ACC-3; ECACC 98070106; Leibniz Institute, German Collection for Microorganisms and Cell Cultures, Braunschweig). The cells were routinely cultivated in the same cultue medium and at the same conditions as described for connective tissue fibroblasts. The non-adherent cells were routinely cultivated as suspended mass cultures and were regularly subcultured in fresh culture medium twice a week. By adding 1.5 vol% dimethyl sulfoxide to the culture medium, the cells were differentiated over a period of 5 to 6 days into so-called functional neutrophils or neutrophilic granulocytes which have the characteristic feature to undergo an oxidative burst upon stimulation by a phorbol ester (see below).

Basic Principles of the Experimental Design

For the present study with cultivated cells, the experiments without the Medic Amber were carried out over a period of 4 weeks and thereafter the experiments with the Medic Amber for another 3 weeks. The tests were started not earlier than 3 days after the Medic Amber was switched on. The distance between the Medic Amber and the external temperature-controlled mini incubator (Cultura M; Almedica, Switzerland) was 90 cm and was completely free without any barriers. The total exposure time to the actively transmitting mobile phone including WLAN was 4 hours at 37 ± 1 °C in the temperature-controlled external mini incubator. A second cell culture in the second mini incubator served as unexposed control and was wrapped in several layers of aluminium foil and incubated for the same time period at a completely different place.

Cell Regeneration/Wound Healing of Connective Tissue Fibroblasts

Connective tissue fibroblasts were seeded at a density of 100,000 cells/ml into the four individual compartments of a silicone 4 well-culture insert (ibidi, Gräfelfing, Germany). The single compartments of the inserts are separated by a 500 µm thick silicone bar. Due to the special adhesion area, an insert adheres firmly to the bottom of a culture dish and forms a distinct cell-free space (artificial wound), which the cells can close by migration and proliferation.

Upon reaching confluency within 24 hours after cell seeding, the silicone inserts were carefully removed with tweezers to achieve a sharp edge of the cell-free space between the compartments. The normal culture medium was replaced by Leibowitz L-15 medium supplemented with 10 % growth mixture and 0.5 % gentamycin. This culture medium keeps the pH value constant at environmental air conditions. Cell cultures were transferred to two separated mini incubators at 37 °C without gassing for mobile phone radiation exposure and unexposed control cells, respectively.

For each experiment, two culture dishes with the cells were placed on the actively transmitting mobile phone with the display switched-off. This corresponds to the real situation of a radiation direction towards the user.

After the exposure period was finished, L-15 medium was replaced by the normal culture medium and the cell cultures were incubated at standard conditions for another 16 hours to allow the cells to migrate and proliferate into the cell-free space. Then, cells were fixed with 100 % methanol, stained with Giemsa's azur eosin methylene blue solution (Merck, Darmstadt, Germany) and were air-dried. The width of the remaining cell-free space was measured by micrographical methods at 4 different cell layer edges with triplicate measurements at each edge for each cell culture. Therefore, for each experimental situation 24 data points were taken in a single experiment. The resulting mean value with and without the Medic Amber in comparison to the corresponding unexposed control cell culture was used for the final evaluation.

Oxidative Burst of Functional Neutrophils

On day 5 of the differentiation process induced by the addition of 1.5 vol% dimethylsulfoxide, the differentiated cells were exposed to the radiation from the actively transmitting mobile phone including WLAN for 4 hours just one day before the actual tests were conducted. After exposure, the cells were transferred back to the standard incubator and incubated for another 20 hours before the actual start of the testing. The experimental setup was performed in such a way that two culture flasks with the cells were placed on the actively transmitting mobile phone with a switched-off display and two culture bottles on the back of the mobile phone near the antenna (Figure 1).

Figure 1: *Experimental setup during exposure with duplicate samples on the mobile phone display (= direction of radiation towards the user) and beneath the mobile phone (= direction of radiation away from the user).*

The basal metabolic activity of the functional neutrophils was determined by a color reaction based on the activity of the mitochondrial dehydrogenases. In an additional experimental approach, the differentiated cells were induced to generate ROS by adding phorbol-12-myristate-13-acetate (Sigma-Aldrich, Deisenhofen, Germany) to the reaction mixture [17-20]. The color change (= optical density) of the reaction was measured continuously as a differential measurement $\Delta OD = 450 - 690$ nm with an Elisareader (BioTek SLx 808 with software Gen 5/3.00), recorded for 30 min at selected time points and evaluated by Microsoft Excel. A total of three independent experiments was conducted over a period of 3 weeks.

