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Cytotoxicity of Carbon Nanotubes

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The industrial applications of carbon nanotubes (CNTs) for creating of new kinds of materials are limited because of their potential toxicity. There are many data concerning CNTs influences on the living body—from quite negative ones to the possibility of CNTs use in medicine. In a given work, the cell toxicity of CNTs on the example of healthy hepatocytes and Eirlich adenocarcinoma cells (EAC) is studied. As shown, the cells' contact with CNTs suspension leads to the radicals' release from cells. This radical release depends on both the time of cells' contact with suspension and the CNTs concentration. As also shown, the influence of CNTs on EAC is essentially higher than that on healthy hepatocytes.

Промислове використання вуглецевих нанорурок (ВНР) для виготовлення нових композиційних матеріялів обмежене їх потенційною токсичністю. В літературі є дані, які уможливлюють тлумачити вплив ВНР на живий організм від вкрай неґативного до можливого використання їх в медицині. Дану роботу присвячено вивченню токсичности ВНР на прикладі здорових клітин печінки (гепатоцитів) та клітин аденокарциноми Ерліха (ЕАК). Показано, що контакт клітин з суспензією ВНР призводить до викиду ними перекисних радикалів, а рівень викиду цих радикалів залежить від часу контакту і концентрації ВНР. Також було встановлено, що вплив ВНР на ЕАК є більш явним у порівнянні з гепатоцитами.

Промышленное использование углеродных нанотрубок (УНТ) для создания новых композиционных материалов ограничено их потенциальной токсичностью. В литературе представлены данные, которые позволяют рассматривать влияние УНТ на живой организм как крайне негативное, так и позволяющее их использование в медицине. Данная работа посвящена изучению токсичности УНТ на примере здоровых клеток печени (гепатоцитов) и клеток аденокарциномы Эрлиха (ЭАК). Показано, что контакт клеток с суспензией УНТ приводит к выбросу клетками перекисных радикалов, уровень чего зависит от времени контакта и концентрации УНТ. Также показано, что влияние УНТ на ЭАК — более выраженное, чем на гепатоциты.

Key words: carbon nanotubes, cells, cytotoxic effect.

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1. INTRODUCTION

Carbon nanotubes (CNTs) are extensively used in different fields of science and industry last decade. Such a multidisciplinary application of CNTs raises the question of their safety for human health. There is no accurate data of CNTs influence on living body. A lot of information in literature varies from their toxicity to possible use in pharmacology. Thus, this study is carried out to evaluate the CNTs effect on cells of different nature.

2. MATERIALS AND METHODS

Multiwall CNTs (Fig. 1) were synthesized by CVD method on equip-

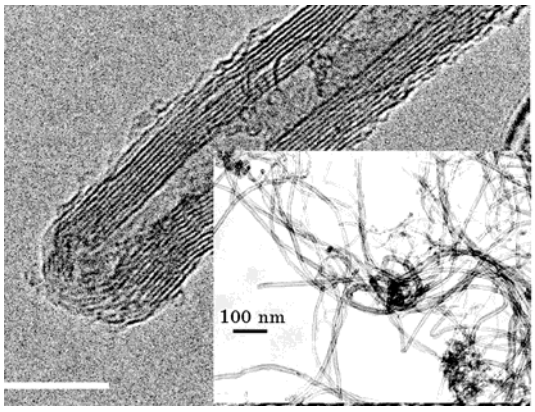

Fig. 1. Multiwall CNTs (TEM data).

ment with manufacturing capacity 1.0–1.5 kg per day [1]. The composite of oxides like Al_2O_3–MoO_3–Fe_2O_3 was used as catalytic agent. Propylene was the source of carbon. CNTs were obtained as agglomerates of entangled tubes with 20–500 μm in size. Additives were eliminated by CNTs treatment with hydrofluoric acid. Ultrasound drying of CNTs resuspended in saline solution was carried out using UZDN-2 dispergator. Size distribution function of particle agglomerates was determined on laser correlation spectrometer 'ZetaSizer-3' with type 7032 s multicomputing correlator (Malvern Instrument, Great Britain).

