Research Results in Pharmacology

Research Results in Pharmacology 9(4): 43–51
UDC: 615.212.7
DOI 10.18413/rrpharmacology.9.10055

Research Article

Polyelectrolyte microcapsules as a tool to enhance photosensitizing effect of chlorin E6

Ekaterina P. Brodovskaya[1], Larisa A. Tararina[2], Mikhail N. Zharkov[1], Irina A. Khutorskaya[1], Denis E. Yakobson[1], Amina M. Al-khadj Aioub[1], Igor V. Maev[2], Andrey V. Zaborovsky[2], Dina V. Yunina[2], Sergei V. Tsaregorodtsev[2], Sergey N. Gerasimchuk[2], Gleb B. Sukhorukov[3,4], Nikolay A. Pyataev[1]

1 National Research Ogarev Mordovia State University, 68 Bolshevistskaya St., Saransk 430005 Republic of Mordovia, Russia
2 A.I. Yevdokimov Moscow State University of Medicine and Dentistry of the Ministry of Healthcare of the Russian Federation, 20|1 Delegatskaya St., Moscow 127473 Russia
3 School of Engineering and Materials Science, Queen Mary University of London, Mile End Road London E1 4NS UK
4 Skolkovo Institute of Science and Technology, 30 Bolshoi Boulevard St., Bldg 1, Moscow 121205 Russia

Corresponding author: Sergei V. Tsaregorodtsev (sergiotsar@yandex.ru)

Academic editor: Tatyana Avtina ♦ **Received** 15 June 2023 ♦ **Accepted** 01 October 2023 ♦ **Published** 15 November 2023

Citation: Brodovskaya EP, Tararina LA, Zharkov MN, Khutorskaya IA, Yakobson DE, Al-khadj Aioub AM, Maev IV, Zaborovsky AV, Yunina DV, Tsaregorodtsev SV, Gerasimchuk SN, Sukhorukov GB, Pyataev NA (2023) Polyelectrolyte microcapsules as a tool to enhance photosensitizing effect of chlorin E6. Research Results in Pharmacology 9(4): 43–51. https://doi.org/10.18413/rrpharmacology.9.10055

Abstract

Introduction: Photodynamic therapy is a promising method of tumors treatment using photosensitizers and light of a certain wavelength. PS modification improves and enhances the phototoxic effect with decreased dark cytotoxicity.

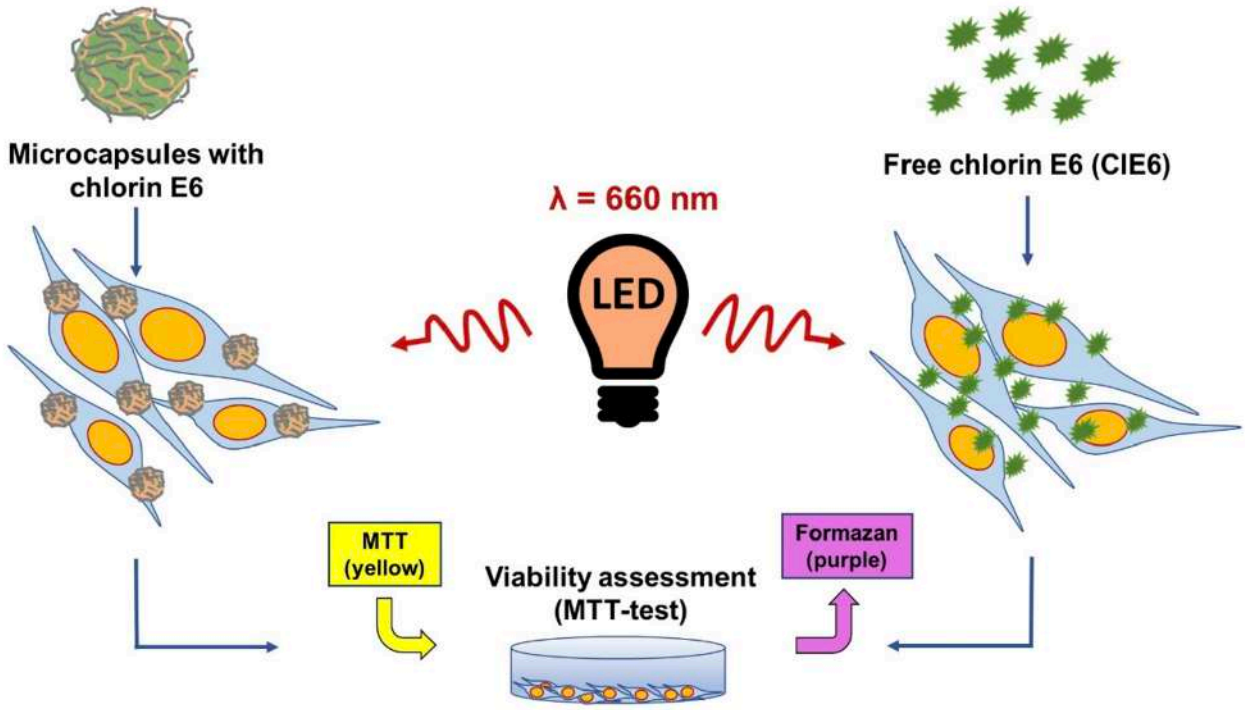
Materials and Methods: We compared the photosensitizing effect of polyelectrolyte microcapsules with chlorin E6 (ClE6) and free ClE6 at equivalent concentrations on murine fibroblast culture L929 using *in vitro* tests. Microcapsules were prepared layer by layer, sequentially depositing oppositely charged polyelectrolytes onto spherical $CaCO_3$ particles. Cellular uptake of capsules was assessed using confocal microscopy. MTT test was used for a study of cell viability, and the relative amount of ROS was determined by the fluorescent method.

Results: Microcapsules with ClE6 (in all tested concentrations) after exposure to red light (660 nm) reduced cell viability from 20% to 5%, while these capsules did not have dark cytotoxicity. Free ClE6 at the same concentrations as in the capsules after irradiation reduced viability from 65% to 35%. The level of ROS in the group of cells with capsules was 2 times higher compared to the group with CLE6.

Discussion: The most probable mechanism of toxicity increase is creation of a higher ROS concentration and effect localization in the area of microcapsule interaction with the cell membrane. ROS production activation may stem from capsules providing a higher local PS concentration in the cell or nearby than the drug's free form.

Conclusion: The inclusion of chlorin E6 in polymer capsules reduced dark toxicity and increased the photosensitizing effect compared to the free form of ClE6.

