Oxidative and Mutagenic Effects of Low Intensity GSM 1800 MHz Microwave Radiation

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Aim: Despite a significant number of epidemiological studies on potential carcinogenicity of microwave radiation (MWR) from wireless devices and a bulk of experimental studies on oxidative and mutagenic effects of low intensity MWR, the discussion on potential carcinogenicity of low intensity MWR is ongoing. This study aims to assess oxidative and mutagenic effects of low intensity MWR from a typical commercial model of a modern smartphone. Materials and Methods: The model of developing quail embryos has been used for the assessment of oxidative and mutagenic effects of Global System for Mobile communication (GSM) 1800 MHz MWR from a commercial model of smartphone. The embryos were exposed in ovo to 0.32 µW/cm², discontinuously — 48 s — On, 12 s — Off, during 5 days before and 14 days through the incubation period. Results: The exposure of quail embryos before and during the incubation period to low intensity GSM 1800 MHz has resulted in expressive statistically significant oxidative effects in embryonic cells, including a 2-fold increase in superoxide generation rate and 85% increase in nitrogen oxide generation rate, damages of DNA integrity and oxidative damages of DNA (up to twice increased levels of 8-oxo-dG in cells of 1-day old chicks from the exposed embryos). Finally, the exposure resulted in a significant, almost twice, increase of embryo mortality. Conclusion: The exposure of model biological system to low intensity GSM 1800 MHz MWR resulted in significant oxidative and mutagenic effects in exposed cells, and thus should be recognized as a significant risk factor for living cells.

Key Words: radiofrequency, microwaves, reactive oxygen species, oxidative effects, mutagenic effects, carcinogenesis, embryo.

Since 2011, when the World Health Organization (WHO) classified radiofrequency radiation (RFR) as a possibly carcinogenic to humans (group 2B) [1], the discussion on the risks from modern wireless devices for human health persists. Epidemiological data of independent from the industry studies inevitably demonstrate the increased risk of some types of tumors in heavy users of wireless devices [2]. Also, a significant amount of experimental studies demonstrates plausible biological mechanisms of low intensity RFR and particularly microwave radiation (MWR) potential carcinogenicity. During last years, dozens of studies assessed potential oxidative and mutagenic effects of low intensity RFR/MWR and a majority of them has demonstrated statistically significant adverse effects of the exposure for living cells (see, for example, reviews [3, 4]). Nevertheless, neither International Commission on Non-Ionizing Radiation Protection (ICNIRP) nor national regulatory bodies over the world have reacted properly on the growing body of scientific evidence on potential risks of chronic exposure of human body, especially human brain, to modulated MWR from modern wireless devices [5]. This regulatory uncertainty coupled with technological advantages and customer benefits of the wireless technologies leads to a lack of understanding of real risks of MWR over-exposure among the public. That is why demonstrative experimental studies on reliable biological models using real commercial wireless devices are extremely important both for deeper scientific understanding of the phenomena and for changing in risk perception of MWR among the regulatory bodies and the public.

Here we demonstrate that a typical modern wireless device, a smartphone, which meets all official safety limits does produce significant adverse effects in classical biological model, a developing quail embryo, and thus does provide significant risk for consumers’ health, including the risk of oncogenic transformation of living cells.

Materials and Methods

Biological model of developing quail embryos in ovo was used for the experiments. Two groups of fresh hatching eggs of Japanese Quail were formed for each experiment (n = 15). One group was the control, and the other was exposed to MWR. Incubation of the embryos in ovo was carried out in two foam plastic incubators designed for the experiments, free of metal covers. Thus, MWR was neither shielded nor reflected on the incubators structures. Hatching eggs were incubated in conditions close to optimal.

A commercial model of smartphone Huawei 5YII of the Global System for Mobile communication (GSM) 1800 MHz standard assigned to a local mobile con-
nector provider was used as a typical source of low intensity MWR from a modern wireless device. The muted and silenced smartphone was activated due to auto-redial computer program, which guaranteed a discontinuous activation of the phone as a source of MWR (48 s — ON, 12 s — OFF). The phone was placed on a plastic setup 3 cm over the surface of hatching eggs of the exposed group. MWR intensities were assessed by the RF Field Strength Meter (USA).

In order to maximize the time of MWR exposure we started irradiation of quail embryos of the exposed groups in ovo 5 days before the incubation at the room temperature. Then the exposure of embryos in ovo was continued during 14 days of incubation. The embryos of control groups were subjected to the same procedures as the exposed ones except for the MWR exposure. The exposed and control embryos were incubated at the same conditions in two separate incubators placed 2 m from each other.

The average intensity of MWR on a surface of hatching eggs of exposed groups was 0.32 µW/cm². A calculated specific absorption rate (SAR) value for quail embryos in our experiments was about 3.8 µW/kg. The radiofrequency background in the laboratory during the experiments was 0.001 µW/cm².

