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SHORT COMMUNICATIONS

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EFFECT OF THE VISIBLE LIGHT IRRADIATION OF FULLERENE-CONTAINING COMPOSITES ON THE ROS GENERATION AND THE VIABILITY OF TUMOR CELLS

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Aim: **To study the effect of fullerene-containing composites, irradiated by visible light, on the radical oxygen species (ROS) generation in thymocytes, ascitic cells from Erlich's tumor and leukemia cells L1210; to investigate viability of these cells in the presence of fullerene-containing composites under irradiation conditions.** ***Materials and Methods:*** **The viability of cells was evaluated by staining with 0.4% solution of the trypan blue; ROS were detected with the use of electron paramagnetic resonance (EPR) spectroscopy and spin traps; solutions of fullerene-containing composites were irradiated with mercury-vapor lamp.** ***Results:*** **We demonstrated that under irradiation conditions fullerene-containing composites increase the rate of ROS generation and decrease the number of viable tumor cells.** ***Conclusions:*** **The obtained data allow to consider the fullerene-containing composites as potential agents for photodynamic therapy.**
Key Words: **fullerene-containing composites, visible light irradiation, thymocytes, ascitic cells from Erlich's tumor, leukemia cells L1210, ROS, spin traps, EPR spectroscopy.**

The search of new substances, which have high biological activity and can be used in bionanotechnology and medicine, was in a focus during last years. This is why representatives of novel allotropic carbon form — fullerenes, which have unique physical-chemical properties, are actively investigated [1]. In particular, the molecule of C_{60}-fullerene has a shape of almost symmetrical sphere with the surface composed from hexagonal and pentagonal units, in junctions of which 60 conjugated atoms of carbon are located. Because of small sizes and hydrophobic properties, fullerenes are able to be inserted in biological membranes [2–3]. They possess the reduction ability and significant antioxidative potential, compared with other molecules such as quinone and vitamin E [4]. From other side, fullerenes possess unique photophysical properties: under the influence of UV or visible light irradiation, C_{60} molecule is able to shift to the exitation triplet state and generate singlet and other active forms of oxygen [4–5]. Such characteristics of fullerenes evidence on their potential use in regulation of free-radical peroxidation processes.

The low solubility of fullerenes in the water limits their use in biological studies. This problem can be solved by few ways: by formation of fullerene-cyclodextrane or fullerene-calixarene complexes, solubilization by polyvinilpyrrolidone, or covalent modification of fullerene surface with different functional groups [6–7].

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Abbreviation used: ApA – aminopropylaerosyl; C_{60}-AntrIpA – fullerene-antracenaliminopropylaerosyl; C_{60}-ApA – fullerene-aminopropylaerosyl; EPR – electron paramagnetic resonance; ROS – reactive oxygen species

To optimize solubilization of C_{60} fullerenes in water, increase the contact area with substrate, provide C_{60} fullerenes distribution in the contact zone and the interaction specificity, we have established immobilized C_{60} fullerenes localized on spherical particles of the silicon dioxide (aerosyl) — highly dispersed chemically inactive and non-toxic substance. Other components can be added to such composites (in particular, structures that entrape the visible light (porphyrine, antracenalimin) and increase photosensibilizing effect of the fullerens).

The main goal of the study was to investigate the effect of C_{60}-containing composites irradiated by visible light on the rate of ROS accumulation in thymocytes, ascitic cells from Erlich's carcinoma and L1210 leukemia cells, and to evaluate the viability of these cells in the presence of irradiated C_{60}-containing composites.

In experiments Wistar rats (with body weight of 120–150 g) and outbreed rats (with body weight of 20 g), maintained on standard chow diet, were used. All experiments were carried out according to the rules of local Ethic Committee˝Ω.

On day 8–12[th] after intraperitoneal transplantation of ascitic Erlich's carcinoma and leukemia L1210 to experimental animals, tumor cells were obtained. Thymocytes were obtained by grinding rat thymus in RPMI 1640 medium. Cells ($2–4 \times 10^6$/ml) were incubated in RPMI 1640 medium with 8 mM $NaHCO_3$, 20 mM HEPES, 5% serum, streptomycin and penicillin (100 µg and 100 units per 1 ml of the medium, respectively) with and without C_{60}-containing composites. The number of viable cells was counted in Goryaev's chamber after staining with 0.4% trypan blue solution.