Graphical Abstract



Keywords

polyelectrolyte microcapsules, photodynamic therapy, chlorin E6, phototoxicity, ROS – reactive oxygen species

Introduction

Photodynamic therapy (PDT) is one of the most rapidly developing methods of tumors treatment. This method features low invasiveness and insignificant impact on healthy cells (Casas 2020; Chen et al. 2020; Ye et al. 2020). The PDT principle is based on selective effect of optical radiation on biological objects (malignant tumor cells, microorganisms, blood cells, etc.) treated with a photosensitizer (PS). PS is activated by light with appropriate wavelength (mainly in the red and near infrared regions) and in the presence of endogenous molecular oxygen induces generation of reactive oxygen species (ROS) such as singlet oxygen, superoxide anion, etc. (Donohoe et al. 2019; Zhou et al. 2020; Ming et al. 2021). ROS cause irreversible photo-oxidative damage to lipids, DNA and proteins, with subsequent cell death by several ways including apoptosis, necrosis or autophagy (Castano et al. 2005; Bacellar et al. 2015; Donohoe et al. 2019; Zhou et al. 2020; Ming et al. 2021). Potential ROS targets are the membranes which can undergo lipid peroxidation, protein cross-linking, loss of ionic homeostasis (Kessel 1977) and membrane organelle functions impairment (Spikes 1984). ROS are also known to cause DNA breakage and alkali-labile damage, also inactivating enzymes involved in DNA repair (Bisland et al. 1999).

Chlorin E6 (ClE6) is widely used as a clinical PS drug, which under exposure to red light (RL) (maximum absorption 660 nm) leads to the death of cancer cells due to the induction of apoptosis. Currently available data on the clinical use of ClE6 derivatives show its high photodynamic activity in various malignant neoplasms and some non-neoplastic diseases (Juzeniene 2009). Modern ClE6-based PS feature relatively rapid clearance. However, major drawbacks to PS use are still drug resistance in some tumors and hypoxia in the tumor, which may cause insufficient ROS formation (Duan et al. 2018; Rosin et al. 2018). ROS lifetime in aqueous medium is several microseconds and their effect is limited by intracellular diffusion (Moan 1990). Thus, the effects of singlet oxygen are very limited, which requires high PS concentration and may consequently increase dark PS cytotoxicity.

All this necessitates a search for new, more stable and efficient PS or modification of those already studied, such as ClE6. In our study, polyelectrolyte microcapsules with ClE6 in the nucleus were synthesized, and their photosensitizing action was studied. A greater phototoxic effect of the encapsulated drug form was shown *in vitro* due to specific interaction of the developed microcapsules with cells and creation of a higher local PS concentration compared to its free form.

Materials and Methods

Synthesis and characterization of polyelectrolyte microcapsules with ClE6

Materials for polyelectrolyte microcapsule synthesis: sodium carbonate ($NaCO_3$) (99.9%, Vecton), calcium chloride ($CaCl_2$) (99.9%, Vecton, Russia), polystyrene sodium sulfonate (PSS) (M ≈ 50 kDa, Sigma-Aldrich, USA), polyallylamine hydrochloride (PAH) (M ≈ 17.5 kDa, Sigma-Aldrich, USA), sodium chloride (NaCl) (99.9%, Vecton, Russia), dimethyl sulfoxide (DMSO) (99.9%, Diaem, Russia), 38% hydrochloric acid (99.8%, Vecton, Russia), and chlorin E6 (ClE6) (99.9%, Cayman Chemical Company, USA).

Polyelectrolyte microcapsules were prepared layer by layer, sequentially depositing oppositely charged polyelectrolytes (PAH and PSS) onto spherical calcium carbonate particles (Volodkin et al. 2004). The ClE6 PS was loaded into $CaCO_3$ particles by incubating them with an aqueous solution of the drug for 3 h at 10°C. After ClE6 loading, the spherical $CaCO_3$ particles were washed several times with deionized water. In the next step, polyelectrolyte layers were alternately deposited on the ClE6-loaded spherical nuclei. Upon polymer shell formation, the microcapsules were washed several times with a 0.9% NaCl solution. In order to avoid large aggregates, the suspension of the capsules obtained was passed through a metal filter with a pore size of 5 μm. The resulting microcapsules had the following composition:

$$CaCO_3@\ ClE6/(PAH/PSS)_4.$$

The number of capsules in 1 ml of the suspension was calculated in the Goryaev chamber. Capsule concentration in the suspension was 610 million ml^{-1}.

Morphology of the microcapsules obtained was investigated using a transmission electron microscope(FEI Tecnai Osiris, USA with an operating voltage of 200 kV, equipped with a SuperX EDS system for ultrafast element mapping.

Electrokinetic potential of the polymer capsules was measured with a Stabino particle charge analyzer (Microtrac Inc., Germany).

Amount of the ClE6 PS incorporated into the microcapsules and bound to fibroblasts was measured fluorimetrically with a Varioskan LUX multimodal reader (Thermo Scientific, USA). ClE6 was extracted from the capsules using a mixture with a 0.5% aqueous hydrochloric acid solution and DMSO in a volume ratio of 1:2, respectively. Fluorescence intensity at λ=665 nm under photoexcitation at λ=405 nm was measured for the extracts obtained. ClE6 amount in the test solution was calculated on a calibration curve, for which linearity range of ClE6 concentration was 3.5 to 550 ng/mL and correlation coefficient r* = 0.9995. Given microcapsule concentration and drug concentration in the suspension, average ClE6 amount per capsule was calculated to be $5.6 \cdot 10^{-12}$ g.

In order to determine the amount of the PS bound to cells in free or encapsulated form, after 24-h incubation with the test substances, fibroblasts were washed, removed from the plate surface and resuspended in the ClE6 extraction mixture. The remaining assay procedures were performed similarly with the microcapsule suspension.

Cell culture

The study focused on the cell culture of mouse fibroblasts L929, which was obtained from the Tissue Culture Collection of the N.F. Gamaleya Research Institute of Epidemiology and Microbiology of the Ministry of Health of the Russian Federation (Culture Certificate #67/01-08-184 dated December 1, 2015). The cells were grown in vials on the complete culture medium DMEM (PanEco, Russia) with a 10% fetal bovine serum (FBS, Corning, USA) and antibiotics added: 100 μg/mL of penicillin and 100 μg/ml of streptomycin (PanEco, Russia) under standard conditions: 5% CO_2, temperature 37°C, humidity 5% (an incubator by Thermoscientific, USA). The cells were counted with a 0.4% trypan blue solution (PanEco, Russia) in the Goryaev chamber. Nanomaterials cytotoxicity was studied as per the procedures of International SOP ISO SO 10993-5:2009 Tests for In Vitro Cytotoxicity.

Confocal microscopy

Features of interaction of the polyelectrolyte microcapsules with the fibroblasts were studied using Zeiss laser scanning microscope 880 with Airyscan module (Germany). Thus, the cells and microcapsules could be visualized simultaneously by their specific green (due to acridine orange, which was used to stain the fibroblasts for better visualization) and red (due to ClE6 in capsules) fluorescence, which was excited by a diode laser with a wavelength of 405 nm and maximum power of 30 mW. Images (sections) were recorded in several focal planes, on the basis of which 3D structures of the objects under study were built.