Analysis of DNA single- and double-strand breaks in 38-h embryo cells was performed using an alkaline Comet assay as described [6, 7] with slight modifications [8]. Briefly, embryos were separated from the yolk membrane using paper rings, and embryonic cells were dissociated by careful trituration of whole embryos in PBS to achieve about 5 • 10⁴ of cells per ml. The frosted microscope slides were first covered with a layer of an agarose gel (Sigma-Aldrich, Munich, Germany). Then, 1–2 • 10⁴ of cells were embedded into 75 µl of 1% low melting point agarose (Sigma-Aldrich) at 37 °C and the gel was cast over the first agarose layer on ice for 10 min. Slides were immersed into a lysis solution (2.5 M NaCl, 100 mM ethylenediamine tetra-acetic acid (EDTA), 10 mM Tris, 10% dimethylsulphoxide (DMSO), 1% Triton X-100, pH 10, all reagents Sigma-Aldrich) and kept for an hour at 4 °C. After cell lysis, the slides were placed in a horizontal gel electrophoresis for 20 min at 0.8 V/cm. After lysis, the slides were centrifuged at 3,000 rpm for 10 min. The level of lipid peroxides in the embryo tissues was measured in supernatants by spectrophotometer Specoll 11 (Germany) at λ = 532 nm.

Superoxide dismutase (SOD) activity was assessed using the assay based on a competition of SOD and nitro blue tetrazolium (NBT) for superoxide [15]. Superoxide was produced in the reaction medium in a reaction of NADH with phenazine methosulfate in the presence of oxygen. A decrease of hydrazine tetrazolium level (which formed in a reaction of superoxide with NBT) due to a presence of SOD of the sample was detected spectrophotometrically at λ = 540 nm.

Catalase activity assessment in the embryo tissues was made using a reaction of decomposition of hydrogen peroxide (H₂O₂; 0.03% solution) added into the samples. The determination of the hydrogen peroxide...
residual in the sample was carried out using its reaction with molibdate ammonium (4% solution) [16]. Molibdate ammonium produces a color complex with H₂O₂, and its level was assessed spectrophotometrically (λ = 410 nm).

Ceruloplasmin activity in embryo cells was assessed in express test with parafenilendiamine as described in [17]. Briefly, the reaction was carried out at 60 °C for 10 min and stopped by the addition of a 25% solution of NaOH. The reaction yield was evaluated spectrophotometrically (λ = 440 nm).

All experiments were carried out according to the permission of the Bioethics Commission of Bila Tserkva National Agrarian University.

The data were expressed as the mean ± standard error of the mean (M ± m). Student’s t-test was used for the statistical analysis. A significant difference was considered as p < 0.05.

RESULTS

MWR intensity from the smartphone varied significantly during the time of exposure, from 0.05 to 20 µW/cm² with average intensity 0.32 ± 0.05 µW/cm² that is far below the ICNIRP safety limit in 450 µW/cm².

Smartphone radiation exposure produced significant oxidative effect in 38-h embryo cells. Level of TBARS in exposed embryo cells was statistically significantly higher as compared to the control (61.1%, p < 0.05), ceruloplasmin activity increased by 110% (p < 0.05), and catalase activity was 60.2% higher than in control, although the last number was not statistically significant as compared to the control group (Fig. 1).

This MWR exposure of quail embryos 5 days before the incubation and 38 h during the incubation period also resulted in a statistically significantly increased level of DNA strand breaks in cells of 38-h embryos detected by alkaline comet assay. The percentage of DNA in comet tails was by 17.5% (p < 0.05) higher in cells of exposed embryos as compared to control embryos (Fig. 2).

In cells of exposed 10-day embryos there were detected significantly increased rates of generation of both superoxide radical and nitrogen oxide radical (Fig. 3). Superoxide radical generation rate was by 140–210% (p < 0.05–0.001) higher in cells of brains, hearts and livers of 10-day exposed embryos as compared to control embryos. Nitrogen oxide generation rate increased in cells of 10-day embryos after the smartphone radiation exposure by 18.7–84.7% (p < 0.01–0.001) as compared to the control.

Significantly increased levels of free radical generation also were observed in cells of 1-day old chicks from the exposed embryos. Particularly, superoxide generation rate was by 180–217% (p < 0.001) higher in cells of brains, hearts and livers of chicks from the exposed embryos as compared to control embryos. Nitrogen oxide generation rate in cells of brains, hearts and livers of 1-day chicks from the exposed embryos was by 25.0–87.7% (p < 0.001) higher than in control.

![Fig. 2. Alkaline comet assay of cells from 38-h quail embryos: a — microscopic pictures (× 20) of control cells; b — microscopic pictures (× 40) of exposed cells (GSM 1800 MHz, 0.32 µW/cm², discontinuously, 48 s — ON, 12 s — OFF); c — percentage of DNA in tails of comets. *p < 0.05 as compared to the matched control.](image)

![Fig. 3.](image)
Also, level of 8-oxo-dG, a marker of oxidative damages of DNA, was statistically significantly, 63.4–119.4% ($p < 0.01–0.001$) increased in cells of brains, hearts and livers of chicks from smartphone radiation exposed embryos as compared to the control chicks (Fig. 4).