Statistical Analysis

Statistical analysis was done by using the non-parametric two-tailed Wilcoxon-Mann-Whitney test**.**

Results and Discussion

As shown in Figure 2 for one representative experiment, the use of the Medic Amber with an active mobile phone including WLAN resulted in a significant improvement of cell migration and proliferation in comparison to the exposed and unprotected cell cultures. Thus, an increased closure of the cell-free space was examined (Figure 3). However, even under the influence of the Medic Amber, the values of the unexposed control without mobile phone radiation were not quite achieved. When setting the unexposed controls as 100 %, the summarized data gave the following results for closure of the cell-free space (mean values ± standard deviations):

- Unexposed control: 100 ± 6.5 %
- Exposed and protected cells by the Medic Amber: 80.1 ± 7.4 %
- Exposed and unprotected cells: 63.6 ± 7.5 %

The results show that the Medic Amber was able to compensate a significant part of the mobile phone radiation in comparison to the exposed and unprotected situation ($p \leq 0.05$; Wilcoxon-Mann-Whitney test).

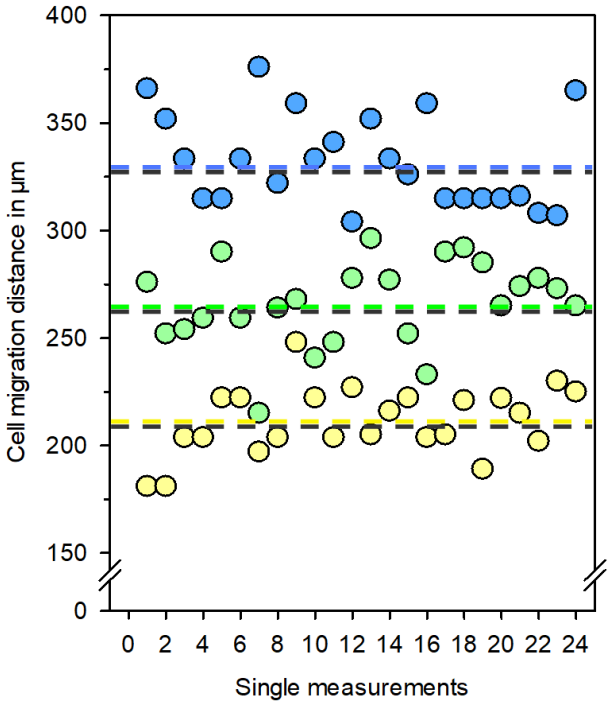

Figure 2: *The results of one representative experiment demonstrating the effect of the Medic Amber on the closure of a cell-free space in a layer of connective tissue fibroblasts by migration and proliferation. Depicted is the migration distance of the cells of all single measurements. Blue data points = Unexposed control cells; green data points = Exposed and protected cells by the Medic Amber; yellow data points = Exposed and unprotected cells. The mean values for each experimental situation are given by the dashed lines in the appropriate color. The protective effect of the Medic Amber against mobile phone radiation can be clearly seen in direct comparison to the unprotected situation.*

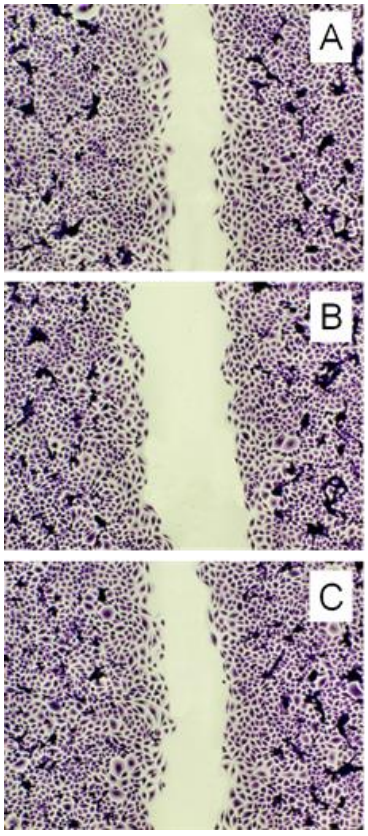

Figure 3: *Micrographs of cell regeneration/wound healing after 16 hours of migration and proliferation of connective tissue fibroblasts. (A) Unexposed control culture; (B) Mobile phone-treated culture without protection; (C) Mobile phone-treated culture with Medic Amber protection. Note the different cell-free spaces within the dense cell layer. Olympus IX 50 inverted microscope with planachromate 10x and Olympus E-10 digital camera at 4 megapixel resolution at bright field illumination.*

As shown in detail in Figure 4, the use of the Medic Amber with an active mobile phone including WLAN resulted in a significant improvement in both basal cell metabolism of functional neutrophils and formation of ROS when compared to the experimental situation without any protection ($p \leq 0,05$; Wilcoxon-Mann-Whitney test).