Composites were established on the basis of aminopropylaerosyl (ApA) — silicon dioxide, in the surface layer of which aminopropyl groups (0.8 mM/g) were introduced; consequently, total negative charge of the composite was lowered. C_{60}-containing composites of two types were synthesized: 1) fullerene-aminopropylaerosyl (C_{60}-ApA), that consists from 0.12 mM/g of C_{60} fullerene, immobilized by "stitching" to amino groups on the surface [8]; 2) Fullerene-antracenaliminopropylaerosyl (C_{60}-AntrIpA) composite includes also antracinalimin (0.2 mM/g), introduced by azometine condensation of the aldehyde group of antracenal and amino group of the surface. Upon addition of composites to incubation medium, the concentration of the silicon dioxide in the sample was 0.02%, and C_{60} fullerene — 2.4×10^{-5} M.

For detection of the ROS EPR spectroscopy and spin trap technique were used [9]. Spin trap (1-hydroxo-2,2,6,6-tetramethyl-4-oxopiperidine) at the concentration of 2×10^{-3} M possesses high level of affinity to singlet oxygen and superoxide radical, which have been detected by EPR method at room temperature. EPR spectra of this radical represents the triplet with following characteristics: g = 2.005, A_N = 16 Hz, ΔH_{pp} = 0.4 Hz. Irradiated C_{60}-containing composite solution was immediately added to studied cell suspension (3×10^6 cells/ml) to final concentration of C_{60}-containing composites of 10^{-5} M, and then EPR spectra was recorded in special quartz cuvette (V = 200 µl). C_{60} containing composites were irradiated in glass tube with mercury-vapor lamp (power 24 W) for 2 min.

As one may see from Table 1, irradiation of 10^{-5} M C_{60}-ApA or C_{60}-AntrIpA solutions was followed by ROS formation with the rate of 6.3 and 11.0 nM/ml per min as well as ROS generation in cell suspensions. Thymocytes accumulated ROS more intensively compared with other cells, possibly, due to normal functioning of the antioxidant defense system. The rate of ROS formation in thymocytes in the presence of 10^{-5} M C_{60}-ApA or C_{60}-AntrIpA increased by 2.6 times and by 3.4 times compared with thymocytes, incubated without composites. In the presence of C_{60}-ApA or C_{60}-AntrIpA the rate of ROS formation increased by 4-fold and 5-fold in Erlich's carcinoma and by 4.6-fold and 6–fold in leukemia L1210 cells, respectively, compared to the cells, incubated without composites. The obtained results showed that modification of fullerenes, in particular with antracenal, promotes the photosensibilizing effect of fullerene, that increases the rate of ROS generation [10].

Table 1. The rate of ROS accumulation in the presence of irradiated C_{60}-containing composites in the suspension of thymocytes, ascitic cells from Erlich's tumor and leukemia cells L1210

Cells/treatment	The rate of ROS accumulation (nM/ml per min)
10^{-5}M C_{60}-ApA	6.3 ± 0.2
10^{-5}M C_{60}-AntrIpA	11.0 ± 0.4
Thymocytes	2.1 ± 0.2
+ 10^{-5}M C_{60}-ApA	5.4 ± 0.3
+ 10^{-5}M C_{60}-AntrIpA	7.8 ± 0.3
Ascitic cells from Erlich's tumor	1.4 ± 0.1
+ 10^{-5}M C_{60}-ApA	6.1 ± 0.4
+ 10^{-5}M C_{60}-AntrIpA	7.2 ± 0.2
Leukemia cells L1210	1.2 ± 0.1
+ 10^{-5}M C_{60}-ApA	5.5 ± 0.3
+ 10^{-5}M C_{60}-AntrIpA	6.8 ± 0.4

The influence of the irradiated C_{60}-containing composites on cells viability was studied upon 4 h or 24 h of incubation (Table 2); upon 4 h of incubation, we did not observe significant changes in the number of thymocytes, ascite Erlich's tumor cells and leukemia cells L1210 as well as in those in thymocytes incubated for 24 h. However, upon 24 h of incubation with irradiated C_{60}-ApA, the number of Erlich's tumor cells decreased by 19%, and with C_{60}-AntrIpA — by 34% compared with the control, whilst the number of L1210 — by 20%, and with C_{60}-AntrIpA — by 23% compared with the control.