And finally, level of embryo mortality was statistically significantly increased in GSM 1800 MHz exposed group of embryos, from 42.1% in control group to 80% in the exposed group ($p < 0.05$).

**DISCUSSION**

The used biological model, a developing quail embryo, and the methods of analysis applied have allowed us to demonstrate statistically significant adverse biological effects of GSM 1800 MHz radiation from a commercial model of modern smartphone. There were detected expressive statistically significant oxidative effects and damages of DNA integrity in cells of 38-h embryos under the GSM 1800 MHz exposure in intensity three orders of magnitude lower than official “safety limits”. The adverse effects under the smartphone radiation exposure were persistent during the embryogenesis and included a 2-fold increase in level of superoxide generation rate and up to 85% increase in nitrogen oxide generation rate in tissues of 10-day embryos and 1-day old chicks. Also, in 1-day old chicks from the exposed embryos there were demonstrated statistically significant oxidative damages of DNA. Finally, the smartphone emission applied to quail embryos 5 days before the incubation and during 14 days of incubation period resulted in a significant, almost twice, increase of embryo mortality as compared to the control embryos.

Superoxide and nitrogen oxide, which significant overproductions were detected in our experiments, both are free radical species. Thus, we could state on the free radicals overproduction in living cells as the first step response of the cell on GSM modulated MWR exposure. On the other hand, it is not clear yet the mechanisms of the free radicals’ overproduction in the cell under low intensity MWR exposure. Previously both mitochondrial and NADH oxidase pathways of superoxide generation were experimentally proven to be activated under low intensity MWR [18, 19]. As we used the spin trap for the ESR detection of superoxide specifically in mitochondria, our data support a mitochondrial pathway of superoxide overproduction. It is still unclear the site of interaction of modulated MWR with mitochondria structures/electron transport chain (ETC) complexes of mitochondria. At least three sites of superoxide generation in ETC are...
known at the moment: complex I [20], complex II [21], and complex III [22].

As for a persistent statistically significant overproduction of nitrogen oxide in the exposed embryonic cells, the question is if this is an additional expression of NO-synthases under the MWR exposure or a direct activation of the NO-synthese molecules presented in the cells at the moment of irradiation. For example, direct interaction of MWR with NADH oxidase was demonstrated previously [19]. On the other hand, a significant overproduction of NO· may itself lead to disturbing in ETC and increase a generation of superoxide in the cell [23]. The increased levels of superoxide and nitrogen oxide in embryonic cells due to low intensity GSM 1800 MHz MWR exposure can result in a significant activation of peroxidation processes [24]. Also, significantly increased levels of antioxidant enzymes’ activities detected in the exposed 38-h embryos cells is a typical feature of the first stage of the oxidative stress. Next stages of oxidative stress development typically are accompanied by significant depression of antioxidant enzymes activities. And the dramatic consequence of the increased levels of O2·- and NO· in the exposed embryonic cells was a pronounced oxidative damages of DNA, which resulted in a 2-fold increased level of 8-oxo-dG in cells of 1-day old chicks from the exposed embryos.

These findings are in line with our previous research data [8, 14, 24, 25] and data from the other laboratories over the world on expressive oxidative and mutagenic effects of low intensity MWR exposure [18, 26–28]. A huge pathogenic potential of oxidative stress in the cell, including its role in carcinogenesis [29, 30], allowed us to hypothesize that overproduction of free radical species, namely superoxide and nitrogen oxide, in MWR exposed living cells is one of the key mechanisms for the next pathological transformation of cells [24]. The persistent oxidative damages of DNA could be a first step of mutagenic and carcinogenic processes [30]. Thus oxidative damages of DNA resulted in alters of transcription rate, replication errors and genomic instability [31]. In turn these processes are associated with carcinogenesis. And in different cancer tissues, an increased level of oxidative damages of DNA were reported [30].

Recently we have demonstrated that oxidative effects of low intensity MWR significantly depend on the GSM modulation of the signal [25]. Thus, except of urgent necessity to reevaluate current out-of-date official safety limits on RF/MWR intensity, additional biologically proven restrictions on different types of modulated signals should be elaborated and implemented by international and national regulatory bodies.

In conclusion, statistically significant persistent oxidative and mutagenic effects in model biological system, a developing quail embryo, as well as significantly increased embryo mortality have been detected under low intensity GSM 1800 MHz MWR from a typical modern commercial smartphone. Thus the data confirm that modulated MWR from modern wireless devices should be recognized as a significant risk factor for living cells, and out-of-date official safety limits should be urgently reevaluated in line with current biological research on the issue.

**CONFLICT OF INTERESTS**
The authors declare no conflict of interests.

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**REFERENCES**