Japan Journal of Medicine
2019; 2(2): 338 - 343 . doi: 10.31488/jjm.1000137

Research article

Cellular Effects Following Exposure to Mobile Phone Radiation and its Compensation

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Received: January 15, 2019; Accepted: February 25, 2019; Published: February 28, 2019

Abstract

The effect of non-thermal radiation on biological systems is still discussed controversial. In this present study we investigated the non-thermal effects of an actively transmitting 1800 MHz mobile phone on cultured connective tissue fibroblasts (L-929). Cell vitality was examined morphologically and by using a colorimetric assay. For an exposure period of 2 h followed by a 22 h period of normal incubation, only 8.8 ± 6.1 % (mean value ± standard deviation; n = 5) of the cells survived for total radiation with an intensity of 1.8 W/m^2 at the level of the cells and 45.3 ± 5.3 % (mean value ± standard deviation; n = 13) of the cells survived for non-thermal radiation with an intensity of 1.25 W/m^2 at the level of the cells. The exposure to non-thermal radiation was produced by placing a double wall corrugated board between the mobile phone and the cell layers. Cell vitality after exposure was independent from the mobile phone orientation, i.e. whether the display or the reverse side was directed towards the cells. In addition, we examined a device for its efficacy to compensate the non-thermal radiation of an actively transmitting mobile phone. At the same experimental conditions as mentioned above the device increased cell vitality to 82.4 ± 7.5 % (n = 6) or by the additional use of a specific mobile phone cover to 95.5 ± 6.1 % (n = 5). The use of an inactive dummy device caused no protective effect (n = 4). The results demonstrate that non-thermal radiation affects vitality of cultured connective tissue fibroblasts. The study also demonstrates that this specific device is able to compensate this radiation to a large extent (> 95 %).

Keywords: electromagnetic field; 1800 MHz; mobile phone radiation; non-thermal effect; oxidative stress; L-929; fibroblasts; cell death; cell culture

Introduction

Mobile data communication has become an integral part of everyday life. Today, nearly all technical devices work with mobile data transmission. Therefore, the electromagnetic tolerance of this technology is an important part of current research. In the next few years, the new 5G net-work will be implemented. The effect on humans remains still not clear to this day. In addition to the known thermal effects which are aimed at the warming of the body tissue, non-thermal effects also occur in biological systems and have already been discussed in several publications [1-5]. Although the mechanism of action of non-thermal effects continues to be controversial [6,7], there are some innovative theoretical approaches that require further investigation.

In 2011, the World Health Organization (WHO) classified high-frequency electromagnetic fields as potentially carcinogenic (2B) [8]. Since then, the application and expansion of high-frequency fields has increased dramatically and makes the relevance of past research very difficult when compared to current technological advances. The latest measuring methods in the areas of whole-body dosimetry and cell biology show the current status. Current standards of the IEEE and the ICNIRP [9,10] are still based on the thermal compatibility of biological

organisms.

The main problem of studies of whole multi-cellular organisms such as rats, mice, drosophila and others is the complexity of the test systems. There are numerous unknown variables which are difficult to be established. In contrast to these models, cultivation of eukaryotic cells can be standardized and provides the opportunity to vary different factors depending on the experimental needs. Prompted by this background, we conducted a study in order to clarify whether high-frequency electromagnetic fields created by a commercially available mobile phone might cause non-thermal effects on cultured connective tissue cells. Moreover, we also studied the question whether a newly constructed device might be able to compensate the non-thermal effects of mobile phone radiation influencing biological systems, but not the thermal effects or the technical functionality.

Materials and Methods

Cell culture

In the present study, cultured connective tissue fibroblasts (cell line L-929; Leibniz-Institut, Deutsche Sammlung für Mikroorganismen und Zellkulturen, Braunschweig, Germany) as a standard cell line for toxicological studies were taken at passages 22 to 50 over a total experimental period of 8 months. Cells were routinely cultivated in the moist atmosphere of an incubator at 37 °C and gassed with 5 % CO_2 and 95 % air to yield a constant pH value. Culture medium was RPMI 1640 with 10 % fetal calf serum and standard amounts of gentamycin. All cell culture reagents were from Capricorn Scientific, 35085 Ebsdorfergrund, Germany.

Experimental design

For the tests, cells were seeded from 80 to 90 % confluent mass cultures at a density of 20,000 cells/well into 24 wells in the middle part of a 96 well-plate (200 µL culture medium/well). After 24 hours to ensure cell attachment and metabolisation, culture medium was exchanged to 200 µL/well of Leibowitz L-15 medium containing 10 % fetal calf serum and standard amounts of gentamycin. This culture medium guarantees a pH value at 7.4 even at normal atmospheric conditions. Plates were transferred to a Cultura M mini incubator and cultivated further at 37 ± 1 °C without CO_2 gassing. A commercially available and actively transmitting 1800 MHz mobile phone (SAR value 1.510) at continuous operation mode was used to conduct the different test conditions. Radiation intensities were measured at the level of the cells at the same conditions used for the assays with an Aaronia Spectran HF-4060 equipped with a calibrated area antenna of 1 cm^2. An intensity of 1.8 W/m^2 was measured for the actively transmitting mobile phone without an corrugated cardboard and 1.25 W/m^2 when the same corrugated cardboard as used for the cell experiments was placed between the mobile phone and the cells.

In adition, the cardboard had also marked effects on the local temperature. When using the exposure times as in this study, the transmitting mobile caused a surface temperature at the level of the cells of approximately 46 °C, but when we used the cardboard we measured a value of 37.5 to 38 °C at the same position. So we concluded that our experimental design omitted thermal effects. The temperature in the incubator was keep constant at 37 ± 1 °C.

