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Combined Action of Doxorubicin and Magnetic Field on Free Calcium Content in Human Cells

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DMYTRO B. MIROSHNYK[1], IGOR F. KOVALENKO[2], YURIY G. SHCKORBATOV*[1]

[1] V.N.Karazin Kharkiv National University, 4 Svobody Sq., Kharkiv, 61022, Ukraine

[2] Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine, 23, Pereyaslavskaya str., Kharkov, 61015, Ukraine

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ABSTRACT

Objective: To investigate the effect of the magnetic field and doxorubicin on of free calcium content in exfoliated cells of human buccal epithelium. Methods: The intracellular content of free calcium was determined by staining cells with calcium probe Fluo-3. **Results:** Decrease in calcium content was statistically significant if cells exposed in magnetic field (25 mT) for 20 and 60 minutes, 30 minutes exposure did not cause statistically significant change of Ca^{2+}. Doxorubicin in concentration 2 μg/ml (exposure time 2 hours) caused a decrease of Ca^{2+} in cells. The simultaneous exposure to the doxorubicin and the magnetic field caused the additive effect of lowering of free calcium concentration in cells when cell were exposed to the magnetic field for 20 and 30 minutes, but 60 minutes magnetic field exposure had no such effect. **Conclusion:** Magnetic field at certain regime enhance the doxorubicin-related decrease of Ca^{2+}in cells.

INTRODUCTION

Doxorubicin (DOX) is an antitumor antibiotic of a wide spectrum of action, with the application of which, however, is connected with a number of side effects and for which there are a number of contraindications [1]. A number of methods have been proposed to increase the effectiveness of anticancer antibiotics and to reduce their side effects. Among such methodsis the combined application of anticancer antibiotics with magnetic field (MF). The pulsed magnetic field (PMF) was applied to modulate the potency of cisplatin, carboplatin or doxorubicin. If carboplatin was applied to A-431 cells, its effectiveness increased after a 1 hour PMF application. Cytotoxicity of carboplatin and daunomycin, but not cisplatin against HT-29 cells was measured, if PMF was simultaneously applied [2]. In combination with one hour of whole body PMF exposure, the PMF enhanced effectiveness of antibiotics against A-431 and HT-29 cells growing as xenografts in immune deficient mice [3]. The positive effects of magnetic field on efficacy of antitumor antibiotics are reported in many studies reviewed in [4]. The ROS production induced by MF is essential in the enhancement of doxorubicin by magnetic field [5]. Calcium ions, being an intracellular mediator in the transmission of various signals [6], play a significant role in cell responses to stress factors, in cell death and apoptosis [7,8]. The purpose of this work was to investigate cell effects of doxorubicin and static magnetic field on the content of free calcium ions in cytoplasm and cell nucleus.