MTT test

Microcapsule cytotoxicity was measured with the MTT colorimetric test. The cells were incubated in 96-well plates at a concentration of 8,000 cells/well for 24 h under standard conditions. Then the culture medium was removed and replaced with a medium containing microcapsules with ClE6 (capsClE6) at concentrations of 5, 10, 20 capsules/cell; microcapsules without ClE6 (caps) at concentrations of 5, 10, 20 capsules per cell; ClE6 solution at concentrations of 13, 26, 52 pg per cell or 1.05, 2.1, 4.2 μg/ml, which corresponds to the ClE6 concentration included in the microcapsules equivalent to 5, 10, 20 capsules/cell. In the case of induction of photodynamic reactions with ClE6, we used a LED lamp with a wavelength of 660 nm and maximum power of 60 W. The lamp was fixed at a distance of 20 cm from the cells in a 96-well plate irradiated for 15 min. Light power density was measured with the optical sensor ThorLabs PM100D (Germany) to be 125 mW/cm^2. Fibroblast viability was determined without irradiation and 24 h after irradiation. To this end, an MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl) tetrazolium bromide solution at a concentration of 5 mg/mL was added to each well. Absorbance was measured with a Varioscan Lux EIA reader (Thermoscientific, USA) at a wavelength of 570 nm versus the reference wavelength of 650 nm. Cell viability was assessed by the ratio of the test sample absorbance to the control absorbance (medium cells) expressed as a percentage.

ROS production study

ROS were studied by identifying the fluorescent agent

2′,7′-dichlorodihydrofluorescein diacetate (H_2DCFDA) (Moiseeva et al. 2023). L929 cells were seeded in a 96-well plate at a concentration of 1x10^4/well. After 24-h incubation under standard conditions (5% CO_2 and 37^{o}C), test capsClE6 and empty microcapsules were added with a quantity of 5, 10, 20 capsules/cell and a ClE6 solution at 13, 26, 52 pg/cell. After 24 hours, the cells were washed twice with Hank's solution (PanEco, Russia). The prepared work solution of 2',7'-dichlorodihydrofluorescein diacetate (H_2DCFDA, Lumiprobe, Russia) was added at a concentration of 10 mM, 100 µl into all wells. They were incubated in the dark for 45 min at 37^{o}C. Then the H_2DCFDA solution was removed and Hank's solution was added. As a positive control, 0.6 and hydrogen peroxide (H_2O_2) solutions were added. The cell plate was then irradiated with RL for 15 min. After 3-h incubation, fluorescence intensity was recorded on a Varioscan Lux plate reader (Thermoscientific, USA) at 485 nm excitation and 535 nm emission. The higher fluorescence intensity, the more ROS were formed in the cell.

Fluorescence microscopy

Penetration and distribution of PS in free and encapsulated form in the fibroblasts were assessed with a BM35FXT fluorescent microscope (ICOE, China). Characteristic ClE6 red fluorescence (with intensity maximum at λ=665 nm) was observed when the samples were illuminated with violet light (with intensity maximum at λ=400 nm), corresponding to PS absorption maxima in the visible spectrum.

In order to assess cytotoxicity mechanism, after incubation with the microcapsules and ClE6 solutionL929 fibroblasts were stained with a mixture of acridine orange and ethidium bromide dyes before and after exposure to RL under the protocol (McGahon et al. 1995).

Statistics

Statistical data was processed using one-way analysis of variance (ANOVA test) in the GraphPad Prism 8.0 software. Differences were considered significant at $p<0.05$. The results are presented as Mean±SD.

Results

Figure 1 shows a bright-field image and chemical element distribution maps (Ca, C, S) for polymer microcapsules.

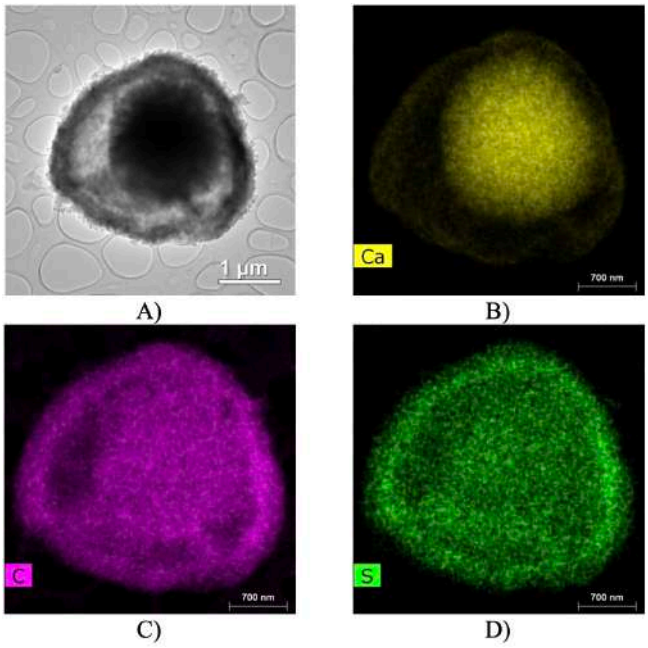


Figure 1. TEM image (**A**) and chemical element distribution maps (**B, C, D**) of a ClE6 polymer microcapsule.

Figure 1 shows that the polymer capsules have a rounded shape and an average size of 3±0.5 µm. Analysis of the calcium distribution map showed that the capsules contained $CaCO_3$ nuclei. High carbon and sulfur densities within the polymers confirm presence of formed capsule shells. The microcapsule electrokinetic potential was 19±2 mV (in a 0.9% NaCl solution, pH=7.4).

Confocal microscopy

In order to check whether the polymeric microcapsules were internalized by cells or simply adsorbed on their membrane, detailed images of the fibroblasts incubated with capsules (at a concentration of 20 capsules/cell) for 24 h were obtained and analyzed with confocal microscopy (Fig. 2).

The 3D reconstructions obtained clearly show that after 24h incubation, the main part of the polymer microcapsules adheres to the cell membrane, but there are also cells with internalized capsules. The number of capsules absorbed by one cell varies from 1 to 5.