All tests were conducted with unexposed control cells in a 96-well plate at the same cultivation conditions, but approximately 10 meters distant from the active mobile phone in an Cultura M mini incubator at 37 ± 1 °C without CO_2 gassing. Cell vitality was checked by morphological observation of the cell cultures and by enzymatic activity. For this purpose, cell culture medium was removed and replaced by 180 µL fresh culture medium and 20 µL of XTT (Xenometrix AG, Allschwil, Switzerland) and incubated for 120 minutes in the incubator at 37 °C. XTT is the sodium salt of 2,3-bis[2-methoxy-4-nitro-5-sulfo-phenyl]-2H-tetrazolium-5-carboxyanilide and has a yellowish color. XTT is cleaved to an orange formazan by a complex cellular mechanism which occurs in viable cells only, and is related to NAD(P)H production by glycolysis. Therefore, the amount of formazan dye formed directly correlates to the number of metabolically active cells in the culture [11,12].

After 120 minutes, optical density was measured as a differential measurement ΔOD = 450 – 690 nm after a 4 second shaking interval using an ELISA reader (BioTek Slx808; Bad Friedrichshall; Germany).

Statistical analysis

Statistical analysis of all test assays was done using two-tailed Wilcoxon-Mann-Whitney test.

Compensatory device

A device was used to test its potential in compensating the effects of radiation exposure on connective tissue cell cultures. This device is sold commercially under the name memonizerMOBILE by the manufacturer (Memon bionic instruments GmbH, DE 83026 Rosenheim, Germany).

The function and method of action of the device has already been described in detail elsewhere [13] and will only be referenced here. In short, this device is a multi-layered metamaterial that is capable of compensating the non-thermal effects of radiation. Various phyllosilicates are used and have already proven their protective function against the influences of electromagnetic fields on biological systems in various experiments [14]. The device has dimensions of 35 x 13 x 0.25 mm (L x W x H) and is placed directly in the vicinity of the charging socket of the mobile phone or the antenna. Natural finite impulse response (FIR) frequencies are supported in living organisms by the resonance frequencies of the phyllosilicates in the infrared (IR), near infrared (NIR) and ultraviolet (UV) range. Artificial electromag-netic frequencies block these

natural areas and can be compensated by corresponding resonances of the phyllosilicates [15-17].

Prior to the experiments with the memonizerMOBILE as presented here, we conducted a set of related experiments by using a conventional router as the source of non-thermal electromagnetic radiation ± a memonizerWLAN. In these experiments, an active and inactive version of the device was tested in a way that the experimentator did not know what kind of device he tested (blind study). In each experiment it was easily possible to identify the active device. Thus, we concluded that it seems to be not necessary in the experiments presented here to use the setup of a blind study furthermore. In addition, we also did some experiments with the memonizerMOBILE by using an inactive dummy in order to see if the material itself might influence the results.

Results

Effect of total vs. non-thermal mobile phone radiation

In order to evaluate the effect of thermal and non-thermal radiation on the cells, the actively transmitting mobile phone was directly placed on the lid of the culture plate without and with a double wall corrugated board (thickness: 7 mm) between mobile phone and cell layer to eliminate the influence of thermal effects by microwaves. Cells were exposed to mobile phone radiation as follows: 0.5 h of radiation exposure + 23.5 h of normal incubation; 1 h of radiation exposure + 23 h of normal incubation; 2 h of radiation exposure + 22 h of normal incubation; 4 h of radiation exposure + 20 h of normal incubation; 24 h of radiation exposure without additional normal incubation. The additional normal incubation period ensures that cellular repair might become active.

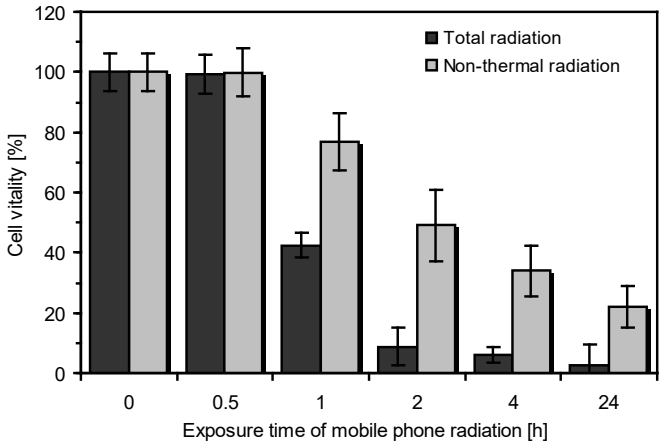

Figure 1. Effect of total and non-thermal radiation of an actively transmitting mobile phone on connective tissue cells in culture. Data represent mean value ± standard deviation (n = 5 and 13).

As depicted in Figure 1, the total effect of radiation on cell vitality was time-dependent when the actively transmitting mobile phone was directly placed on the lid of the culture plate. At exposure times ≥ 1 h, the reduced cell vitality was statistically significant when compared to untreated control and to the non-thermal radiation effect ($p < 0.01$; Wilcoxon- Mann-Whitney test) The ET50, i.e. the exposure time causing 50 % loss in cell vitality was calculated to be 0.75 h and cell vitality for 2 h exposure time (which was taken in further experimental approaches) was 8.8 ± 6.1 % (mean value ± standard deviation; n = 5). Examination of the non-thermal radiation effect by placing a double wall corrugated board between active mobile phone and cell layers also caused a time-dependent loss in cell vitality, but the time-vitality curve decreased much slower. Thus, the ET50 was calculated to be approximately 2 h and cell vitality for 2 h exposure time was 45.3 ± 5.3 % (mean value ± standard deviation; n = 13). The reduced cell vitality was also statistically significant ($p < 0.01$) at exposure times ≥ 1 h.

Morphological examination of exposed cells showed similar alterations in cell shape as already described for wireless DECT base radiation at 1.885 GHz [13] such as cell rounding, detachment and intracellular vacuolization which might be related to oxidative stress (Figure 2) which was also visualised by time-lapse video micrography (not depicted).

