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Comparison of cytotoxic and genotoxic effects of plutonium-239 alpha particles and mobile phone GSM 900 radiation in the *Allium cepa* test

Dmitry S. Pesnya*, Anton V. Romanovsky

I.D. Papanin Institute for Biology of Inland Waters, Russian Academy of Sciences, 152742 Borok, Nekouz, Yaroslavl region, Russia

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ABSTRACT

The goal of this study was to compare the cytotoxic and genotoxic effects of plutonium-239 alpha particles and GSM 900 modulated mobile phone (model Sony Ericsson K550i) radiation in the *Allium cepa* test. Three groups of bulbs were exposed to mobile phone radiation during 0 (sham), 3 and 9 h. A positive control group was treated during 20 min with plutonium-239 alpha-radiation. Mitotic abnormalities, chromosome aberrations, micronuclei and mitotic index were analyzed. Exposure to alpha-radiation from plutonium-239 and exposure to modulated radiation from mobile phone during 3 and 9 h significantly increased the mitotic index. GSM 900 mobile phone radiation as well as alpha-radiation from plutonium-239 induced both clastogenic and aneugenic effects. However, the aneugenic activity of mobile phone radiation was more pronounced. After 9 h of exposure to mobile phone radiation, polyploid cells, three-groups metaphases, amitoses and some unspecified abnormalities were detected, which were not registered in the other experimental groups. Importantly, GSM 900 mobile phone radiation increased the mitotic index, the frequency of mitotic and chromosome abnormalities, and the micronucleus frequency in a time-dependent manner. Due to its sensitivity, the *A. cepa* test can be recommended as a useful cytogenetic assay to assess cytotoxic and genotoxic effects of radiofrequency electromagnetic fields.

1. Introduction

In the past few years the use of cell phones has become widespread. Many studies have been carried out to assess potential hazards to human health and the environment due to the increased exposure to radiofrequency electromagnetic fields (RF-EMF) emitted by mobile phones and other wireless devices.

Most of the attention on possible adverse effects of electromagnetic radiation has been focused on human health [1–5]. Moreover, some studies suggested higher risk for malignant brain tumors in people with ipsilateral mobile phone use [6–8]. Recently, the IARC classified radiofrequency electromagnetic fields as possibly carcinogenic to humans (Group 2B) [9].

Additionally, the mobile phone radiation seems to have effects upon different biological species: bacteria [10], protozoa [11], plants [12,13], insects [14,15], amphibians [16], birds [17] and mammals [18–21]. These reports have contributed to a better knowledge of the interactions between EMF and living organisms.

Amongst many biological targets, the DNA molecule has received the greatest attention with respect to potential RF-EMF damage [22]. Alterations of DNA in somatic cells are one of the key events in the process of carcinogenesis and any agent with genotoxic activity may also be suspected to be carcinogenic [23–25]. However, genotoxic data from different non-mammalian species are very limited. Information on invertebrates and plants is particularly lacking.

Along with other plant species, *Allium cepa L.* has been used to evaluate DNA damages, such as chromosome aberrations, micronuclei and disturbances in the mitotic cycle. The *A. cepa* test is now frequently used for environmental monitoring [26] and also for assessing effects of ionizing and non-ionizing radiation [13,27].

In the present study the effects of RF-EMF on the mitotic index, and on the frequencies of chromosomal abnormalities and micronuclei were investigated in root meristematic cells of *A. cepa L.*, in comparison with the effects of alpha particles.

2. Materials and methods

2.1. The A. cepa test

The *A. cepa* test was used to analyze genotoxic effects [28,29].

We used onion bulbs (*A. cepa L.*, $2n = 16$) of the Stuttgarten-Risen variety, average weight 25 g. The bulbs were placed in glass jars with their basal ends dipping in distilled water, and germinated at room temperature ($24 \pm 3\,^{\circ}C$). When the newly emerged roots were 0.50–1 cm in length, they were used in the experiments.