MATERIALS AND METHODS

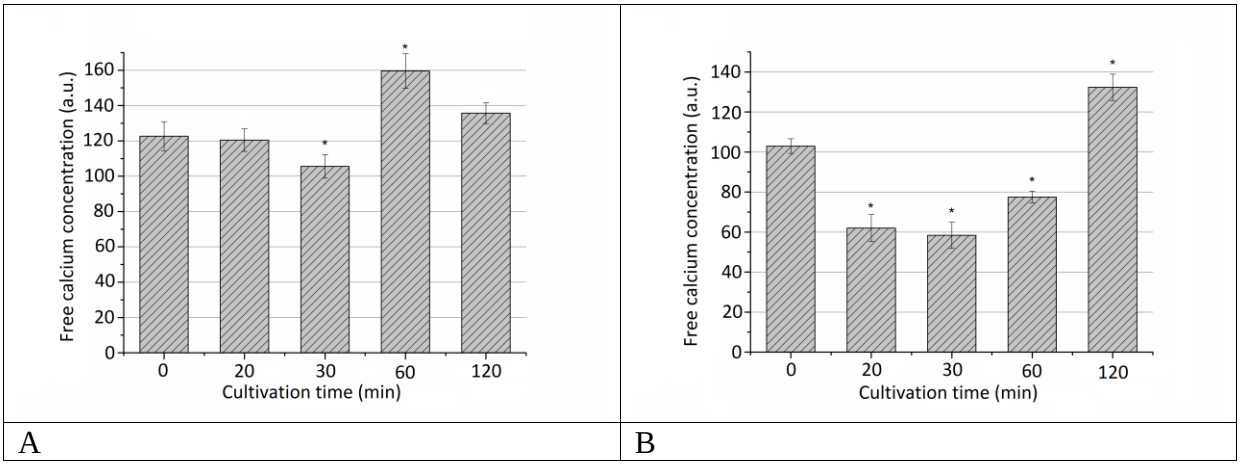
The buccal epithelium cells of male donor (25 years) were used. Donor of cells was informed about the purpose of the experiments and gave his informed consent to take part in experiments. Cells were obtained by scraping from the surface of the donor's cheeks using a sterile spatula. The cells were then placed in a buffer solution of the following composition: 3.03 mM phosphate buffer, pH 7.0 with addition of 2.89 mM $CaCl_2$ [9]. Exfoliated cells of buccal epithelium retain their viability in the above mentioned solution for at last eight hours[10]. As a source of doxorubicin, the medical preparation “Doxorubicin EBEVE” (Ukraine) was used. Cells were placed in the doxorubicin (2 μg/ml) dissolved in the mentioned above medium for two hours. This time was chosen, as it was previously shown that cell show response to doxorubicin in this time, for instance, in cells the significant changes in the activity of the p53 and the amount of free radicals were observed after 2 hours of exposure to doxorubicin [10]. Doxorubicin was used in concentration 2 μg/ml. This

concentration was applied based on our previous experiments, as one that causes a significant reduction in the viability of the buccal epithelium cells and an increase in the number of heterochromatin granules in cell nuclei [11].

A static magnetic field (MF) with the magnetic induction of 25 mTwas created by a magnet (permalloy, 26 x 9 x 1.8 cm). The effect of MP on cells was as follows: 10 µl of cell suspension in a buffer solution previously described in an Eppendorf tube was placed above the magnet pole of the north. If combined effects of doxorubicin and magnetic field on cells were carried out, the total time of cell placement in doxorubicin solution was always 2 hours, where the last 20, 30 or 60 minutes of the cell experiment were additionally influenced by a magnetic field with a magnetic induction of 25 mT. Evaluation of the change in activity in cells of Ca^{2+} ions was performed after the doxorubicin and magnetic field exposure by measuring fluorescence of calcium probe Fluo 3 [13], AM dye with a final dye concentration of 10 µmol. Fluo-3, AM ether (Thermo Fisher Scientific, USA, Catalog number: F1241) was used. The suspension of cells (5 µl) was placed on a microscope slide and 5 µl ofFluo-3was added. Drop of cell suspension was then covered with a coverslip. After 10 minutes cells were photographed to determine the calcium content. Determination of calcium content in arbitrary units was measured using a LSM 510 META (Carl Zeiss) confocal microscope. An argon laser with a wavelength of 488 nm and a power of 10 mW was used to excite the fluorescence. To fix the fluorescence, an emission filter of 518 - 572 nm was used. An aperture diaphragm (pinhole) was 356 µm.