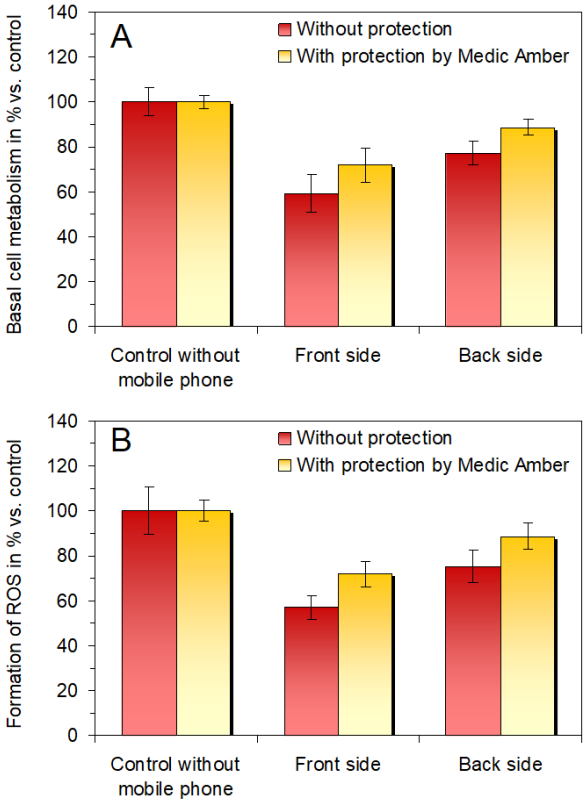

Figure 4: *(A) Effect of the Medic Amber on basal cell metabolism of functional neutrophils. The protective effect of the Medic Amber can be clearly seen in comparison to the unprotected situation. The front side of the mobile phone which is directed towards the user produces much more radiation than the back side - despite the fact that the display is being switched off. (B) Effect of the Medic Amber on the formation of reactive oxygen species (= ROS) of functional neutrophils of the innate immune defense against microbial pathogens. The protective effect of the Medic Amber can be clearly seen in comparison to the unprotected situation. The data represent mean value ± standard deviation of 3 independent experiments.*

Nevertheless, even under the influence of the Medic Amber, the values of the unexposed control without mobile phone radiation were not quite achieved, i.e. a pronounced, but not a complete compensation of the mobile phone radiation was measured. On the other hand, a considerable improvement in the compensation with increasing operating time of the Medic Amber in the premises used (3 weeks in total). Was noticed. This observation correlates with the descriptions of the effect on the manufacturer's homepage.

The present investigation clearly demonstrates two different aspects of mobile phone radiation and its compensation. The first aspect is that the radiation of an actively transmitting mobile phone including WLAN causes not only a reduced cell vitality [21-25], but also reduces cell migration and proliferation as well as the formation of superoxide anion radicals by cells which are part of the innate immune defense system. All the observed cellular effects seem to be mainly related to oxidative stress [8]. In their review, Yakymenko et al. [8] concluded that low-intensity radiofrequency radiation acts as an oxidative agent for living cells with a high pathogenic potential, Moreover, this oxidative stress "should be recognized as one of the primary mechanisms of the biological activity of this kind of radiation". In the present test systems the exposure time to mobile phone radiation might be too short to induce massive oxidative alterations in the cells. But it is strong enough to reduce the degree of characteristic features of cultivated organ-specific cells such as a decreased cell regeneration/wound healing process of connective tissue fibroblasts [26-28] or a decrease in the formation of ROS by functional neutrophils. However, the latter finding is in contradiction to the findings of Vlasova et al. [29] who found that extremely high frequency electromagnetic radiation promoted the antimicrobial activity of neutrophils. Further investigations could shed more light on this phenomenon by the addition of antioxidants to the culture medium during mobile phone exposure.

The second aspect is the fact that the Medic Amber is able to reduce the effect of mobile phone radiation. From the experimental setup it cannot be decided what the primary cellular target of the Medic Amber might be. On one hand, it is conceivable that the induced oxidative stress by mobile phone radiation is markedly reduced by the device; on the other hand, one can imagine that the cell membranes are strengthened by the

Medic Amber which also results in a better resistance of the cells against any kind of mobile phone radiation.

Conclusion

As shown here, cell regeneration/wound healing of connective tissue fibroblasts and activity of functional neutrophils as the first defense of the innate immune system against invading microbial pathogens, is significantly decreased by mobile phone radiation. The use of the Medic Amber was able to attenuate these unwanted effects. Based on the results of both tests, the use of the Medic Amber can be recommended to reduce the effects of mobile phone radiation.

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