After the treatment, root-tips were fixed in a solution of ethanol (96%) and glacial acetic acid (3:1, v/v) for 48 h, washed with distilled water, and then stained with aceto-orcein for 1 h. The squash technique was applied to prepare samples for

* Corresponding author at: Dmitry Sergeevich Pesnya, Avenue Frunze, h.2, ap.77, 150030 Yaroslavl, Yaroslavl region, Russia. Tel.: +7 4852 524196.
E-mail addresses: 24pesnya@gmail.com (D.S. Pesnya), imnement@gmail.com (A.V. Romanovsky).

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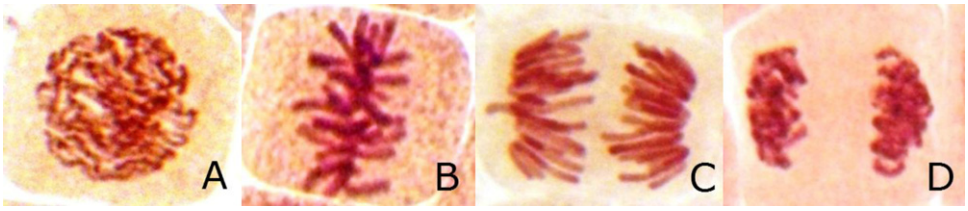

Fig. 1. Stages of mitosis in the meristematic cells of *Allium cepa* (400×): (A) prophase; (B) metaphase; (C) anaphase; (D) telophase.

the study of the mitotic index (MI), mitotic and chromosomal abnormalities and micronuclei.

Experiments were repeated two times. In each experiment five groups of bulbs were prepared: a negative control group (distilled water), a positive control group (20 min of exposure to plutonium-239 α-particles), a sham-exposed group (9 h of sham-exposure to mobile phone radiation), and "9 h" and "3 h" groups (9 and 3 h of exposure to mobile phone radiation). Six replicates were performed for each group. From each of six bulbs we took three roots for microscopic analyses [30].

2.2. Microscopic observations

MI, mitotic and chromosome abnormalities and micronuclei were analyzed on the same slides. The MI was calculated as a number of dividing cells per 700 cells. Phases of mitosis are shown in microphotographs (Fig. 1).

Chromosomal aberrations – chromatid (single) and chromosome (double) bridges, and fragments – were scored in 100 ana/telophases per slide. Mitotic abnormalities (lagging chromosomes and stickiness) were scored in 1000 mitotic cells per slide. Micronucleus frequency was expressed as the number of interphase cells with micronuclei per 3000 cells for every slide. All examinations were done with a light microscope at 300× and 400× magnification.

2.3. Standard source of alpha particles, and treatment

A standard source of α-particles was obtained from RITVERC GmbH Scientific Production Association "V.G. Khlopin Radium Institute", Russia. The source is delivered in the form of a stainless-steel backing on which a thin shift of active plutonium-239 is deposited. Half-life: 24,000 years; alpha-particle energy (MeV): 5.155; intensity (%): 73.4; nominal activity (Bq): 3.0×10^4. Measurements were performed by use of a dosimeter-radiometer DRBP-03 (Russia). The level of incident alpha-particle flux-density (alpha-particle fluence) was $0.19 \pm 0.02\ cm^{-2}\ s^{-1}$ at the place where the experiments were performed. The characteristic mean alpha-particle flux-density at the surface of plutonium-239 source was $164.8 \pm 0.45\ cm^{-2}\ s^{-1}$ (data are mean values of three separate measurements ± SD).

Group of six non-germinated bulbs was put on the surface of the alpha-particle source for 20 min (positive control). After treatment they germinated in distilled water, as described above.

