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SHORT COMMUNICATIONS

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CATALYTIC SYSTEM OF THE REACTIVE OXYGEN SPECIES ON THE C_{60} FULLERENE BASIS

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***Aim*: To study the ability of fullerenes C_{60} to catalise the reactions of generation of reactive oxygen species (ROS) in water solution after photoexcitation and to affect the vitality of tumor cells *in vitro*. *Methods*: The number and vitality of cultured Ehrlich carcinoma cells or rat thymocytes were determined using tripane blue, ROS levels were registered using the methods of electron paramagnetic resonance (EPR) spectroscopy and spin traps, photoirradiation of water solution of fullerenes C_{60} with visible light was carried out using mercury lamp. *Results*: Irradiation of water solution of fullerenes C_{60} (10^{-5} M) was accompanied with generation of ROS with the rate of 10 nMol/ml/min. After addition of irradiated C_{60} solution to suspension of thymocytes or ascite cells, the decrease of the number of vital cells by 67 and 58%, respectively, has been registered. *Conclusion*: Photoirradiated water solutions of fullerenes C_{60} may be potentially useful for photodynamic therapy of tumors as ROS catalytic system.**

***Key Words*: aqueous C_{60} fullerene solution, irradiation by visible light, ROS, anticancer therapy, spin trap, EPR spectroscopy.**

Taking into account that malignant tumors possess increased sensitivity to oxidative stress that is linked with the changes in generation of reactive oxygen species (ROS) one may propose the use of molecules of fullerenes C_{60} as regulators of these processes.

Fullerenes belong to new allotropic form of carbon that possesses unique physicochemical properties. They have a number of advantages compared to other known biologically active compounds, i.e.: 1) those inert compounds have no toxic effects and may bind to biological molecules; 2) they reveal biological activity upon concentrations that are by few orders lower than those of natural antioxidants; 3) they may be easily extracted from water solutions and may be used multiply [1–2]. Fullerenes and their derivatives act as effective absorbents of active oxygen–containing radicals whose generation increase upon development of pathological state. From other hand, as a result of photoexcitation the ability of fullerenes to catalyse the production of singlet oxygen, play a role of prooxidants and destructors of biological molecules, in particular nucleic acids, become apparent [2–6]. The abovementioned data point to the possibility of application of fullerenes as new pharmacological compounds for the development of selective photodynamic therapy of tumors.

In this work photoexcited water solutions of fullerenes C_{60} are proposed to be used for increase of the levels of ROS and controlling the rate of tumor cell growth.

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Abbreviation used: EPR spectroscopy — electron paramagnetic resonance; ROS — reactive oxygen species.

The samples of water solutions of fullerenes C_{60} were prepared in Chemical Laboratory of Ilmenau Technical University (Ilmenau, Germany) by transfer of fullerenes C_{60} from organic solvent (for example, toluene) to the water with the next ultrasound dispersion of the molecules in the solution. For that purpose, fullerenes C_{60} of purity > 99.5% were used. That method allowed to receive maximal concentration of solution of fullerene C_{60} ($5 \cdot 10^{-4}$ M): the final dark–brown water solution of fullerenes C_{60} possesses properties of molecular–colloid system stable for 12–18 months if stored at the temperature 4–40 °C without stabilizators.

In the work, Wistar rats weighing 120–150 g and nonbred mice weighing 20 g received from the vivarium of R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, NAS of Ukraine (Kyiv, Ukraine) were used. Animals received standard feeding. All animals procedures were carried out according to the rules of local Ethic Committee.

Ascite cells were isolated from mice at days 8–12 after intraperitoneal transplantation of Ehrlich's carcinoma. Rat thymocytes were obtained by passaging thymus from 4 layers of neulone. Then cells were washed by 10 min centrifugation at 600 g in the buffer (3 mM Na_2HPO_4, 5 mM KCl, 120 mM NaCl, 10 mM glucose, 10 mM HEPES, pH 7.4, 4 mM $NaHCO_3$, 1 mM $CaCl_2$, 1 mM $MgCl_2$) and resuspended in the same buffer. The number of cells was calculated in Goryaev's chamber using tripane blue.

To register ROS, EPR and spin traps techniques were applied [7]. Spin harboring compound 1–hydroxy–2,2,6,6–tetramethyl–4–oxypiperedine at the concentration of $2 \cdot 10^{-3}$ M possessing high affinity to singlet oxygene and superoxide radical–anion binds them and turns into nitroxyl radical that has been registered by EPR at the room temperature. Its EPR spectrum is presented by a triplet with the characteristics as follows: g = 2.005, A_N = 16 G, ΔH_{pp} = 0.4 G.