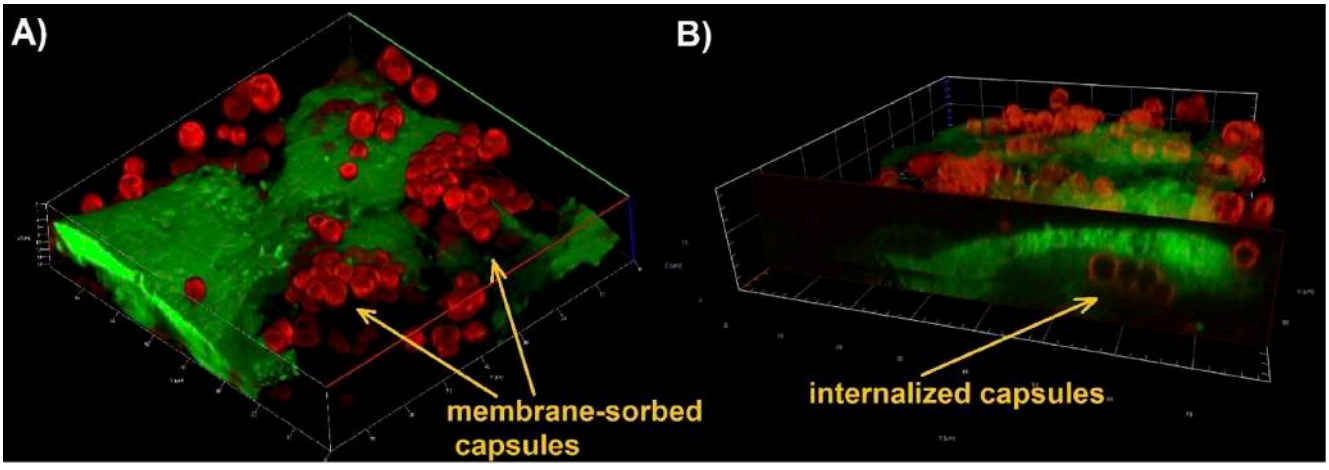


Figure 2. Confocal fluorescent 3D images of fibroblasts (green) incubated with polymer ClE6 microcapsules (red): **A**) top view and **B**) side view (longitudinal section).

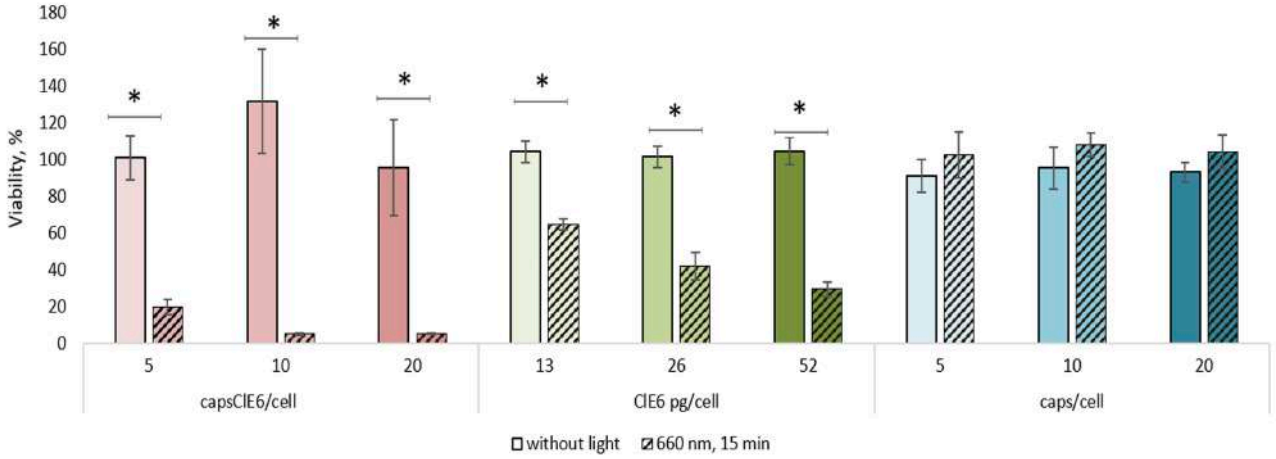


Figure 3. MTT test before and after 660 nm lamp irradiation for 15 min of L929 cells incubated with chlorin microcapsules (capsClE6), chlorin ClE6, and chlorin-free microcapsules (caps) at doses of 5, 10, and 20 microcapsules/cell. ***Note:*** * – $p<0.05$.

MTT test

According to MTT test results, capsules without ClE6 did not affect L929 viability at concentrations of 5-20 capsules/cell both without and after RL treatment (Fig. 3).

The microcapsules with nuclei-embedded ClE6 did not exhibit dark cytotoxicity. After RL exposure for 15 min and after 24-h incubation, cell death was 95% for microcapsules at a concentration of 10-20 per cell, with statistically significant difference in cell viability at dark cytotoxicity and RL exposure ($p<0.05$). At a concentration of 5 capsules/cell, cell death was about 20%.

The ClE6 solution had no dark cytotoxicity at the concentrations under study. During RL exposure, death was 35, 53, and 65% for 13, 26, 52 pg/mL or 1.05, 2.1, 4.2 μg/mL ($p<0.05$), respectively.

ROS level detection and fluorescence microscopy

Results of ROS detection in cells are presented in Figure 4.

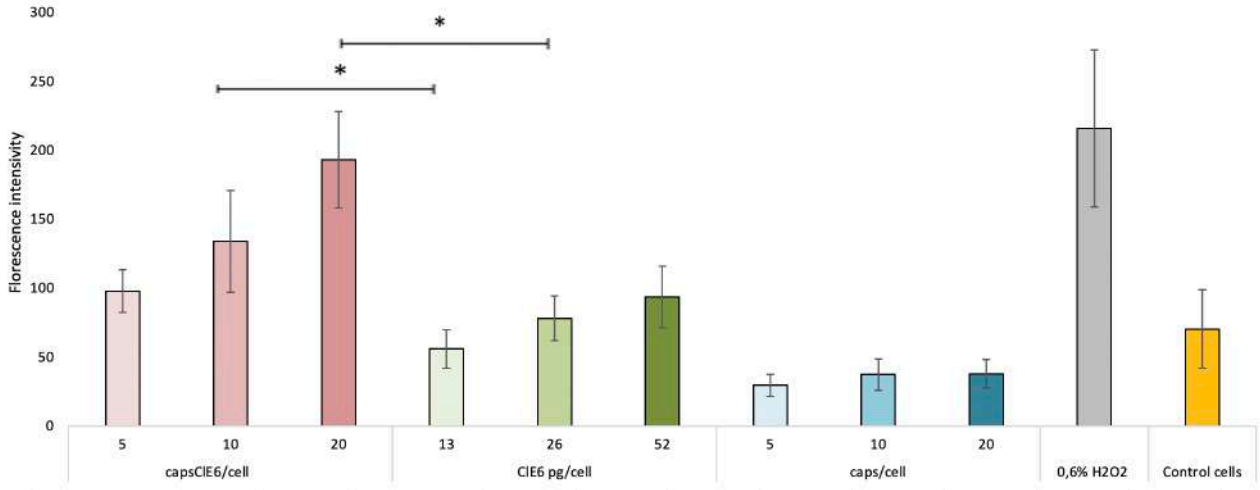


Figure 4. Fluorescence intensity of L929 cells during ROS formation under 660 nm light for 15 min after their incubation with chlorin ClE6, microcapsules with chlorin E6 (CapsClE6), microcapsules without chlorin (Caps) at doses of 5, 10 20 capsules/cell. Untreated cells as a negative control. H_2O_2 as a positive control. Results are presented as ±SD (n=8). ROS levels were analyzed by preincubation with H_2DCFDA 3 hours after RL irradiation. ***Note:*** * – $p<0.05$