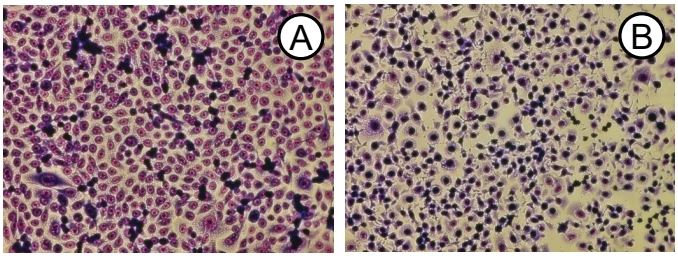

Figure 2. Morphological alterations observed in connective tissue fibroblasts after a 2 h exposure to non-thermal radiation followed by 22 h of normal incubation (B) in comparison to untreated cell cultures (A). Cells were stained with Coomassie-Giemsa solution. Bright field microscopy with an Olympus IX50 inverted microscope equipped with an Olympus 20x Planachromate and an Olympus E-10 camera at 4 megapixels.

Effect of mobile phone orientation

Based on the results described in the last chapter, we used the same radiation and additional normal incubation parameters with the double wall corrugated board between active mobile phone and cell layers. However, in one experimental series the display of the mobile phone was placed towards the underlying cell layers and in a second series the reverse side of the mobile phone was directed towards the cells. The cause for these experiments was to examine whether the display which becomes quite hot when the mobile phone is actively transmitting, might also increase both, thermal and non-thermal radiation.

As shown in figure 3, there was no significant difference between both mobile phone orientations indicating that the display is mainly emitting microwaves and minor non-thermal radiation. Thus, for easier handling, the active mobile phone was always placed with its reverse

side towards the cell layers in further experiments.

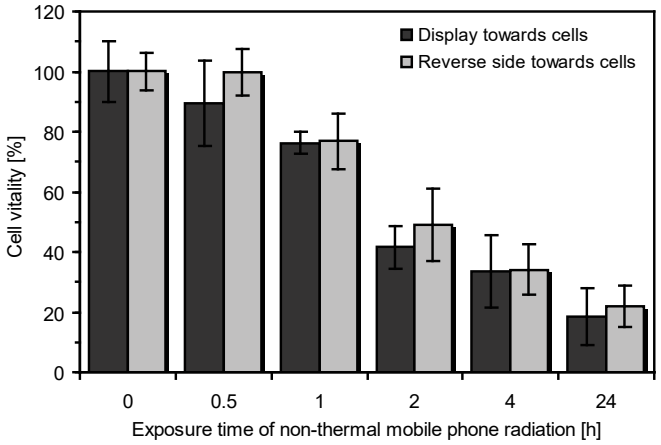

Figure 3. Effect of actively transmitting mobile phone orientation either with the display towards the cell layers or with the reverse side towards the cell layers on connective tissue cells in culture. A double wall corrugated board was always placed between mobile phone and cell layers. Data represent mean value ± standard deviation (n = 3 and n = 13).

Efficacy of the device to compensate non-thermal mobile phone radiation

Based on the results described so far, we investigated whether the compensatory device is able to protect connective tissue cells from non-thermal mobile phone radiation. In all experimental series, cells were exposed to 2 h of the actively transmitting mobile phone followed by a 22 h period of normal incubation. A double wall corrugated board was placed between active mobile phone and cell layers and the mobile phone orientation was always with the reverse side towards the cell layers. The compensatory device was either placed on the rechargeable battery unit in the mobile phone or in the specially designed clamp of the mobile phone cover. In order to examine whether a protection might be due to the presence of the device material alone, an inactive dummy of the device was used to conduct the same experiments.

As depicted in Figure 4, the use of the compensatory device resulted in an enhanced cell vitality of 82.4% ± 7.4% (mean value ± standard deviation; n = 6) in comparison to cells exposed to the actively transmitting mobile phone without the device (cell vitality: 45.3% ± 5.3%; mean value ± standard deviation; n = 13). The use of the compensatory device clamped in a specially designed mobile phone cover caused a cell vitality of 95.5% ± 6.1% (mean value ± standard deviation; n = 5) which means that this combination was able to compensate the non-thermal radiation of the actively transmitting mobile phone by a great extent which did not differ significantly from untreated conrols.

Finally, we observed no significant difference between the effect of the non-thermal mobile phone radiation on cells without protection of the compensatory device (cell vitality: 45.3 ± 5.3%; mean value ± standard deviation; n = 13) or when using an inactive dummy of the device (cell vitality: 50.5 ± 7.6; mean value ± standard deviation; n = 13) or when using an inactive dummy of the device (cell vitality: 50.5% ± 7.6%; mean value ± standard deviation; n = 4).

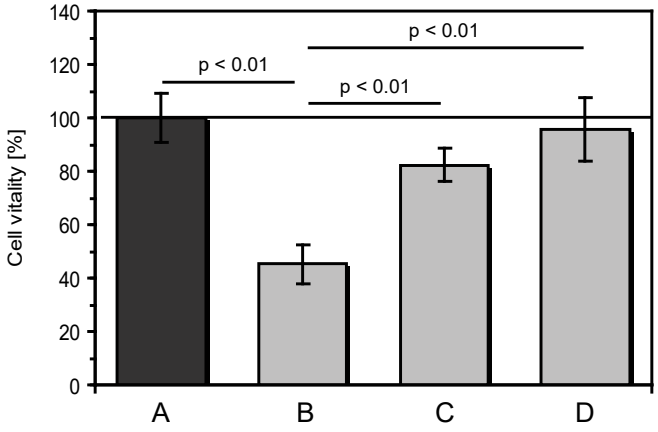

Figure 4. Graphical presentation of the efficacy of the device to compensate non-thermal mobile phone radiation. (A) Untreated control cells with normal incubation for 24 hours (n = 13); (B) Cells exposed for 2 hours + 22 hours normal incubation (n = 13); (C) Cells exposed for 2 hours + 22 hours normal incubation with the device placed on the rechargeable battery unit in the mobile phone (n = 6); (D) Cells exposed for 2 hours + 22 hours normal incubation with the device clamped in the specially designed mobile phone cover (n = 5). Data represent mean value ± standard deviation. Statistical significance is indicated between the groups (p < 0.01; Wilcoxon- Mann-Whitney test.