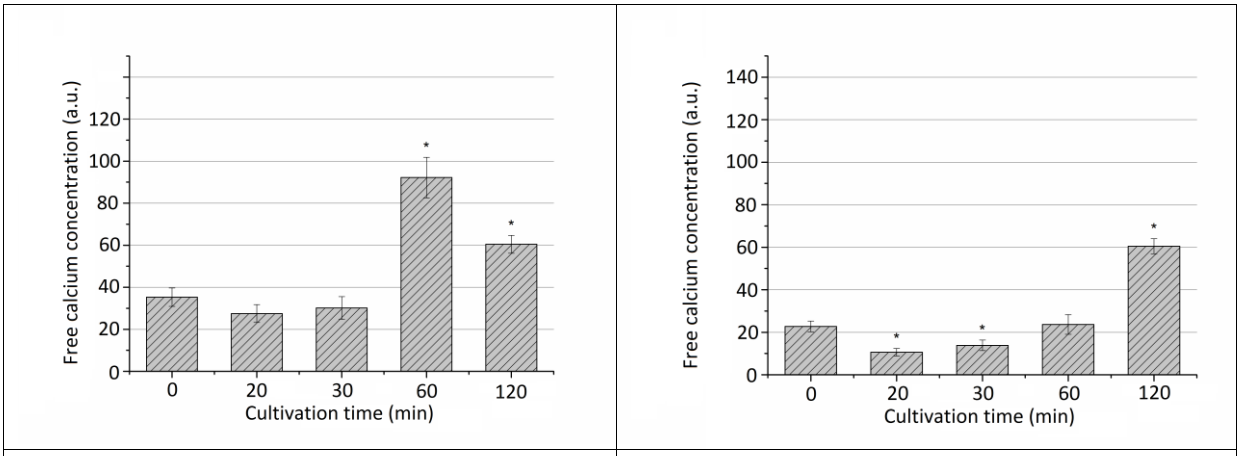
RESULTS

The content of calcium ions in the conditions of survival culture of buccal epithelium varies in cells in different periods after obtaining cells from the donor's cheek. The concentration of calcium ions in cell nuclei was significantly higher than in the cytoplasm (Fig. 1, 2). As can be seen in cell nuclei (Fig. 1) and cytoplasm (Fig. 2) of both donors concentration of calcium ions decreases relatively to the initial level in the first period of cultivation (20 and 30 minutes of cultivation), and then increases again (60 minutes of cultivation). These changes indicate the instability of the cells state in a survival culture.



A B

Fig. 1. Concentration of free calcium in nucleus in different periods of cultivation of cells (A - cells of donor A, B - cells of donor B).



A B

Fig. 2. Concentration of free calcium in cytoplasm in different periods of cultivation of cells (A - cells of donor A, B - cells of donor B).

Under the influence of the static magnetic field for 20 and 60 minutes the free calcium concentration decreases as compared to the initial level in nuclei and in cytoplasm of cells of both donors (Fig. 3, 4). The decrease in the free calcium concentration is also observed after 20 minutes in nuclei and cytoplasm of cells of donor B (Fig. 4B, 5B). This decrease coincides with the decrease in calcium concentration in the cells of donor B in the control at that time, although in control in the cells of the donor A the level of free calcium at this time does not change (Fig. 2A, 3A). After a 30-minute exposure to the magnetic field free calcium concentration does not change comparatively to the initial level in the nuclei and cytoplasm of the donor A cells (Fig. 3A, 4A), but it decreases it in the nuclei of the cells of donor B (Fig. 3B).