After cell irradiation from the ClE6 microcapsule, more ROS formed compared to the same concentrations of free ClE6. There was a higher level of fluorescence and, correspondingly, ROS level was also higher. The microcapsules themselves did not contribute to ROS formation, so fluorescence level of microcapsules without ClE6 was comparable to the control. The higher level of ROS formation under 660 nm light on ClE6 microcapsules can be explained by the larger amount of the drug in cells compared to the batch with free-form PS. This is confirmed by the assays of cell-bound ClE6 and fluorescence microscopy studies (Fig. 5).

As shown by red fluorescence characteristic of ClE6 in Figure 5 (A), free drug molecules penetrate into cells and are evenly distributed in the cytoplasm. The cell group incubated with capsules (Fig. 5 (C)) show brighter fluorescence from fibroblast-bound capsules, indicating a higher drug concentration. Furthermore, for some cells from this group, weak PS fluorescence is also visible in the cytoplasm, possibly due to partial recovery from

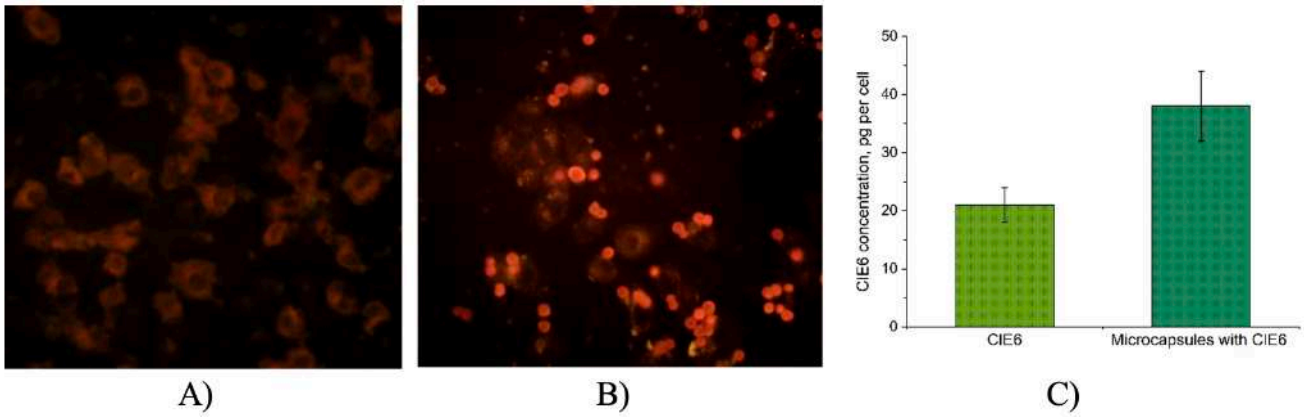


Figure 5. Fluorescence images of fibroblasts incubated for 24h with free form (**A**) and encapsulated form (**B**) of the drug with a ClE6 concentration of 52 pg/cell. Average amount of ClE6 per cell after 24-h incubation with test substances and washing from the cell-unbound drug (**C**).

capsules and drug penetration into cells. Cell-bound PS assay results confirm fluorescence microscopy findings (Fig. 5 (C)). After 24-h incubation with free-form ClE6, about 50% of initially added drug amount penetrates into cells. In the case of microcapsule-treated fibroblasts, binding efficiency of ClE6 to cells was 73%.

Double staining with fluorescent dyes (acridine orange and ethidium bromide) was performed to study mechanisms of cell death from phototoxic action of microcapsules and ClE6. Fluorescence microscopy results are shown in Figure 6.

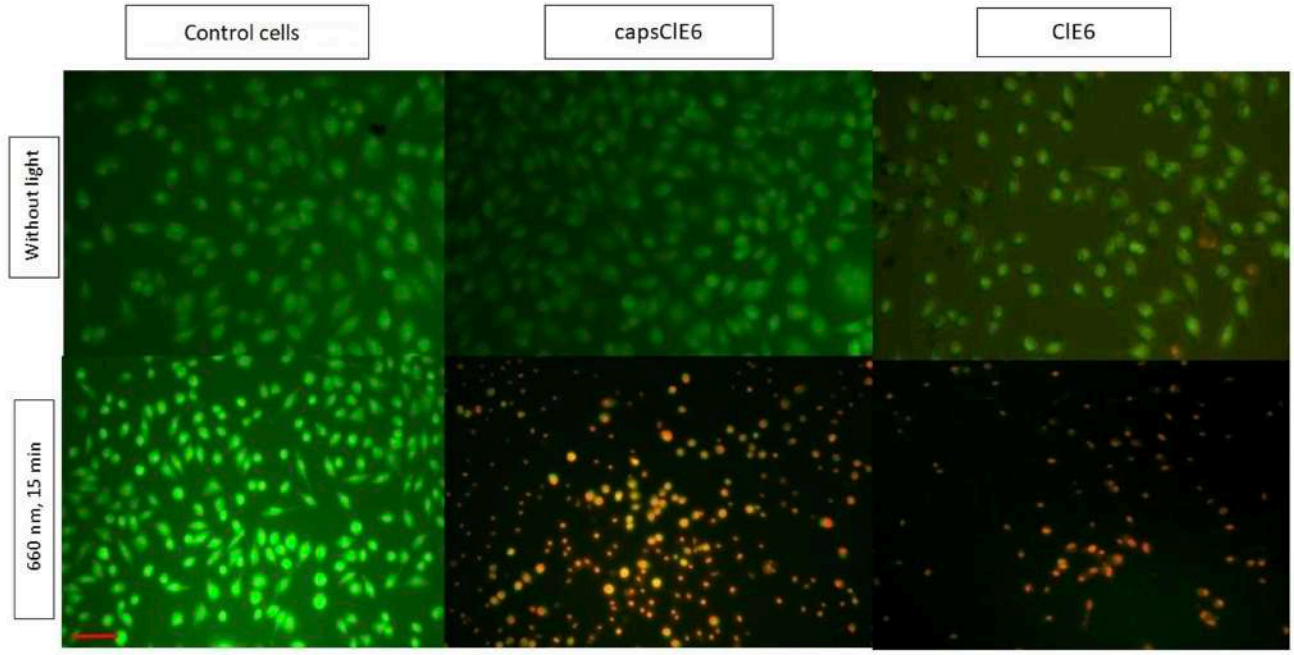


Figure 6. Double staining of L929 with acridine orange/ethidium bromide (scale bar – 100 μm). Green colored nuclei – living cells, orange colored nuclei – dead cells.