Discussion

The first main question of the present study was to evaluate the effect of non-thermal 1800 MHz radiation of an active mobile phone in operation (talking) mode in relation to exposure time on cultured connective tissue fibroblasts as a sensitive biological test system. Our results showed that only 2 hours of continuous exposure to non-thermal radiation caused reduced cell vitality by more than 50 % in comparison to untreated control cells. The effect of the total radiation including the portion of microwaves caused more than 90 % loss in cell vitality. This effect is in accordance with other reports on the time-dependent effect of high-frequency radiation on cultured cells of different origin [18-21]. New in our study is the possibility to distinguish between the portion of thermal and non-thermal radiation by placing a double wall corrugated board between the actively transmitting mobile phone and the cell layers. The difference between both, total radiation and non-thermal radiation, is more than 40 % in cell vitality.

Somehow surprising was the result that cell vitality was not influenced by the orientation of the actively transmitting mobile phone. One should expect that the orientation with the display directed towards the cell layers, as in normal use of the mobile phone, would decrease cell vitality much more than the reverse side directed towards the cells. Lewicka et al. [22] investigated the changes taking place in human blood platelets under the effect of electromagnetic radiation emitted by LCD monitors. They found adverse effects within blood platelets' oxygen

metabolism which might cause physiological dysfunction of the organism. However, it is conceivable that most of the radiation emitted by displays is thermal which has been blocked in our test sytem by use of the cardboard.

Reactive oxygen and nitrogen species ubiquitously exist in complex biological systems, tissues or cells to participate in various cellular signaling pathways. They play an important homeostatic role in regulating the signal transduction involved in cell proliferation and cell survival [23]. Oxidative stress takes place when an excess of reactive oxygen and nitrogen species occurs. Then, endogenous detoxification of the reactive intermediates or repair of the oxidized biomolecules such as nucleic acids, lipids or proteins is not sufficient enough to avoid cell damage. Severe oxidative stress can cause cell death, and even a moderate action of oxidizing species can trigger apoptosis, while more intense stress may cause necrosis [24-27]. Cell death after electromagnetic radiation has been attributed in most publications to be related to increased levels of reactive oxygen and nitrogen species in a variety of species including human, tissues and cells (for review, see [28]).

Although the examination of oxidative stress itself was not a main point of interest in this study, we applied the subsequent normal incubation period after radiation exposure to allow the cells to regulate endogenous signaling for apoptotic or necrotic processes. As already described in detail by Kerr at al. [29] and El-Schich et al. [30], apoptosis shows characteristic morphological changes in single cells such as an increase in cell volume followed by a decrease and the formation of small, roughly spherical cytoplasmic fragments (apoptotic bodies). These typical signs were also observed in our study with cultivated connective tissue fibroblasts after mobile phone radiation suggesting that the cells also underwent apoptosis induced by oxidative stress as shown for cultivated fibroblasts [19,20]. Especially a time-lapse video demonstrated the initial increase in cell volume followed by shrinkage of the cells and the formation of apoptotic bodies after detachment (not shown here). The high pathogenic potential of the induced reactive oxygen species and their involvement in cell signaling pathways explains a wide range of biological and health effects of electromagnetic radiation which include both non-cancer and cancer pathologies [5,31-33].

The second main question of this study was to evaluate whether a special device might be able to compensate non-thermal mobile phone radiation during continuous operation. The results to this question are very convincing demonstrating that this device is actually able to compensate 1800 MHz non-thermal mobile phone radiation to a large extent without influencing the transmitted information. The efficacy of this device has been already shown in a previous publication [13].

It is planned to transfer the study settings to field experiments with humans in their normal living environment in order to see whether the results of the in vitro study as presented here can be assigned to a real life situation.

Conclusions

The results of our study demonstrate that exposure of cultured connective tissue cells to the non-thermal radiation of an actively transmitting mobile phone causes a significantly reduced cell vitality. However, this unwanted biological effect can be extensively compensated (> 95 %) by using the device either alone or with a specifically designed mobile phone cover.

Conflict of Interest

The authors declare no conflict of interests.

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To cite this article: Dartsch PC, Dochow T. Cellular Effects Following Exposure to Mobile Phone Radiation and its Compensation. Japan Journal of Medicine. 2019: 2:2.

Journal of Complementary and Alternative Medical Research

3(2): 1-9, 2017; Article no.JOCAMR.33849
ISSN: 2456-6276

Cellular Effects Following Exposure to Wireless DECT Base Radiation and Presentation of a Device for Their Compensation

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Authors' contributions

This work was carried out in collaboration between both authors. Author PCD designed the study, performed the experiments and wrote the first draft of the manuscript. Author TD wrote the chapter describing the operation principle of the compensation device. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JOCAMR/2017/33849
Editor(s):
(1) Nawal Kishore Dubey, Centre for advanced studies in Botany, Banaras Hindu University, India.
Reviewers:
(1) José Martínez Reyes, University of the Ciénega of Michoacán State, México.
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(3) Adlina Binti Suleiman, University of Malaysia, Malaysia.
Complete Peer review History: http://www.sciencedomain.org/review-history/19610

Original Research Article

Received 1st May 2017
Accepted 11th June 2017
Published 19th June 2017

ABSTRACT

Background: Wireless telecommunication sources working with frequencies ranging from 0.9 to 2.5 GHz are still increasing rapidly. Among these are digitally enhanced cordless telecommunication (DECT) phones which have been considered to emit only a weak radiation when an active DECT base and handset are separated from each other.
Aim of the Study: Prompted by this background this study investigated the cellular effects of DECT base radiation and its possible compensation by a specially designed device, named memonizerCOMBI Standard A.
Materials and Methods: Connective tissue fibroblasts (L-929) were exposed to the radiation of an

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active commercially available DECT base with a frequency of 1.885 GHz for 24 hours ± memonizer COMBI beneath the incubator. Unexposed cells in another incubator placed with a distance of about 10 m in the same laboratory rooms served as corresponding controls. Cell vitality was checked by enzymatic measurement of the activity of mitochondrial dehydrogenases by XTT.

