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Research Article

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Protective Effect of QiOne® 2 Pro on Cultured Intestinal Epithelial Cells after Mobile Phone Radiation

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ABSTRACT

QiOne® 2 Pro is a specific device which creates a static field that stimulates water molecules to undergo a transition into the coherent state. Since our body consists of about 70 to 85% of water (depending on age), this coherent state of the water molecules might increase the cellular resistance against exogenous reliabilities such as electromagnetic fields. In this study, the protective effect of QiOne® 2 Pro against mobile phone radiation was examined by using the cultured intestinal epithelial cells. Unprotected cells and untreated control cells served as point of reference. The cell regeneration process as well as the integrity of the intestinal epithelial barrier was investigated by measuring the transepithelial electrical resistance.

Mobile phone radiation caused a reduced cell regenerative activity by approximately 60%, whereas the values were about 15% for QiOne® 2 Pro protected cells and untreated controls, respectively. Moreover, mobile phone radiation caused a rupture on the epithelial barrier in unprotected cells by cell death caused due to the oxidative stress with a complete loss of morphological integrity on the barrier. In contrast, untreated controls and QiOne® 2 Pro protected cells did not show any morphological change on the cell layers with an epithelial barrier of a 10-fold higher transepithelial electrical resistance than the unprotected cells.

Overall the results clearly demonstrate the sensitivity of intestinal barrier against oxidative stress generated by mobile phone radiation. In addition, the results also show that the QiOne® 2 Pro device is able to reduce unwanted cellular effects of mobile phone radiation.

Keywords

Mobile phone radiation
Coherent water
Intestinal epithelial cells
IPEC-J2
Cell regeneration
Transepithelial electrical resistance
Cell culture

ABBREVIATIONS

TEER: Transepithelial Electrical Resistance

INTRODUCTION

The intestinal epithelium with single cell layer thickness has two essential tasks. The first one is to create a physical barrier between the contents of the intestinal lumen and the rest of our body. The second one is to ensure an efficient absorption of essential nutrients from the gut lumen and to produce mucus, anti-microbial peptides and cytokines with both protective and immune-regulatory properties. Thus, a reduced barrier function may have far reaching consequences, not only for intestine, but also for systemic health [1]. This barrier function can be destroyed by oxidative stress [2] as generated by mobile phone radiation.

Despite the ubiquity of electromagnetic field emitters, the influence on living organisms and cells has become the subject of numerous research studies with contradictory results [3-6].

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However, it has been demonstrated that oxidative stress plays a key role in the events causing cell death [7-11]. Although the gastrointestinal tract does not play a key role in the mobile phone radiation studies, there are some articles dealing with the effect of electromagnetic radiation on the intestine. For example, in an experimental animal study Sieroń et al. [12] demonstrated that electromagnetic radiations modified the redox balance in the gastrointestinal tract of rats. This might enhance the sensitivity against intestinal oxidative stress by injury or disease with all its unwanted health consequences.

According to the manufacturer, Qi Blanco UG (haftungsbeschränkt) from Germany, the QiOne® 2 Pro device contains a grid chip which forms a static field that stimulates water molecules to undergo a transition into the coherent state. Since our body consists of about 70-85% of water (depending on age), the coherent state of the water molecules positively influence the cells of our whole body. Moreover, QiOne® 2 Pro is stated to neutralize the unwanted effects of mobile phone radiation.

In this present *in vitro* study, current bioassays were used to investigate whether the use of QiOne® 2 Pro might result in a protective effect on cultured intestinal epithelial cells against mobile phone radiation and the arising oxidative stress.

MATERIAL AND METHODS

QiOne® 2 Pro device

QiOne® 2 Pro was provided by Qi Blanco UG (haftungsbeschränkt), D-97711 Maßbach, Germany, for the duration of the experiments. The device is a second generation grid chip of a specifically developed gold alloy with eightfold strength which creates a static field that stimulates water molecules to enter a coherent state in your body.

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. No distinction was made between thermal and non-thermal radiation, because both are also present in reality when making a call and have an effect on the human body.

Cultivation of intestinal cells

The investigations presented here were conducted with IPEC-J2 cells (ACC-701; Leibniz Institut, DSMZ, Braunschweig, Germany). The cells were grown in Dulbecco's Modification of Eagles Medium (DMEM; 1.0 g/L glucose) with 10% growth mixture and 0.5% gentamycin and cultivated in an incubator at 37°C in an atmosphere of 5% CO_2 and 95% air at nearly 100% humidity. The cells were cultivated as mass cultures and were regularly subcultured twice a week with fresh culture medium. For the experiments, cells were taken from 80-90% confluent mass cultures.