As seen from the figure, cell nuclei are stained green when incubated with microcapsules, indicating that cells are alive and microcapsules did not have dark cytotoxicity. After 660 nm light irradiation for 15 min, cell nuclei were stained yellow and orange, indicating apoptosis and necrosis development. We counted the number of living, apoptotic and necrotic cells per 200 cells/HPF. Findings are presented in Figure 7.

A large accumulation of ClE6 microcapsules on the cell membrane caused death by necrosis due to release of singlet oxygen from microcapsules under RL, which in turn caused oxidation of cell membrane lipids. Therefore, 24 h later, we can mostly observe necrotic cells and some apoptotic cells. Upon exposure to free ClE6 for 24 h, apoptosis was mainly observed, with cells in necrosis stage also found, but these cells were fewer in number than the capsClE6 series. In the case of microcapsules, cell death was higher, possibly due to higher concentration of singlet oxygen on the cell membrane.

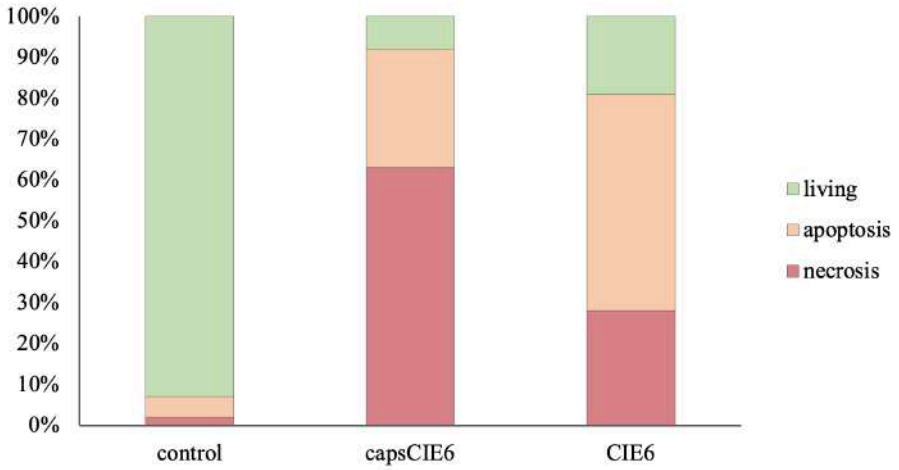


Figure 7. Evaluation of the quantitative ratio of viable, apoptotic and necrotic cells after 660 nm light exposure for 15 min of L929 cells incubated with microcapsules containing chlorin (capsClE6) and with chlorin E6 (ClE6).

Discussion

The idea of using microcapsules with PS is based on the fact that encapsulation can influence spatial PS distribution inside and outside a cell and thus localize its effects. Therefore, it was necessary to evaluate capability of adhesion on the cell membrane and internalization of the capsules.

Polyelectrolyte microcapsules, like other micron-sized objects, are known to undergo cellular uptake by phagocytosis. Capsules can be internalized by cells even without specific ligands on the particle surface (Javier et al. 2008). However, some substances and polymers, such as hyaluronic acid, can stimulate capsule uptake by cells (Soleymani et al. 2020). Micron particles can also enter cells by caveolae-mediated endocytosis (Rejman et al. 2004) or lipid raft (De Geest et al. 2006). Polyelectrolyte capsules captured by cells are usually localized in endosomes or lysosomes, sometimes in the cytoplasm.

Also, polymeric microcapsules adhere quite well to the cell membrane of fibroblasts. Degree of adhesion depends on the polymer's chemical nature, other characteristics, and specific composition of the cytoplasmic cell membrane (Lanza et al. 2000). Particle sorption on the membrane normally occurs due to interaction of charged polymers in the microcapsule shell with proteins on the cell surface. Negatively charged polyelectrolytes (polyglutamate, polymethacrylate) show most adhesion to the cell membrane, while particles with an outer shell of polyethylene glycol or poly-2-hydroxypropylmethacrylamide did not show significant sorption towards cell cultures. Microcapsules with an outer shell of dextran sulfate showed significant sorption towards L929 fibroblasts (Zharkov et al. 2021). Also worth noting is that microcapsules may be surrounded by proteins contained in the medium or synthesized by cells themselves. This creates a so-called "protein corona" facilitating particle adsorption on the cell membrane.

In the present paper, microcapsules with an outer layer of biocompatible polystyrene sulfonate with negative charge and ensuring capsule interaction with fibroblasts using the above mechanisms have been studied.

Results of cytotoxicity evaluation of microcapsules containing ClE6 confirmed the assumption on its possible enhanced efficacy in microencapsulation. Microcapsules had significantly greater phototoxicity than free-form ClE6 at a concentration of 4.2 μg/mL. There is evidence in literature that nanostructured ClE6 forms have higher photosensitizing activity than the free substance. For instance, Ting Yin et al. (2016) showed that supermagnetic nanoparticles with ClE6 in their composition, being exposed to laser, caused death of 60% of MGC-803 cells at chlorin concentration of 20 μg/mL, while chlorin itself had minor phototoxic effect at concentrations of 1-10 μg/mL. On glial cells (C6), NpPEG-Ce6-Gd nanoparticles conjugated with ClE6 significantly affected the cells whose viability decreased to 40% at the highest concentration (1.25 μg/mL) (Xu et al. 2021). This phenomenon was explained either by potentiation of PS effect by active agents incorporated into particles or by more efficient PS transport into a cell.

In our case, the most probable mechanism of toxicity increase is creation of a higher ROS concentration and effect localization in the area of microcapsule interaction with the cell membrane. This is supported by results of evaluating ROS concentration, which was twice higher in the microcapsule group than in the free chlorine group. ROS production activation may stem from capsules providing a higher local PS concentration in the cell or nearby than the drug's free form.

In the assessment of the mechanisms of cell death affected by ROS, it should be noted that their excessive formation may cause both cell apoptosis and necrosis. ROS interaction with nuclear or cytoplasmic DNA normally triggers apoptosis process. However, in the case of damage to the outer cytoplasmic membrane of cellular or membrane organelles such as lysosomes, cell death by necrosis seems most likely. The second mechanism prevailed in our case because microcapsules as the main ROS source were localized in the premembrane region and, in the case of cellular internalization, in lysosomes.

Conclusion

A method has been developed for the synthesis of polyelectrolyte microcapsules contained ClE6 in the core. Using confocal microscopy, it was found that the resulting capsules are predominantly sorbed on the

membrane of L929 fibroblasts. The inclusion of chlorin E6 in polymer capsules reduced dark toxicity and increased the photosensitizing effect compared to the free form of ClE6 at irradiation of red light (660 nm). It was found that encapsulated chlorin E6 generates more ROS compared to its free form due to the production of a higher local concentration of the drug.