Results: The results clearly demonstrate that exposure to DECT base radiation caused a significantly reduced cell vitality by 47.6 ± 7.4% (mean value ± standard deviation; *P* = .01; Wilcoxon-Mann-Whitney test). Reduction in cell vitality was accompanied by marked morphological changes in the cells such as intracellular vacuolization, rounding and detachment which are similar to alterations observed during oxidative stress by the presence of reactive oxygen species. Reduction in cell vitality after DECT base radiation exposure was compensated by use of memonizerCOMBI by two-thirds yielding a reduction in cell vitality by only 17.5 ± 8.1% (mean value ± standard deviation; *P* = .01 vs. exposed cells without memonizer; Wilcoxon-Mann-Whitney test).

Conclusions: The results indicate that exposure of cultured connective tissue cells to DECT base radiation at a frequency of 1.885 GHz causes a significantly reduced cell vitality which can be extensively compensated by using a memonizerCOMBI device.

Keywords: Electromagnetic radiation; health effects; DECT base; memonizerCombi; cell death; cell culture.

1. INTRODUCTION

The continuous increase of wireless telecommunication sources, such as mobile phones, digitally enhanced cordless telecommunication (DECT) phones, routers and many others have caused a dramatic increase in environmental levels of electromagnetic radiation [1-4]. All these sources emit radiation in a wide spectrum of frequencies with different characteristics ranging from 0.9 to 2.5 GHz. Although the energy of this type of radiation is weak compared to ionizing radiation, recent research provides strong evidence that electromagnetic radiation is able to affect biological and biochemical processes and might lead to oxidative stress, cell death, cellular dysfunction and even carcinogenesis [see, for instance refs [5-11].

Due to its world-wide importance with more than 5 billion users [12], mobile phone technology has been extensively investigated for its health effects at the cellular, experimental animal and epidemiological level [see, for instance refs [6,7, 13-16]. Epidemiological and experimental research on DECT base and handset radiation exposure which might be also potentially harmful to millions of people is very limited [17].

Given the limited available data, the objective of the present study was to investigate the effects of radiation emitted from an active DECT base on cultured connective tissue cells with their wide-spread distribution within the body and the use of a newly created device for the compensation of DECT base radiation.

2. MATERIALS AND METHODS

2.1 DECT Phone

The active base of a commercially available DECT phone (Gigaset 4010 Classic; Siemens, Germany) was used for the experiments described here. Analysis of the frequency characteristics gave a sharp peak at 1.885 GHz with - 46.47 dBm (Fig. 1).

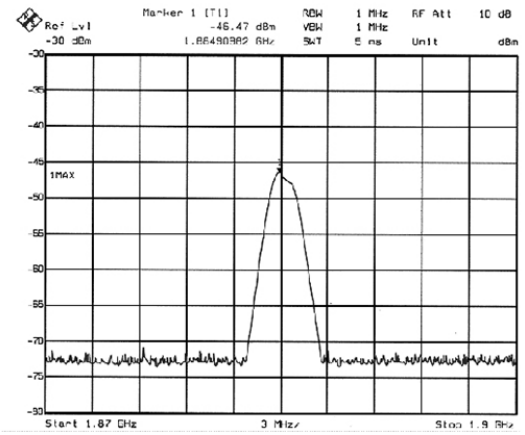

Fig. 1. Analysis of frequency for the active DECT base (Siemens Gigaset 4010 Classic) ranging from 1.87 GHz to 1.90 GHz

The main peak is found at 1.885 GHz with -46.47 dBm

2.2 Device for Compensation of DECT Base Radiation

The device which was tested for its potential to compensate DECT base radiation was a

memonizerCOMBI Standard A (COT-STD.A). This device is commercially available from memon® bionic instruments GmbH, D-83026 Rosenheim, Germany.

2.3 Operation Principle of the Device

Certain mineral groups are able to compensate negative health effects caused by non-ionizing radiation. These are used as so-called metamaterials in the technological application. It is known that such phyllosilicates have ionic clathrate hydrates which can interact with natural and artificial electrons and have properties of typical monochromatic infrared and terahertz frequencies as well as cyclotron resonances [18]. They show octahedral quantum resonances [19] at terahertz frequencies, UV, IR and other lower and higher frequencies. Phyllosilicates are able to protect DNA molecules from ionizing radiation and to act as catalysts in RNA synthesis [20]. Protective mechanisms against biotic and abiotic influences have also been discovered [21].

Typical resonances for living organisms are within certain FIR frequencies, supported by octave resonances of the phyllosilicate metamaterial in the IR, NIR and UV spectra. Artificially generated electromagnetic waves block access to natural octave resonances for living organisms and water clathrate systems. Restoration of this natural field is achieved by using the phyllosilicate metamaterial [22]. These are, together with water molecules, able to transfer their own or induced terahertz resonances [23] and thus to compensate biological effects generated by artificial, non-thermal, non-ionizing radiation.

Selected electromagnetic resonance spectra have led to a marked increase in the activity of ornithine decarboxylase (ODC) in L-929 cells in similar experiments [24]. The technology used in this experiment utilizes specially induced natural resonance spectra in phyllosilicate metamaterials, which are believed to lie within the terahertz gap and can be transferred to living systems. The hypothesis of the EM wave transmutation [22] also stated that the quantum states of such phyllosilicates and their particular resonances in biological evolution could have been decisive in the creation of the first living cells [25].