2.4. Mobile phone used, measurements and exposure system

It has been reported that effects of RF radiation depend on a variety of physical characteristics such as frequency, polarization and modulation [31]. We used a GSM 900 mobile phone (model Sony Ericsson K550i, provider Beeline-Vimpel, Ltd.) in order to analyze effects of realistic exposure conditions including complex modulation of the GSM signal, geometry and EMF components, which is missing in studies with GSM-simulator models. The SAR of this phone is 1.4 W/kg according to the manufacturer. It was impossible to get the confidential information from the provider on which of the 124 frequency channels they use at the nearest base station. Thus, root meristematic cells may be exposed to any of these frequencies (890–915 MHz). Measurements were performed with an RF-EMF meter PZ-31 (FGUP Specialized Design Office of Radio Equipment, Nizhny Novgorod, Russia). The level of the incident RF power-density was $0.05 \pm 0.01\ \mu W/cm^2$ at the place where the experiments were performed. The characteristic mean power densities – at a distance of 1.5 cm to the surface of the mobile phone – measured for 10 min of exposure are presented in Table 1. These data were collected at the beginning of exposure. The emission was kept going during exposure by use of a recorded human voice.

Six bulbs were placed at the distance of 1.5 cm from the mobile phone as shown in Fig. 2. Roots of *A. cepa* were placed in glass jars and exposed to modulated radiation from the mobile phone.

The experiment was continued for three days, with the following three types of exposure. For the first group the duration of exposure was 3 h per day (in total 9 h), for the second group 1 h per day (in total 3 h). Then, sham-exposure took place, during which the mobile phone was turned off. The duration of the sham exposure was 3 h per day (in total 9 h).

Table 1
Data on the radiation power-density on the different parts of a mobile phone. The mean values of seven separate measurements ± SD are given.

Side	Part	Power density ($\mu W/cm^2$)
Front panel	Upper part (F1)	8.41 ± 1.32
	Middle part (F2)	12.10 ± 1.29
	Lower part (F3)	5.25 ± 0.91
Back panel	Upper part (B1)	4.79 ± 0.77
	Middle part (B2)	6.20 ± 0.81
	Lower part (B3)	4.04 ± 0.52

2.5. Statistical analysis

The differences in the mitotic index, frequency of chromosome aberrations, micronuclei and mitotic abnormalities between treated and control groups were statistically analyzed by the independent Student's *t*-test with Statistica 8.0. Values of $p < 0.05$ were considered as indicative of a significant difference with the negative control (*), with the sham-exposure group ([a]), with the positive control ([b]), and between the "3 h" group and the "9 h" group ([c]).

3. Results and discussion

3.1. Mitotic index

The most frequent abnormalities are shown in Figs. 1 and 3–5, along with normal cells. The results regarding MI and the frequency of abnormalities in root-tip cells of *A. cepa* are summarized in Tables 2 and 3 and in Appendix A. Also there are video recordings of micronuclei after 3 h (Video 1) and 9 h (Videos 2 and 3) of exposure to mobile phone radiation.

Power densities of radiation were different at different exposure location (Table 1 and Fig. 2). However, the differences between values of MI, abnormalities and micronuclei when *A. cepa* bulbs were exposed at different exposure locations were not statistically significant (see Appendix A). Thus, the mean values of summary data ± SD are shown in Tables 2 and 3.

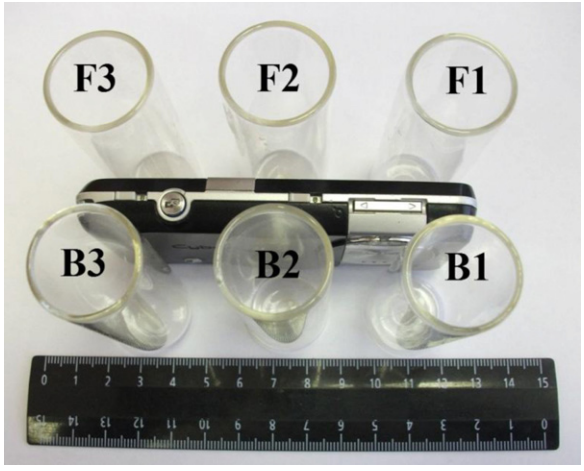

Fig. 2. Schematic presentation of the exposure system.