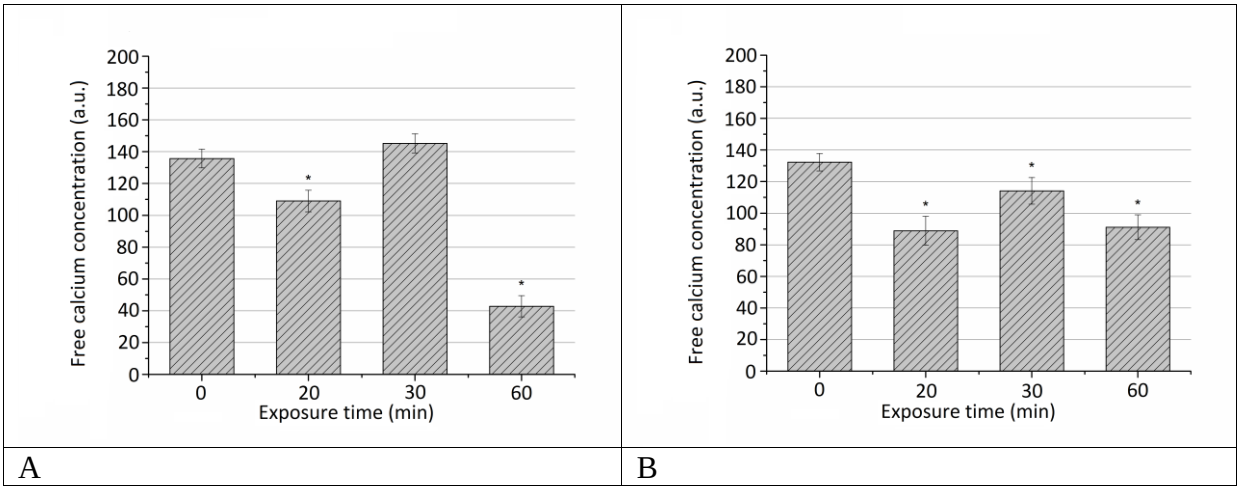


Fig. 3 Concentration of free calcium in cell nuclei in different periods of the influence of the magnetic field (A - cells of donor A, B - cells of donor B).

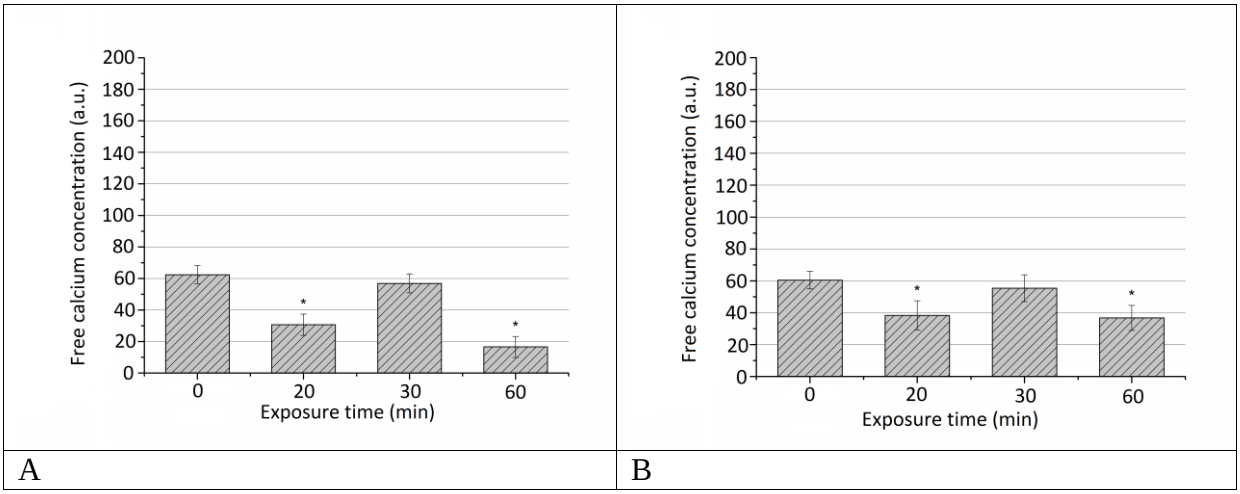


Fig. 4. Concentration of free calcium in cytoplasm in different times of the influence of magnetic field (A - cells of donor A, B - cells of donor B)

Thus, the reaction of calcium concentration in cell to the magnetic field has a "wave-like" shape. At 20 minutes and at 60 minutes there is a significant decrease in free calcium concentration, which drastically differs from the control cells, since in control after 60 minutes of cultivation the increase in the concentration of calcium ions in cell nuclei and cytoplasm is observed (Fig. 1).

The effect of doxorubicin on the calcium ion content was analyzed earlier; in particular, the effect of doxorubicin at a concentration of 0.2 and 2 mg/l on the concentration of calcium ions in nuclei and in the cytoplasm of human buccal epithelium was shown [11]. Fig. 5 and 6 show the results of measuring of free calcium concentration in cells of donors A and B under

conditions of combined action of magnetic field and doxorubicin. Our current experiments show that doxorubicin in concentration of 2 μg/ml reduces the free calcium concentration in the nuclei of the buccal epithelium of donors A and B (Fig. 5). As a control (the "control" column), were used calcium levels after 2 hours of incubation in the medium without doxorubicin and without the influence of the magnetic field. Doxorubicin also lowers the concentration of calcium ions in the cytoplasm of cells of donor A, but does not affect its content in the cytoplasm of the cells of donor B (Fig. 6).