2.4 Cell Culture and Test Procedure

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

In this present study, cultured connective tissue fibroblasts (cell line L-929; Leibniz-Institut, Deutsche Sammlung für Mikroorganismen und Zellkulturen, Braunschweig, Germany) as a standard cell line for toxicological studies were taken at passages 22 to 50 over a total experimental period of 4 months. Cells were routinely cultivated in the moist atmosphere of an incubator at 37℃ and gassed with 5% CO_2 and 95% air to yield a constant pH value. Culture medium was RPMI 1640 with 10% fetal calf serum and standard amounts of penicillin and streptomycin. All cell culture reagents were from GE Healthcare Life Sciences; Freiburg, Germany.

For the tests, cells were seeded from 80 to 90 % confluent mass cultures at a density of 20,000 cells/well into 16 wells in the middle part of a 96 well-plate (200 µL culture medium/well). After 24 hours to ensure cell attachment and metabolization, culture medium was exchanged to 250 µL/well of Leibowitz L-15 medium (Biochrom; Berlin, Germany) containing 10% fetal calf serum and standard amounts of penicillin and streptomycin. This culture medium guarantees a pH value at 7.4 even at normal atmospheric conditions. Plates were transferred to a Cultura M mini incubator and cultivated at 37 ± 1℃ without CO_2-gassing.

The active DECT base was directly placed on the lid of the culture plate and cells were exposed to the DECT base radiation at continuous operation for the next 24 hours. Approximately 10 meters distance in the same laboratory rooms, a second Cultura M mini incubator was taken for the untreated control cells in a 96-well plate at the same cultivation conditions.

After 24 hours of exposure, cell vitality was checked by morphological observation of the cell cultures and by enzymatic activity. For this purpose, cell culture medium was removed and replaced by 120 µL fresh culture medium and 12 µL XTT (Xenometrix AG, Allschwil, Switzerland) and incubated for 120 minutes in the incubator at 37℃.

XTT is the sodium salt of 2,3-bis[2-methoxy-4-nitro-5-sulfo-pheny]-2H-tetrazolium-5-carboxyanilide and has a yellowish color. Mitochondrial dehydrogenases of viable cells cleave the tetrazolium ring of XTT to yield orange formazan crystals which are soluble in aqueous solutions. The intensity of the resulting orange solution correlates directly with cell vitality and metabolic activity [26,27].

After 120 minutes, optical density was measured as a differential measurement ΔOD = 450 minus 690 nm after a 4 second shaking interval using an ELISA reader (BioTek Slx808). The experiments were performed in 4 independent test series on different days with 14 wells per test. Statistical analysis was done using Wilcoxon-Mann-Whitney test.

In a second series of experiments, the same experimental design as described above was used with the modification that the device for compensation was placed directly beneath the Cultura M mini incubator containing the 96-well plate and the active DECT base. The experiments were performed in 6 independent test series on different days with 14 wells per test. Statistical analysis was done using Wilcoxon-Mann-Whitney test.

3. RESULTS

The results of all experiments presented here were consistent and reproducible, considering they were conducted within a time period of several months with breaks between the investigation intervals. This demonstrates that this experimental setup has been established successfully and provides data which can be directly compared with each other.

As depicted in Fig. 2, the exposure of connective tissue fibroblasts to the active DECT base for 24 hours caused a reduced cell vitality in all experiments when compared to untreated control cells. When the results of the single experiments are taken together, this reduction in cell vitality was 47.6 ± 7.4% (mean value ± standard deviation) and was statistically different from untreated control cells as checked by Wilcoxon-Mann-Whitney test (*P* = .01; Fig. 5). The reduced cell vitality after DECT base radiation exposure also resulted in a largely altered morphology of connective tissue fibroblasts (Fig. 3) with

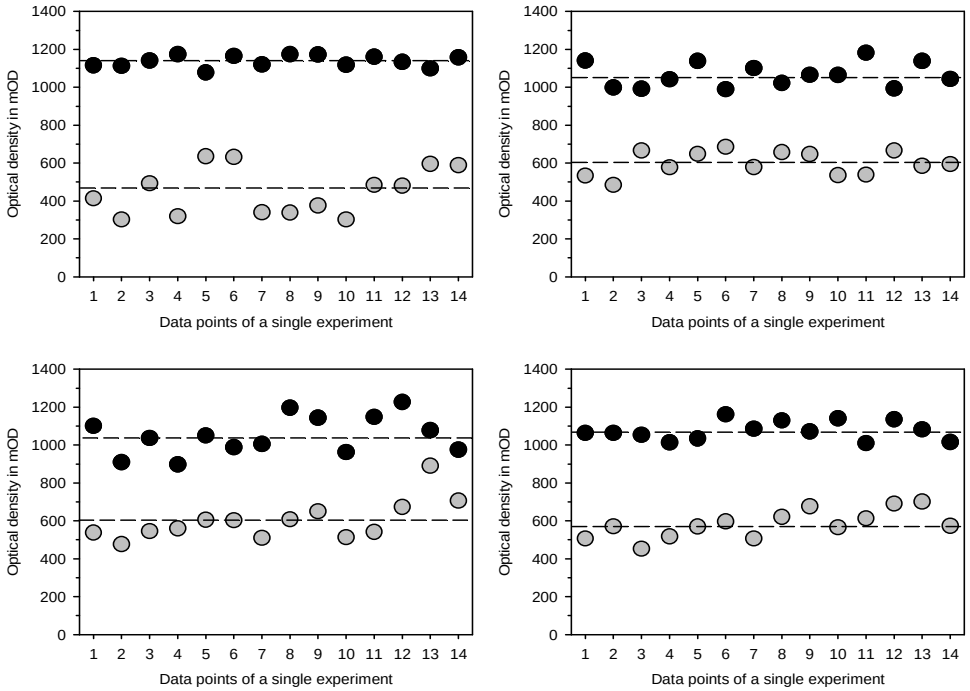

Fig. 2. Original measurements of 4 independent experiments with exposure of connective tissue fibroblasts to the active DECT base for 24 hours (gray circles) in comparison to untreated control cells (black circles)