Cell regeneration after mobile phone radiation

Cells were seeded at a density of 100,000 cells/ml into the four individual compartments of a silicone 4 well-culture insert made (ibidi, Gräfelfing, Germany). The single compartments of the inserts are separated by a 500 µm thick silicone bar with an outer silicone frame of 700 µm. Due to the special adhesion area, an insert adheres firmly to the bottom of a culture dish and forms a distinct cell-free area (artificial wound) in which the cells can colonize by migration and proliferation. Upon reaching confluency within 48 hours after cell seeding, the cells were exposed to the radiation of an actively transmitting mobile phone & WLAN with and without QiOne® 2 Pro. The cell culture dish was placed on the mobile phone display (direction of radiation towards the user) in a mini-incubator for 4 hours at 37°C without gassing (Figure 1). To avoid any pH changes during exposure at normal air conditions, the routine culture medium was replaced by Leibowitz L-15 medium with 1% growth mixture and 0.5% gentamycin. Cells which were cultured in the same way, but without mobile phone radiation, served as corresponding controls. After 4 hours of radiation, cells were incubated for another 8 hours in the same culture medium. Finally, cells were fixed with methanol, stained with Giemsa's azur eosin methylene blue solution (Merck, Darmstadt, Germany), air-dried and examined by micrographs and a specialized software with artificial intelligence from KML Vision, Graz, Austria (IKOSA AI software).

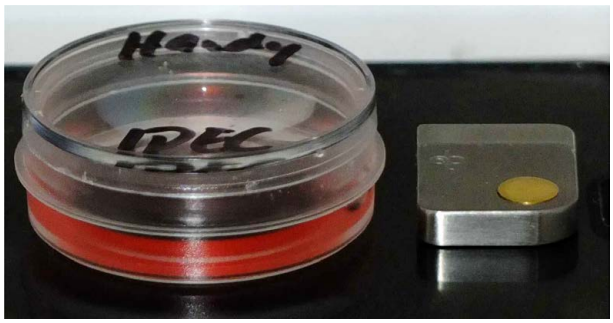

Figure 1: Arrangement of cell culture dish which was placed on the mobile phone display (direction of radiation towards the user) together with a QiOne® 2 Pro device on the right side.

Transepithelial electrical resistance (TEER) after mobile phone radiation

IPEC-J2 cells were grown for 5 days on the surface of a 0.4 µm porous membrane (transwell plate, Corning, Sigma-Aldrich, Deisenhofen, Germany) which yields two seperated compartments within the cell culture dish. As a matter of fact, the cells covering the surface of the membrane represent a physical barrier against the lower compartment. TEER was measured by placing one electrode in the culture medium in the upper compartment and by placing one electrode in the culture medium in the lower compartment. Electrical resistance was directly measured by a portable voltmeter (Millicell ERS-2 Voltmeter, Millipore/Merck, Darmstadt, Germany) as described in detail elsewhere [1,13,14].

Epithelial cell layers were taken for the experiments at an electrical resistance of at least 2,000 Ω/cm^2 representing an intact physical barrier with very good integrity and were exposed to the actively transmitting mobile phone mobile phone & WLAN with and without QiOne® 2 for 4 hours at 37°C as already described. After another 24 hours of cultivation, TEER was measured again and data were compared to each other. Three independent experiments were conducted. As a reference, TEER of the porous membranes without any cell barrier was measured to be 150 to 180 Ω/cm^2.

RESULTS

Cell regeneration

As seen in Figures 2 and 3, mobile phone radiation caused a reduction in cell regenerative activity by leaving a cell-free area