Table 2. The number of viable cells in the presence of C_{60}-containing composites

Cells/treatment	The duration of the incubation (h)	
	4	24
Thymocytes	98 ± 2	97 ± 3
+ C60-ApA	98 ± 2	90 ± 5
+ C60- AntrIpA	94 ± 3	88 ± 3
Ascitic cells from Erlich's tumor	98 ± 2	97 ± 3
+ C60-ApA	95 ± 3	71 ± 5
+ C60-AntrIpA	95 ± 3	66 ± 7
Leukemia cells L1210	98 ± 2	98 ± 2
+ C60-ApA	98 ± 2	80 ± 6
+ C60-AntrIpA	96 ± 3	77 ± 4

We hypothesize that the possible mechanism of ROS accumulation in biosystem in the presence of irradiated C_{60}-containing composites is as follow: upon irradiation with visible light, where C_{60} fullerenes demonstrate strong absorption (450 nm) [11], C_{60} molecule is able to change its state from basic to exited triplet state ($^3C_{60}$). In the presence of oxygen in biosystem, C_{60} fullerene can change from triplet state into the basic state, transferring its energy to oxygen, which in turn changes to the singlet molecule of the oxygen ($^1O_2$). From other side, C_{60} fullerene, existing in triplet exited state, is the acceptor of electrons. This is why in the presence of electron donor (for example, $NADH^+$), its state can be reduced due to electron transfer and the changing to the anion radical ($C_{60}^{\cdot-}$). In the presence of oxygen in biosystem, this anion radical can transfer one electron to O_2 molecule, transforming it to the superoxide anion radical ($O_2^{\cdot-}$). Exactly this ROS (singlet oxygen and superoxide anion radical) were detected in experiment by EPR method and spin trap technique.

In conclusion, it was demonstrated that synthesized C_{60}-containing composites, irradiated by visible light, increase the rate of ROS generation and decrease the number of viable tumor cells *in vitro*. Such effect may be potentially useful for photodynamic therapy of tumors.

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ВЛИЯНИЕ ОБЛУЧЕННЫХ ВИДИМЫМ СВЕТОМ ФУЛЛЕРЕНСОДЕРЖАЩИХ КОМПОЗИТОВ НА ГЕНЕРИРОВАНИЕ РАДИКАЛЬНЫХ ФОРМ КИСЛОРОДА И ЖИЗНЕСПОСОБНОСТЬ ОПУХОЛЕВЫХ КЛЕТОК

Цель: **изучить влияние фуллеренсодержащих композитов, облученных видимым светом, на генерирование радикальных форм кислорода (РФК) в клетках тимоцитов, асцитного рака Эрлиха и лейкоза L1210. Исследовать жизнеспособность этих клеток в присутствии облученных фуллеренсодержащих композитов.** ***Методы:*** **жизнеспособность клеток определяли с использованием 0,4 % раствора трипанового синего; РФК регистрировали методом ЭПР- спектроскопии и спиновых ловушек; облучение водных растворов фуллеренсодержащих композитов в видимом диапазоне осуществляли с помощью ртутной лампы.** ***Результаты:*** **показано, что фуллеренсодержащие композиты при облучении повышают скорость генерирования РФК и уменьшают количество жизнеспособных опухолевых клеток.** ***Выводы:*** **полученные результаты позволяют рассматривать фуллеренсодержащие композиты как потенциальные препараты для фотодинамической терапии.**

Ключевые слова: **фуллеренсодержащие композиты, облучение видимым светом, тимоциты, клетки асцитного рака Эрлиха, лейкоз L1210, РФК, спиновые ловушки, ЭПР-спектроскопия.**