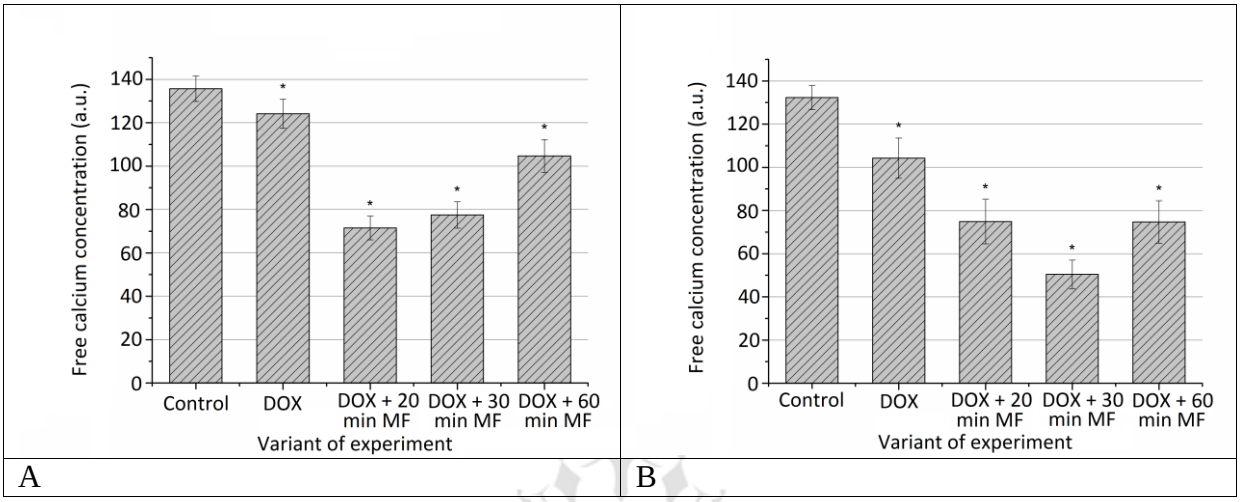


Fig. 5. Concentration of free calcium in cell nuclei after treating of cells with the magnetic field (MF) and doxorubicin (DOX). A - cells of donor A, B - cells of donor B.

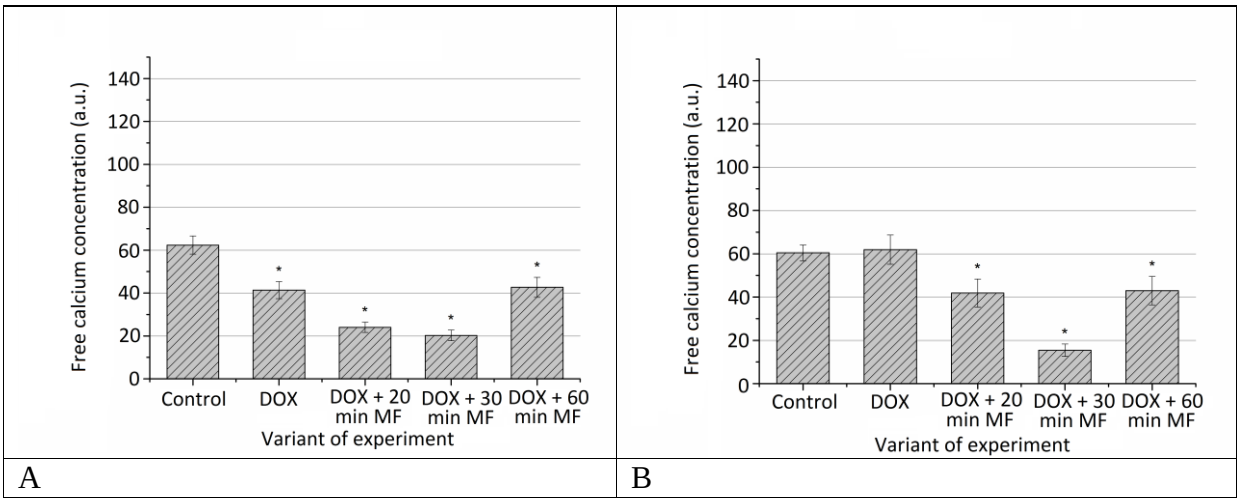


Fig. 6. Concentration of free calcium in cytoplasm after applying of the magnetic field (MF) and doxorubicin (DOX). A - cells of donor A, B - cells of donor B.