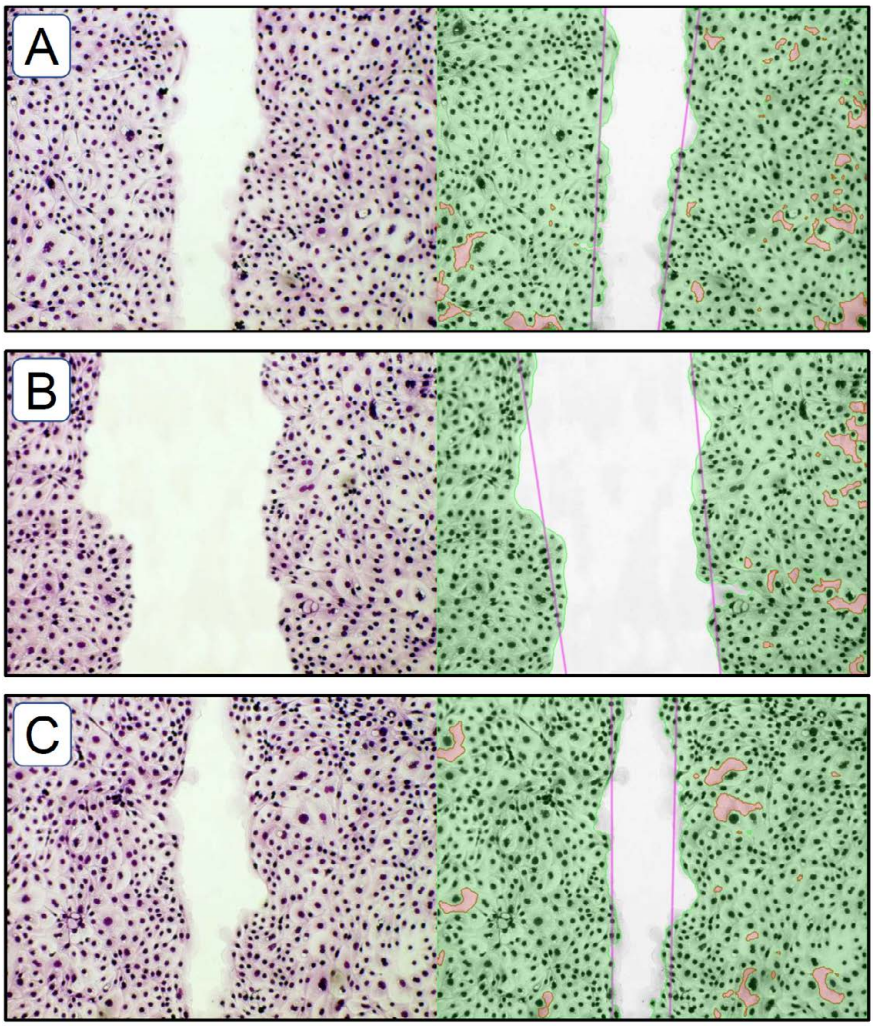

Figure 2: Original micrographs (left part) as well as the evaluation by IKOSA AI software (right part) with marked calculated frontlines of the IPEC-J2 cells closing the cell-free area. (A) Untreated control at the same culture conditions as below, but without any exposure to mobile phone radiation. (B) Unprotected cells after 4 hours of mobile phone radiation with subsequent cultivation for 8 hours. (C) QiOne® 2 Pro protected cells after 4 hours of mobile phone radiation with subsequent cultivation for 8 hours. Note the largely decreased colonization of the cell-free area in (B). Untreated control and QiOne® 2 Pro protected cells show a similar colonization pattern.

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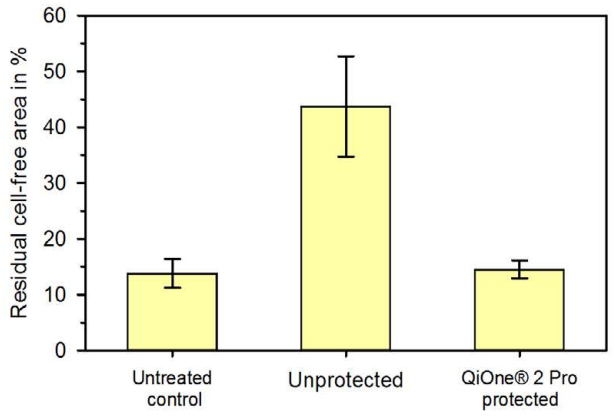

Figure 3: Presentation of the results on cell regeneration after mobile phone exposure with and without QiOne® 2 Pro for 4 hours and subsequent cultivation for 8 hours. Note that the residual cell-free areas of untreated control and QiOne® 2 Pro protected cells do not differ from each other, whereas unprotected cells have a significant higher cell-free area due to a markedly reduced cell activity and even cell death. Data represent mean value ± standard deviation of four independent experiments.

which was 43.7 ± 9.0% (mean value ± standard deviation; n=4) in comparison to the total colonized cell area. In contrast, the residual cell-free area was only 13.8 ± 2.6% for the untreated control and 14.5 ± 1.6% for the QiOne® 2 Pro protected cells (both mean values ± standard deviations; n=4). The difference between the unprotected cells and QiOne® 2 Pro protected cells was statistically significant ($p \leq 0{,}01$; two-tailed Wilcoxon-Mann-Whitney test) demonstrating the effectiveness of the device against mobile phone radiation. Moreover, there was no statistically relevant difference between the QiOne® 2 Pro protected cells and the cells which were not exposed to mobile phone radiation at all.