Each data point given in the diagrams represents the cell vitality in one single well of the appropriate 96-well plate

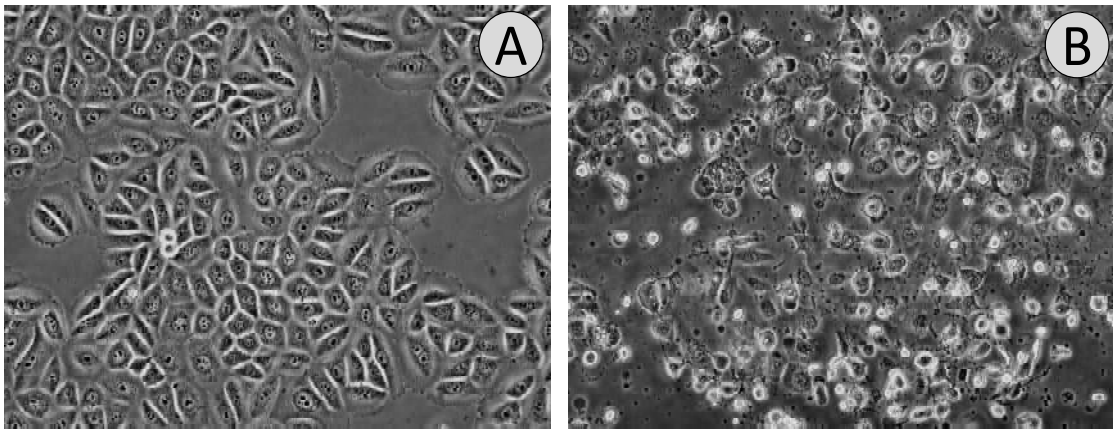

Fig. 3. Micrographs illustrating the alterations in cell morphology of connective tissue fibroblasts which were exposed to DECT base radiation for 24 hours (B) in comparison to untreated control cells (A)

Note the marked cell rounding, detachment and intracellular vacuolization in (B) which might be related to oxidative stress. Phase contrast microscopy at an Olympus IX50 inverted microscope equipped with an Olympus 20x Planachromate and an Olympus E-10 digital camera at 4 megapixels

intracellular vacuolization and rounding of cells with long cytoplasmic protrusions or even detachment. These changes were irreversible, because cells did not achieve normal cell morphology after another 24 hours of incubation in fresh culture medium and without any further DECT base radiation exposure (not depicted).

When a memonizerCOMBI Standard A was placed beneath the incubator in which cells were exposed to DECT base radiation for 24 hours, the reduction in cell vitality was markedly different from previous experiments without the device. Vitality of connective tissue fibroblasts was reduced now only by 17.5 ± 8.1% (mean value ± standard deviation) which is equivalent to a compensation of unwanted cellular effects of approximately two-thirds (Fig. 4 and Fig. 5). The statistical significance of the summarized values between exposed cells ± memonizerCOMBI Standard A was $P = .01$ confirming that the device was able to reduce cellular effects in a quite effective way. However, there was still a statistical difference of $P = .05$ between untreated control cells and exposed cells with memonizerCOMBI Standard A (Fig. 5).

There is another point which should be mentioned here. It took at least 7 days after installation of the device below the Cultura M mini incubator until its information transfer field had developed and was efficient enough to compensate the unwanted cellular effects of DECT base radiation as presented above.

4. DISCUSSION

The fact that wireless telecommunication sources might cause unwanted health effects is still under controversial discussion. However, one should also take the different relevant frequencies under consideration which are ranging from 0.9 GHz to 2.5 GHz and might vary from country to country. Although mobile phones have become the main wireless telecommunication source worldwide, wireless DECT phones are still in use in millions of domestic homes and at workplaces. It has been considered that DECT phones emit only a weak and uncritical radiation when an active DECT base and handset are separated from each other. Indeed, this seems to be not the case under the test conditions as presented here. In the tests, the radiation of an active DECT base reduced cell vitality by approximately 50%.

One might argue that an active DECT base for a continuous period of 24 hours and a distance of only some centimeters between cells and DECT base might be not a realistic situation. This may be right, although there are numerous people who have a DECT base nearby and the handset placed on the table nearly every day. Under these circumstances, the cellular effects of an active DECT base become more prominent. However, the cellular effects in terms of morphology and vitality as observed here are in accordance with previous studies on other cell types [28,29].