It can be seen (Fig. 5, 6) that in relation to the level of free calcium in the variant “doxorubicin only”, under the combined impact of the doxorubicin and magnetic field for 20

and 30 minutes, the concentration of free calcium in the nucleus and cytoplasm decreases. After the combined influence of MF and DOX for 60 minutes, in cells of both donors concentration of free calcium increases relatively to variant "DOX + 30 min MF" but remains lower than in variant "DOX" in cytoplasm and nuclei of cells of donor B and in cytoplasm of cells of donor A. The above data suggest that the magnetic field enhances the effect of doxorubicin on cells, causing an additional decrease in the concentration of free calcium in the cells.

DISCUSSION

It is known that cardiotoxicity of doxorubicin is associated with deregulation of free calcium levels, violated calcium homeostasis and formation of reactive oxygen species [14]. Generally, the violation of the regulation of the concentration of calcium in cell plays an important role in the pathogenesis of cardiomyopathy caused by doxorubicin. Cardiotoxicity caused by doxorubicin is also accompanied by an increase in the intracellular levels of free calcium. The mitochondria of the cells isolated from doxorubicin treated rats have a reduced ability to retain calcium, showing calcium-dependent calcium excretion, which is not observed in mitochondria in rats receiving a saline solution [15]. Doxorubicin reported induce different effects in cells (increase or decrease of free calcium), that may be connected with the difference in experimental conditions. DOX, 0.125 mg/L, for48 hours in human promyelocytic leukemia HL-60 cells may induce a decrease in intracellular calcium content [**Error! Reference source not found.**], but increase of calcium content after treatment of Human Cardiac Progenitor Cells with doxorubicin (100 nM, 5 days) is also reported [16]. Thus, the effect of doxorubicin on the cell is directly related to its effect on the exchange of calcium in the cell. Calcium suppresses the toxicity of doxorubicin in human cancer cells. Addition to the culture of MCF7 cells (the culture of breast cancer cells) of $CaCl_2$ caused a decrease of the doxorubicin-dependent cell death [18].

Based on the above, the decrease in the free calcium concentration in the nucleus and in the cytoplasm under the influence of doxorubicin in our experiments may be interpreted as characteristics that is associated with the toxic effect of doxorubicin and may be among causes of this effect.

The various cell reactions to MF were thoroughly investigated before. The low-intensity static magnetic field (6 mT) induces Ca^{2+} increase in cultured human glioblastoma cells and

reduces damage induced apoptosis in these cells [19]. Reaction of different cells to MF is different, for instance, in HeLa cells after the6 mT MFfor 24 hours exposure the calcium contents increases and not influences apoptosis, butinHepG2 cells the 6 mT MF exposure have no impact on $[Ca^{2+}]_i$ level but increases apoptosis [20]. Thus, the treatment of cells with doxorubicin and magnetic field in our experiments affects the concentration of calcium ions in a living cell. The synergetic decrease of free calcium in cells after combined treatment with doxorubicin and static MF may be related to the enhancement the toxic effect of doxorubicin on cells by magnetic field.

CONCLUSIONS

1. Static magnetic field with a magnetic induction of 25 mT induced a decrease in the content of free calcium in isolated human buccal epithelium cells. The Ca^{2+}changesin a non-monotonic way with cell exposure time and becomes significant at exposure in magnetic field for 20 and 60 minutes.

2. Doxorubicin in concentration of 2 μg/ml, exposure time – 2hours when added to the medium in which cells were incubated, caused a decrease in the content of free calcium in cytoplasm and cell nuclei.

3. The simultaneous exposure to doxorubicin and magnetic field caused an additive effect in decrease of the concentration of free calcium in cells, more pronounced at20 and 30 minutes exposure.

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