Transepithelial electrical resistance (TEER)

As seen in Figure 4, mobile phone radiation caused a rupture on the epithelial barrier by causing cell death due to oxidative stress. In contrast, untreated controls and QiOne® 2 Pro protected cells did not show such massive morphological changes of the cell layers. This situation is reflected by the TEER values measured. For the unprotected cells a TEER of 152 ± 16 Ω/cm² was measured demonstrating the complete

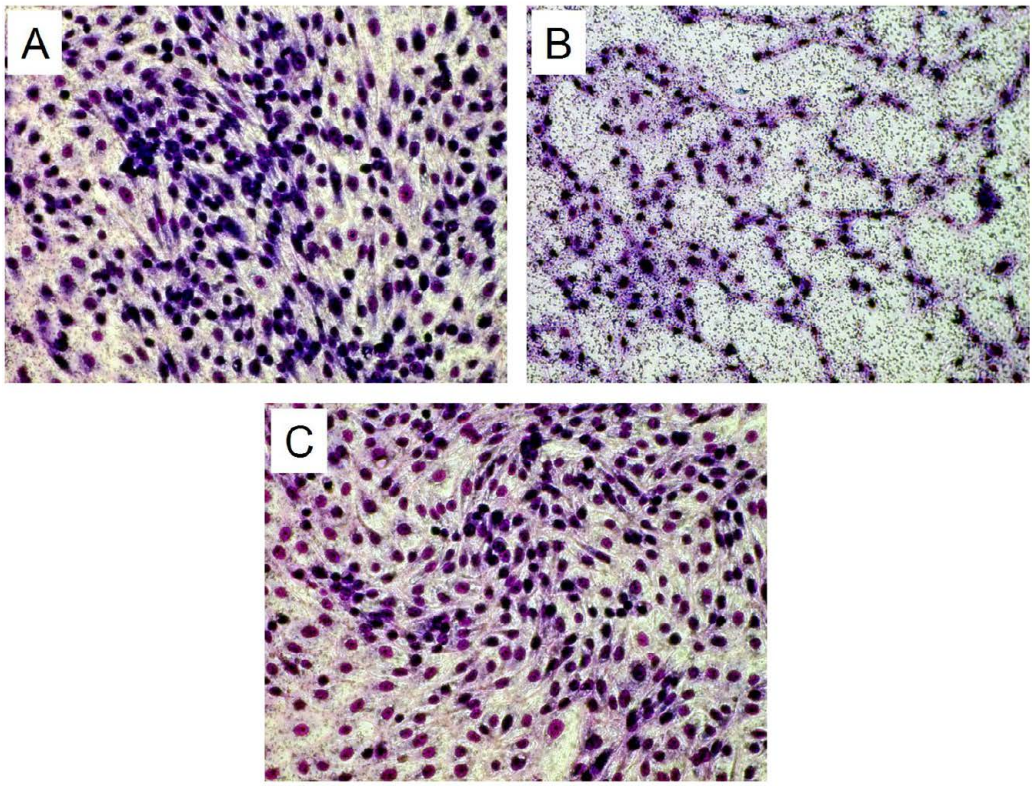

Figure 4: Original micrographs of IPEC-J2 cells establishing a physical barrier on the porous membranes of transwells after 4 hours of mobile phone radiation with subsequent cultivation for 24 hours. (A) Untreated control. (B) Unprotected cells. (C) QiOne® 2 Pro protected cells. The small dark points are the 0.4 µm pores in the membrane and the purple stained structures are the cell nuclei. The cytoplasm of the cells is only weekly stained by the dye. Note the rupture of the epithelial barrier in (B), whereas the barrier integrity in QiOne® 2 Pro protected cells in (C) is nearly similar to the untreated controls in (A).

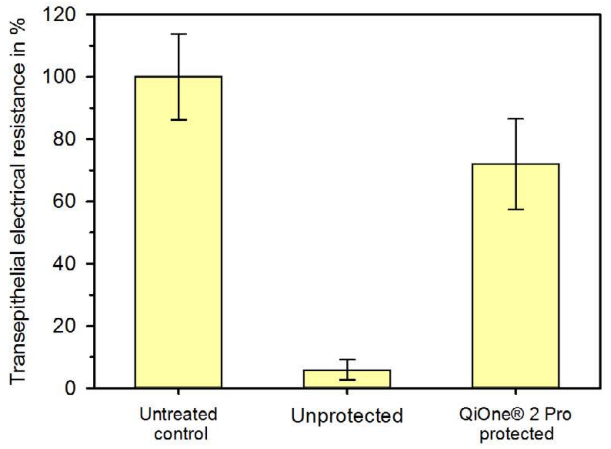

Figure 5: Presentation of the results on transepithelial electrical resistance (TEER) after mobile phone exposure with and without QiOne® 2 Pro for 4 hours and subsequent cultivation for 8 hours. Note that the TEER of untreated control and QiOne® 2 Pro protected cells do not differ from each other, whereas unprotected cells have a significant decreased TEER due to a rupture of the epithelial barrier. Data represent mean value ± standard deviation of three independent experiments.

