INTRODUCTION

Despite the comparatively long history of studying the influence on living systems of such new allotropic forms of carbon as fullerenes and nanotubes, the data on their toxicity for living organisms are very contradictory. Suffice it to note just several works in which the authors postulate a thesis of toxicity of fullerene C$_{60}$ [1–3]. However the results obtained by these authors are at variance with the data of other works about the absence in fullerenes of strikingly pronounced toxicity [4, 5]. Moreover, the finding of toxicity in preparations of fullerene C$_{60}$ in a number of cases may be explained by methodical shortcomings of investigations, connected with the presence in the tested samples of a perceptible enough concentration of tetrahydrofuran used in extraction and purification of fullerene [6].

The contradictoriness of data on toxicity and, in a broader sense, biological activity of fullerenes may also be explained by such of their diverse physico-chemical properties as the ability to generate singlet oxygen with a high quantum yield under the action of light of visible or UV range; high affinity of the fullerene core in respect of electron donors, which determines its ability to be a scavenger of free radicals; high lipophilicity of the C$_{60}$ molecule. In this way, in biological systems fullerene C$_{60}$ can be an antioxidant (in the dark), a prooxidant (upon illumination), or a membranotropic agent.

Investigation to the biological properties of fullerenes is complicated by that they are practically insoluble in polar, acceptable for biological investigations, solvents. One of the most often used means of solubilizing fullerenes is presented by complex formation with hydrophilic polymers—for example, such as N-polyvinylpyrrolidone (PVP) and $\gamma$-cyclodextrin ($\gamma$-CD).

The aim of the present work was an investigation of the influence of C$_{60}$/PVP and C$_{60}$/\$\gamma$-CD complexes in vitro on cell ultrastructure and integral indices of cell metabolism and viability.

EXPERIMENTAL

Complex C$_{60}$/PVP was obtained by an earlier described method [4]. In the quality of a carrier polymer use was made of PVP from Merck (Germany) of mol. mass 25000 or a pharmacopeia PVP preparation Enterodez. The content of fullerene C$_{60}$ in the investigated complexes constituted 0.50–0.67 mass %.

Complex C$_{60}$/\$\gamma$-CD was obtained according to Priyadarsini et al. [7].

Cell culture. In the work use was made of a constant cell line MA-104 (green monkey kidney). Cells were cultivated in medium alpha-MEM (Biolot, Russia).
with addition of 5% fetal calf serum (Sigma, USA) in a CO₂ incubator in an atmosphere of 5% CO₂. The sowing concentration of MA-104 constituted 25 thousand cells/mL. The growth medium in plates with a daily monolayer was changed to a maintenance medium, which also served as solvent for tested compounds. After two days of exposure a part of material was evaluated in a test with fluorescent dye resazurin, the second part was fixed for electron microscopic analysis.

Reduction of fluorescent tetrazolium dye resazurin by cells in culture was used in the quality of an index of integral activity of mitochondrial dehydrogenases and cell viability [8]. Fluorimetric measurements were conducted on a multifunctional plate analyzer Chameleon (Hydex, Finland).

Electron microscopic analysis. MA-104 cells were fixed in 2.5% glutaraldehyde on 0.1 M cacodylate buffer with postfixation in 1% osmium tetroxide. In the course of dehydration and embedding the cells were centrifuged at each change of medium. Ultrathin sections contrasted with lead citrate were analyzed on an electron microscope JEM-100S (Japan).

Ultraviolet irradiation of cells was actualized in sterile conditions with an irradiator of 400 W power at a distance of 30 cm from the plate without plastic cover. The irradiator spectrum 220–400 nm. For removal of heat the plate was immersed by 1/3 into a cuvette with flowing water.

RESULTS

As a result of the work it is established that both fullerene complexes used by us—C₆₀/PVP and C₆₀/γ-CD—prove nontoxic for MA-104 cells in culture in a broad range of investigated concentrations (Table 1). The C₆₀/PVP complex in a concentration up to 5 mg/mL (at a content of C₆₀ 0.5 mass %, i.e. at a concentration of the latter 35 μM) and incubation with cells for 2 day did not cause influence either on monolayer density, or the microscopic structure of cells, or the intensity of respiratory metabolism in the resazurin test, at that analogous data have been obtained for the carrier polymer itself—PVP.

The C₆₀/γ-CD complex in the investigated concentration range (0.1–2.5 mg/mL, which at a C₆₀ content about 2.0 mass % gives a molar concentration of the latter 3–75 μM), and the carrier γ-CD did not cause reliable influence on cell viability and respiratory metabolism.

The ultrastructure of MA-104 cells in control testifies to a active functional state of all organoids and intensely ongoing processes of synthesis of cytosolic proteins (Fig. 1a). The Golgi apparatus is presented by large dictyosomes with a relatively small quantity of peripheral vesicles. The cytoplasmic reticulum is well developed with some prevalence of smooth cisternae. In the cytoplasm one notes many free ribosomes and polysomes. Cells contain a moderate quantity of not large mitochondria with a different quantity of cristae and relatively dense matrix. In the cytoplasm one encounters large vacuoles containing amorphous material, a small quantity of little vesicles and membrane structures. In the nucleus decondensed chromatin prevails, the karyolemma contains a large quantity of pore complexes, the nucleolar material is loosened (nucleus not presented in the figure).

In cells after incubation with PVP (5 mg/mL) one notes a more pronounced degree of chromatin decondensation and nucleolus loosening. The cisternae of the Golgi complex are dilated, the quantity of peripheral vesicles is enlarged. Substantial changes are undergone by sizes of mitochondria, a part of which can be characterized as giant (Fig. 2a). Not infrequently therein against a background of clarified matrix distinctly seen are electron-dense particles. There is also an increase in the quantity and size of vacuoles with a large content inside of little vesicles (Fig. 1b). Within the limits of one ultrathin section the vacuoles may be from three to five. The system of cytoplasmic reticulum is represented prevalently by rough cisternae, a large part of which is insignificantly dilated (Fig. 1b).

The ultrastructure of MA-104 cells after incubation with the C₆₀/PVP complex is similar with that after exposure with PVP in the same concentration. Apart of an increase in the content of peripheral vesicles of the Golgi complex in the cell cytoplasm out of explicit connection with dictyosomes one disclosed in a large quantity are similar vesicles (Fig. 1c). Amazing also is the abundance of mitochondria often of queer shape and gigantic sizes, present in the matrix are electron-dense inclusions (Fig. 2b).