Irradiation of the water solution of fullerenes C_{60} ($5 \cdot 10^{-4}$ M) was carried out in glass tube using mercury lamp with a power of 24 W for 2 min. Immediately after irradiation, the irradiated solution of fullerenes C_{60} was mixed with cell suspension ($3 \cdot 10^6$ cells/ml) in a special quartz cuvette with the volume of 200 μl, and EPR spectrum has been registered.

Quantitative evaluation that was performed earlier [6] has shown that irradiation of fullerene C_{60} in the water solution with visible light may switch it in excited triplet state. The energy of such electron excitation (2.06 eV) is sufficient for initiation of the process of generation of singlet oxygen that, in turn, may initiate the generation of free radicals in the environment.

As it may be seen from our data (Table 1), irradiation of water solution of fullerene C_{60} ($5 \cdot 10^{-4}$ M) with visible light is accompanied with ROS generation with the rate of 10 nMol/ml/min. In cell suspensions ROS generation has been registered, too; in the case if irradiated fullerene C_{60} solution has been added to cell suspension to a final concentration of $2 \cdot 10^{-4}$ M, the increase in the rate of ROS accumulation in the cells has been registered — 3–fold — in thymocyte suspension, 2–fold — in Ehrlich's ascite cells. The difference in these values may be possibly explained by the fact that the ratio of cell surface to total amount of fullerenes C_{60} was lower for ascite cells that are larger than thymocytes.

Then, the cells were incubated with irradiated C_{60} for 2 h at 25 °C and their vitality was evaluated using tripane blue test (Table 2). If in 15 min after addition of C_{60} the number of vital cells remained unaltered, after 2 h incubation this value was decreased by 67% and 58% for thymocytes and ascite cells, respectively. So, fotoexcited fullerenes C_{60} influenced the initiation of cell death.

In conclusion, the presented data have demonstrated that addition of water solution of fullerenes C_{60} that were short–term irradiated with visible light to suspension of thymocytes and Ehrlich ascite cells is accompanied by increased rate of ROS. That effect may be potentially useful for development of photodynamic therapy of tumors with optimization of antitumor action and minimization of negative toxic consequences.

Table 1. The rate of ROS accumulation in water solution of fullerenes C_{60} and cell suspensions

	Water solution of fullerene C_{60} ($5 \cdot 10^{-4}$ M)	Thymocytes	Thymocytes + $2 \cdot 10^{-4}$ M C_{60}	Ehrlich ascite cells	Ehrlich ascite cells + $2 \cdot 10^{-4}$ M C_{60}
Rate of ROS generation, nMol/ml/min	10 ± 0.4	2.5 ± 0.1	7.5 ± 0.3	1.3 ± 0.1	2.6 ± 0.2

Table 2. Influence of irradiated water solution of fullerenes C_{60} on cell vitality

Number of vital cells in the suspension after addition of fullerenes C_{60}	Time after fullerenes C_{60} addition (min)		
	15	60	120
Thymocytes	91 ± 7	60 ± 8	33 ± 6
Ehrlich ascite cells	93 ± 5	52 ± 6	42 ± 5

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КАТАЛИТИЧЕСКАЯ СИСТЕМА РАДИКАЛЬНЫХ ФОРМ КИСЛОРОДА НА ОСНОВЕ ФУЛЛЕРЕНОВ C_{60}

Цель: **исследовать способность фуллеренов C_{60} катализировать реакции образования радикальных форм кислорода (РФК) в водных растворах после фотовозбуждения и влиять на жизнеспособность асцитных клеток Эрлиха.** ***Методы***: **количество и жизнеспособность клеток определяли с использованием трипанового синего, радикальные формы кислорода регистрировали методом ЭПР спектроскопии и спиновых ловушек, облучение водных растворов C_{60} в видимом диапазоне осуществляли с помощью ртутной лампы.** ***Результаты***: **установлено, что облучение водного раствора фуллерена C_{60} (10^{-5} М) видимым светом сопровождается образованием радикальных форм кислорода (со скоростью 10 нмоль/мл/мин). После внесения облученного раствора C_{60} в суспензии тимоцитов или асцитных клеток наблюдается снижение количества жизнеспособных клеток на 67 и 58% по сравнению с контролем соответственно.** ***Выводы***: **обнаруженный эффект предлагается использовать для разработки метода противоопухолевой терапии с применением фуллеренов C_{60} в качестве каталитических систем РФК.**

Ключевые слова: **водные растворы фуллеренов C_{60}, облучение видимым светом, РФК, противоопухолевая терапия, спиновые ловушки, ЭПР спектроскопия.**