loss of barrier integrity. The TEER value for the QiOne® 2 Pro protected cells was 1,837 ± 349 Ω/cm² and for the untreated controls 2,542 ± 389 Ω/cm² (all mean value ± standard deviation). The difference between protected and untreated cells was statistically not significant, whereas the difference to the unprotected cells was highly significant ($p \leq 0,01$; two-tailed Wilcoxon-Mann-Whitney test; n=3). When calculating the relative values by setting the TEER of the untreated controls as 100 ± 13.8%, the QiOne® 2 Pro protected cells had an value of 72.6 ± 14.5% and the unprotected cells of 6.0 ± 3.2% (all mean values ± standard deviations; Figure 5). Again, the data demonstrate the effectiveness of the device against mobile phone radiation.

DISCUSSION

Bhattacharyya et al. [15] have summarized in their review that reactive oxygen species are generated as by-products of normal cellular metabolic activities which can be inactivated by endogenous enzymes such as superoxide dismutase, glutathione peroxidase and catalase. However, reactive oxygen species are generated as a response by a number of traumatic influences acting on our body. Among these are ultraviolet radiation, cigarette smoking, alcohol, nonsteroidal anti-inflammatory drugs, ischemia-reperfusion injury, chronic infections and inflammatory disorders.

Moreover, mobile phone radiation is also known to produce an excess of reactive oxygen radicals [10-13,16-20] which cannot be inactivated by the enzymes mentioned above. Since the epithelial cells of the intestinal barrier have a high turnover rate, they are more sensitive against oxidative stress which can induce oxidative injury and inflammatory responses involving a deficiency of the epithelium and immune/inflammation mediating cells [15].

Prompted by this background cultured intestinal cells were used to examine the effect of oxidative stress on the regenerative potential and the integrity of the epithelial barrier. According to Vergauwen [21] "IPEC-J2 cells are intestinal porcine enterocytes isolated from the jejunum of a neonatal piglet. The IPEC-J2 cell line is unique as it is derived from the small intestine and is neither transformed nor tumorigenic in nature. IPEC-J2 cells mimic the human physiology more closely than any other cell line of non-human origin". The cells were originally isolated in 1989 by Helen Berschneider at the University of North Carolina [22]. The advantage of the IPEC-J2 cell line as an *in vitro* model originates from its morphological and functional similarities with intestinal epithelial cells *in vivo*. IPEC-J2 cells have microvilli on their apical side and tight junctions to act as a barrier and reflecting epithelial functionality [23]. The determination of TEER is a technique that provides information about the uniformity of the IPEC-J2 cell layer on the microporous filter membrane and the integrity of the tight junctions formed between the polarized cells. Therefore, TEER measurements are often used to study epithelial barrier function [1].

The present results with QiOne® 2 Pro complement confirm our previous findings in which we demonstrated that this device was able to protect functional neutrophils against mobile phone radiation [24]. In these experiments, cell viability was checked by the generation of superoxide anion radicals in the course of an oxidative or respiratory burst. Mobile phone radiation in unprotected functional neutrophils caused a reduction in superoxide anion radical generation by approximately 40% in comparison to untreated cells. In contrast, QiOne® 2 Pro protected cells showed a reduction in superoxide anion radical generation by only 16% in comparison to untreated cells. In the present experiments with IPEC-J2 cells the regeneration values for unprotected and QiOne® 2 Pro protected cells were in the same range as in the previous experiments with functional neutrophils. However, the examination of the integrity of a three-dimensional intestinal barrier demonstrated a much higher sensitivity against oxidative stress from mobile phone radiation. This is in accordance to the findings reviewed by Bhattacharyya et al. [15]. The use of QiOne® 2 Pro protected the cells in a significant manner as shown here.

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Although the principles of quantum electrodynamics (QED) are not really accepted in conventional medicine as a method to influence the state of water, the present investigation has shown that coherent water as generated by use of QiOne® 2 Pro obviously has a definite positive impact on cells by increasing their resistance against exogenous traumatic influences such as mobile phone radiation. It has been stated that electromagnetic fields can be coupled to coherent systems resulting in a "self-trapping" which causes a common in phase dynamical oscillation [25-28]. As a matter of fact, the coherent system might be protected against exogenous electromagnetic fields like the inner part of a Faraday cage.

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