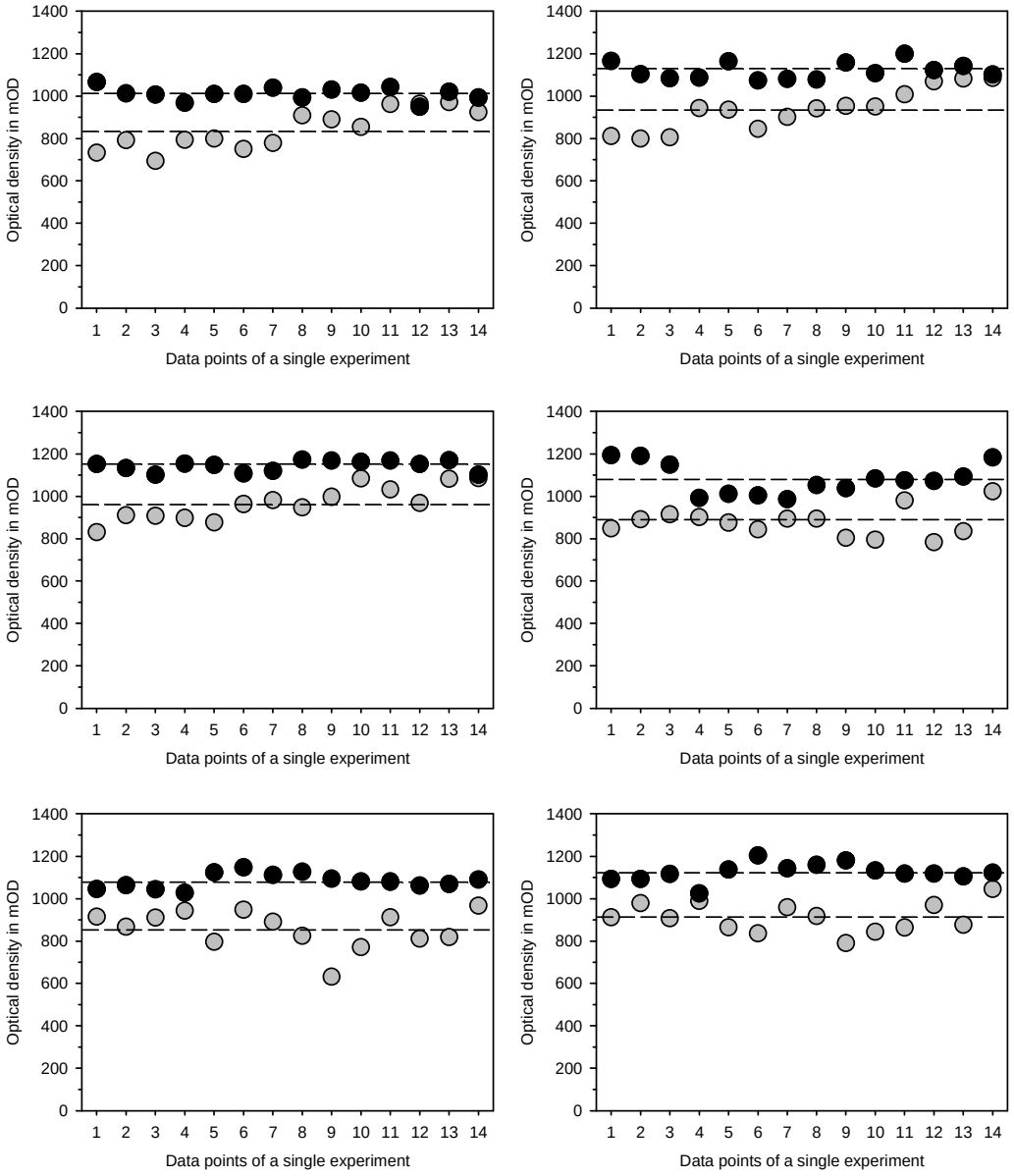

Fig. 4. Original measurements of 6 independent experiments with exposure of connective tissue fibroblasts to the active DECT base with memonizerCOMBI Standard A for 24 hours (gray circles) in comparison to untreated control cells (black circles)

Each data point given in the diagrams represents the cell vitality in one single well of the appropriate 96-well plate

Oxidative stress is a biochemical condition, which is defined by an imbalance between reactive oxygen species and the body's antioxidant protection. The presented morphological results with an active DECT base at a frequency of 1.885 GHz point to previous findings which described that the generation of reactive oxygen species and the resulting oxidative stress seems to be one of the main mechanisms causing cell death by apoptosis (see, for instance [28-31]. Dasdag and Akdag [32] evaluated in their review available in vitro and in vivo studies carried out on the relation between radiofrequency radiation and oxidative stress. The results of their studies indicated that radiofrequency radiation might be a factor which causes oxidative stress.

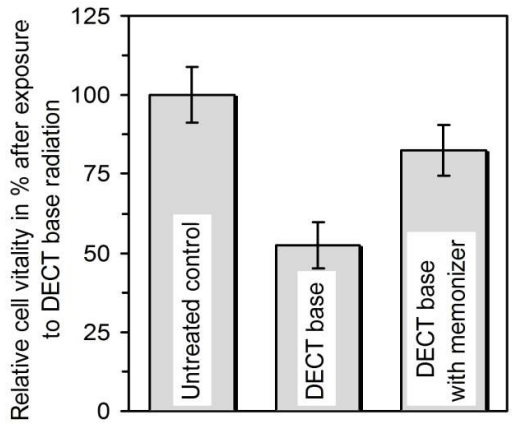

Fig. 5. Summarized presentation of the reduced cell vitality of connective tissue fibroblasts after exposure to DECT base radiation for 24 hours and its compensation by use of a memonizerCombi Standard A device

Data represent mean value ± standard deviation of 4 experiments (untreated control vs. DECT base radiation; P = .01; Wilcoxon-Mann-Whitney test) and 6 experiments (untreated control vs. DECT base radiation with memonizer compensation; P = .05; Wilcoxon-Mann-Whitney test)

Quite surprising and unexpected were the results when a memonizerCOMBI Standard A was placed beneath the incubator in which the cells were exposed to DECT base radiation. As shown here in a number of independent experiments, this device is able to compensate the unwanted cellular effects of the radiation by approximately two-thirds. How the memonizerCOMBI Standard A really acts on the cells and reduces the unwanted effects of DECT base radiation is currently unknown and only a subject of speculation.

Further studies are currently undertaken to come to a closer understanding of the mechanisms how this device compensates DECT base radiation. However, many electro sensitive persons report an improvement of their situation when a memonizerCOMBI Standard A is used over a longer period of time.

5. CONCLUSION

The results indicate that exposure of cultured connective tissue cells to DECT base radiation at a frequency of 1.885 GHz causes a significantly reduced cell vitality which can be greatly compensated by using a memonizerCOMBI device.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENTS

The author gratefully acknowledges the analysis of frequency for the DECT base used here which was done by ROM-Elektronik GmbH, D-86489 Deisenhausen, Germany.

This investigation was funded in part by the sponsorship program of the Bavarian Ministry of Economic Affairs and Media, Energy and Technology (Bayern innovativ).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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http://sciencedomain.org/review-history/19610