Acknowledgements

The authors have received no support to report.

Conflict of interests

The authors have declared that no competing interests exist.

References

- Bacellar IOL, Tsubone TM, Pavani C, Baptista MS (2015) Photodynamic efficiency: from molecular photochemistry to cell death. International Journal of Molecular Sciences 16(9): 20523–20559. https://doi.org/10.3390/ijms160920523 [PubMed] [PMC]
- Bisland SK, Singh D, Gariépy J (1999) Potentiation of chlorin e6 photodynamic activity in vitro with peptide-based intracellular vehicles. Bioconjugate Chemistry 10(6): 982–992. https://doi.org/10.1021/bc990020u [PubMed]
- Casas A (2020) Clinical uses of 5-aminolaevulinic acid in photodynamic treatment and photodetection of cancer: A review. Cancer Letters 490: 165–173. https://doi.org/10.1016/j.canlet.2020.06.008 [PubMed]
- Castano AP, Demidova TN, Hamblin MR (2005) Mechanisms in photodynamic therapy: part two-cellular signaling, cell metabolism and modes of cell death. Photodiagnosis and Photodynamic Therapy 2(1): 1–23. https://doi.org/10.1016/S1572-1000(05)00030-X [PubMed] [PMC]
- Chen J, Fan T, Xie Z, Zeng Q, Xue P, Zheng T, Chen Y, Luo X, Zhang H (2020) Advances in nanomaterials for photodynamic therapy applications: Status and challenges. Biomaterials 237: 119827. https://doi.org/10.1016/j.biomaterials.2020.119827 [PubMed]
- De Geest BG, Vandenbroucke RE, Guenther AM, Sukhorukov GB, Hennink WE, Sanders NN, Demeester J, De Smedt SC (2006) Intracellularly degradable polyelectrolyte microcapsules. Advanced Materials 18: 1005–1009. https://doi.org/10.1002/adma.200502128
- Donohoe C, Senge MO, Arnaut LG, Gomes-da-Silva LC (2019) Cell death in photodynamic therapy: From oxidative stress to anti-tumor immunity. Biochimica et Biophysica Acta. Reviews on Cancer 1872(2): 188308. https://doi.org/10.1016/j.bbcan.2019.07.003 [PubMed]
- Duan X, Chen B, Cui Y, Zhou L, Wu C, Yang Z, Wen Y, Miao X, Li Q, Xiong L, He J (2018) Ready player one? Autophagy shapes resistance to photodynamic therapy in cancers. Apoptosis 23(11-12): 587–606. https://doi.org/10.1007/s10495-018-1489-0 [PubMed]
- Javier AM, Kreft O, Semmling M, Kempter S, Skirtach AG, Bruns OT, Pino P del, Bedard MF, Rädler J, Käs J, Plank C, Sukhorukov GB, Parak WJ (2008) Uptake of colloidal polyelectrolyte-coated particles and polyelectrolyte multilayer capsules by living cells. Advanced Materials 20(22): 4281–4287. https://doi.org/10.1002/adma.200703190
- Juzeniene A (2009) Chlorin e6-based photosensitizers for photodynamic therapy and photodiagnosis. Photodiagnosis and Photodynamic Therapy 6(2): 94–96. https://doi.org/10.1016/j.pdpdt.2009.06.001 [PubMed]
- Kessel D (1977) Effects of photoactivated porphyrins at the cell surface of leukemia L1210 cells. Biochemistry 16(15): 3443–3449. https://doi.org/10.1021/bi00634a023 [PubMed] [PMC]
- Lanza RP, Langer R, Vacanti J (2000) Principles of Tissue Engineering. Academic Press, New York, USA, 995 pp. https://doi.org/10.1016/B978-0-12-436630-5.X5000-4
- McGahon AJ, Martin SJ, Bissonnette RP, Mahboubi A, Shi Y, Mogil RJ, Nishioka WK, Green DR (1995) The end of the (cell) line: methods for the study of apoptosis in vitro. Methods in Cell Biology 46: 153–185. https://doi.org/10.1016/s0091-679x(08)61929-9 [PubMed]
- Ming L, Cheng K, Chen Y, Yang R, Chen D (2021) Enhancement of tumor lethality of ROS in photodynamic therapy. Cancer Medicine 10(1): 257–268. https://doi.org/10.1002/cam4.3592 [PubMed] [PMC]
- Moan J (1990) On the diffusion length of singlet oxygen in cells and tissues. Journal of Photochemistry and Photobiology B: Biology 6(3): 343–344. https://doi.org/10.1016/1011-1344(90)85104-5
- Moiseeva N, Eroshenko D, Laletina L, Rybalkina E, Susova O, Karamysheva A, Tolmacheva I, Nazarov M, Grishko V (2023) The molecular mechanisms of oleanane aldehyde-β-enone cytotoxicity against doxorubicin-resistant cancer cells. Biology 12(3): 415. https://doi.org/10.3390/biology12030415 [PubMed] [PMC]
- Rejman J, Oberle V, Zuhorn IS, Hoekstra D (2004) Size-dependent internalization of particles via the pathways of clathrin- and caveolae-mediated endocytosis. Biochemical Journal 377(Pt 1): 159–169. https://doi.org/10.1042/bj20031253 [PubMed] [PMC]
- Rosin FCP, Teixeira MG, Pelissari C, Corrêa L (2018) Resistance of oral cancer cells to 5-ALA-mediated photodynamic therapy. Journal of Cellular Biochemistry 119(4): 3554–3562. https://doi.org/10.1002/jcb.26541 [PubMed]
- Soleymani M, Velashjerdi M, Shaterabadi Z, Barati A (2020) One-pot preparation of hyaluronic acid-coated iron oxide nanoparticles for magnetic hyperthermia therapy and targeting CD44-overexpressing cancer cells. Carbohydrate Polymers 237: 116130. https://doi.org/10.1016/j.carbpol.2020.116130 [PubMed]
- Spikes JD (1984) Photobiology of porphyrins. Progress in Clinical and Biological Research 170: 19–39. [PubMed]
- Volodkin DV, Petrov AI, Prevot M, Sukhorukov GB (2004) Matrix polyelectrolyte microcapsules: new system for macromolecule encapsulation. Langmuir 20(8): 3398–3406. https://doi.org/10.1021/la036177z [PubMed]
- Xu D, Baidya A, Deng K, Li Y-S, Wu B, Xu H-B (2021) Multifunctional nanoparticle PEG-Ce6-Gd for MRI-guided photodynamic therapy. Oncology Reports 45: 547–556. https://doi.org/10.3892/or.2020.7871 [PubMed] [PMC]
- Ye T, Chen T, Jiang B, Yang L, Liu X, Chen B, Zou Y, Yu B (2020) 5-aminolevulinic acid photodynamic therapy inhibits invasion and metastasis of SCL-1 cells probably via MTSS1 and p63 gene related pathways. Photodiagnosis and Photodynamic Therapy 32: 102039. https://doi.org/10.1016/j.pdpdt.2020.102039 [PubMed]
- Yin T, Huang P, Gao G, Shapter JG, Shen Y, Sun R, Yue C, Zhang C, Liu Y, Zhou S, Cui D (2016) Superparamagnetic Fe3O4-PEG2K-FA@Ce6 nanoprobes for in vivo dual-mode imaging and targeted photodynamic therapy. Scientific Reports 6: 36187. https://doi.org/10.1038/srep36187 [PubMed] [PMC]
- Zharkov MN, Brodovskaya EP, Kulikov OA, Gromova EV, Ageev VP, Atanova AV, Kozyreva ZV, Tishin AM, Pyatakov AP, Pyataev NA, Sukhorukov GB (2021) Enhanced cytotoxicity caused by AC magnetic field for polymer microcapsules containing packed magnetic nanoparticles. Colloids and Surfaces. B, Biointerfaces 199: 111548. https://doi.org/10.1016/j.colsurfb.2020.111548 [PubMed]
- Zhou J, Geng S, Ye W, Wang Q, Lou R, Yin Q, Du B, Yao H (2020) ROS-boosted photodynamic therapy against metastatic melanoma by inhibiting the activity of antioxidase and oxygen-producing nano-dopants. Pharmacological Research 158: 104885. https://doi.org/10.1016/j.phrs.2020.104885 [PubMed]

Author Contributions

- **Ekaterina. P. Brodovskaya**, Candidate of Medical Sciences, Associate Professor of the Department of Anesthesiology and Resuscitation with courses in Valeology, Life Safety and Disaster Medicine of the Medical Institute, National Research Ogarev Mordovia State University; e-mail: kitten-777@mail.ru; **ORCID ID**

- https://orcid.org/0000-0002-1060-9843. Drawing up a research plan and general guidance, designing the experiment, conducting the experiment, analyzing literary data, and writing the text of the article.

- **Larisa A. Tararina**, Senior Lecturer, Department of Pharmacology, A.I. Yevdokimov Moscow State University of Medicine and Dentistry; e-mail: 79104906528@yandex.ru; **ORCID ID** https://orcid.org/0000-0001-8006-698X. Development of the experiment design, conducting the experiment, analysis of literary data, and writing the text of the article.

- **Mikhail N. Zharkov**, Head of Pharmacokinetics and Targeted Pharmacotherapy Laboratories, National Research Ogarev Mordovia State University; e-mail: ykbsn@mail.ru; **ORCID ID** https://orcid.org/0000-0002-8272-1973. Conducting the experiment, analysis of clinical manifestations, and statistical analysis of the data.

- **Irina A. Khutorskaya**, Candidate of Biological Sciences, Engineer of Pharmacokinetics and Targeted Pharmacotherapy Laboratories, National Research Ogarev Mordovia State University; e-mail: alfa200890@yandex.ru; **ORCID ID** https://orcid.org/0000-0001-5553-0525. Conducting the experiment, analysis of clinical manifestations, and statistical analysis of the data.

- **Denis E. Yakobson**, Engineer of Pharmacokinetics and Targeted Pharmacotherapy Laboratories, National Research Ogarev Mordovia State University; e-mail: ykbsn@mail.ru; **ORCID ID** https://orcid.org/0000-0002-7675-0823. Conducting the experiment, analysis of clinical manifestations, and statistical analysis of the data.

- **Amina Al-khadj Aioub**, Engineer of Pharmacokinetics and Targeted Pharmacotherapy Laboratories, National Research Ogarev Mordovia State University; e-mail: amina.aioub1@gmail.com; **ORCID ID** https://orcid.org/0000-0002-2793-6760. Conducting the experiment, analysis of clinical manifestations, and statistical analysis of the data.

- **Igor V. Maev**, Dr. Habil. Med, Full Professor, Member of the Russian Academy of Sciences, Head of the Department of Propaedeutics of Internal Diseases and Gastroenterology, A.I. Yevdokimov Moscow State University of Medicine and Dentistry; e-mail: igormaev@rambler.ru; **ORCID ID** https://orcid.org/0000-0001-6114-564X. Drawing up a research plan and general guidance, designing and conducting the experiment, analyzing literary data, and writing the text of the article.

- **Andrey V. Zaborovskiy**, Dr. Habil. Med., Associate Professor, Head of the Department of Pharmacology, A.I. Yevdokimov Moscow State University of Medicine and Dentistry; e-mail: azabor@mail.ru; **ORCID ID** https://orcid.org/0000-0002-7923-9916. Developing the experiment design, analyzing literary data. and writing the text of the article.

- **Dina V. Yunina**, Lecturer, Department of Pharmacology, A.I. Yevdokimov Moscow State University of Medicine and Dentistry; e-mail: yunina@msmsu.ru; **ORCID ID** https://orcid.org/0000-0001-8901-9557. Developing the experiment design, analyzing literary data writing the text of the article.

- **Sergei V. Tsaregorodtsev**, Lecturer, Department of Pharmacology, A.I. Yevdokimov Moscow State University of Medicine and Dentistry; e-mail: sergiotsar@yandex.ru; **ORCID ID** https://orcid.org/0000-0002-0254-0516. Developing the experiment design, analyzing literary data, and writing the text of the article.

- **Sergey N. Gerasimchuk**, laboratory assistant of the Department of Biological Chemistry, A.I. Yevdokimov Moscow State University of Medicine and Dentistry; e-mail: 1337.serezha@mail.ru; **ORCID ID** https://orcid.org/0009-0001-8340-7011. Analyzing literary data.

- **Gleb B. Sukhorukov**, Dr. Habil. Med., Full Professor, Vladimir Zelman Center for Neurobiology and Brain Rehabilitation, Skolkovo Institute of Science and Technology, Russia; Professor, School of Engineering and Materials Science, Queen Mary University of London, e-mail: g.sukhorukov@skoltech.ru; **ORCID ID** https://orcid.org/0000-0001-6213-0562. Developing the experiment design, conducting the experiment, analyzing literary data, and writing the text of the article.

- **Nikolay A. Pyataev**, Dr. Habil. Med., Associate Professor, Head of the Department of Anesthesiology and Resuscitation with courses in Valeology, Life Safety and Disaster Medicine, National Research Ogarev Mordovia State University; e-mail: pyataevna@mail.ru; **ORCID ID** https://orcid.org/0000-0002-9688-7640. Drawing up a research plan and general guidance, developing the design of the experiment, analyzing literary data, and writing the text of the article.