Obtained autocorrelation function (ACF) was calculated using software PCS-Size mode v 1.61. System with concentration 0.2 wt.% of CNTs in saline solution shows two sizes of particles: 0.01–0.10 μ and 1.0–5.0 μ. Parameters of multiwall CNTs were determined by TEM, x-ray fluorescence analysis, Raman scattering spectrometry, differential thermal analysis, differential thermogravimetric analysis, AFM, and x-ray photoelectron spectroscopy methods. Ash values of purified CNTs were < 1.0 wt.%, specific surface measured by argon was 220 m^2/g, the 5 wt.% losses were determined at 605°C during heating rate 10°C per minute. Amorphous carbon was not present in studied CNTs. Their diameter was 10–20 nm, number of layers 5–10. In addition to main x-ray reflex 002, 100, 101, 110, 112 reflexes were observed that indicated of 3*D* regulating of graphite-like lattice. In this case, interplanar spacing d_{002} is in the range 0.3436–0.3453 nm. Equal intensity of *G* and *D* modes were observed in Raman scattering spectra. According to x-ray photoelectron spectroscopy data, oxygen was determined on CNTs surface and characterized according to type its active centres and oxidized ones (electrochemically or after heat treatment on air) as 0.6, 1.1, 2.3 at.%, respectively. This is considerably lower as compared to carbon fibres [2].

The concentration of oxygen containing centres determined from 1*s*C-electrons band (E_b) is presented in Table [3, 4]. The concentration

TABLE. Relative concentrations of oxygen containing centres on MWNT and their classification by energy of 1*s*O-electrons.

MWNT samples	Relative concentration, %			
	E_b = 286.1–286.3 eV; phenol, alcohol (C–OH)	E_b = 287.3–287.6 eV; carbonyl, quinone (C=O)	E_b = 288.4–288.9 eV; carboxyl, ether (C–OOH)	E_b = 290.4–290.8 eV; carbonate and/or absorbed CO, CO_2
Initial	49.1	17.2	17.2	16.5
Anode oxidation	53.8	19.8	13.6	12.8
Heat treatment	51.1	23.4	15.3	10.2

of oxygen-containing centres was determined by the binding energy of the 1*s*C-electrons.

3. *IN VIVO* EXPERIMENT

Experiments were carried out on white inbred male mice with body weight 20–22 g. Cells of Ehrlich ascetic carcinoma (EAC) (10^6 cells/mice) were injected intraperitoneally (i.p.) in the volume of 0.4 ml of saline solution. Culture of Ehrlich ascetic carcinoma was obtained in cell lines bank IEPOR. All experiments on mice tumour bearing were carried out during 7 days after the EAC transplantation. The CNTs suspension in saline solution was i.p. administered in concentration 0.75 and 1.5 mg per mouse for 24 h.

Fluorescent probe 2′,7′-dichlorofluorescein diacetate (DCFDA, $\lambda_{ex} =$ $= 488$ nm, $\lambda_{em} = 525$ nm) (absorption at 485 nm, emission at 528 nm) was used for measurement of reactive oxygen (ROS) and nitrogen (RNS) species in the EAC cells and hepatocytes.

The obtained EAC cells and hepatocytes were three times washed and precipitated by centrifugation for 5 min at 1 500 r. per minute. Evaluation of ROS and RNS was carried out on analyser Synergy HT Multi-Detection Reader, in plate for 90 min. at 37°C). Reaction mixture is as follows: 250 μl of PBS (phosphate buffer saline), 25 μl cell suspension with concentration of $2 \cdot 10^6$/ml, 25 μl 6 mM DCFDA. Measurement was carried out every 10 minutes during 90 min.

State of the cell surface was examined by SEM. The isolated cells were fixed with 10% formalin, then dehydrated in battery alcohols, coated with gold, and studied with JSM-6490LV (JEOL Japan).

4. RESULTS

Exposure to CNTs in concentration of 0.75 and 1.5 mg per mouse was accompanied by the increase of ROS and RNS production in hepatocytes by 102% and 56% (Fig. 2).

Increasing of free radicals level, which produced by normal cells in 24 hours after contacting with CNTs, could be explained by cells' reaction to exogenous particles. Moreover, findings confirmed hypothesis that the CNTs influence on cells physiology.

In EAC cells, lower dose of CNTs caused slight increase of ROS level (by 20%). Increasing the dose of CNTs (1.5 mg per mouse) led to 39% rise of ROS production (Fig. 3).

Explanation of such disproportion could be found in cell nature. In addition, as at it will be shown below, the surviving of EAC cells after exposure with CNTs is rather low.

After exposure to CNTs in concentration 1.5 mg per mouse, the un-

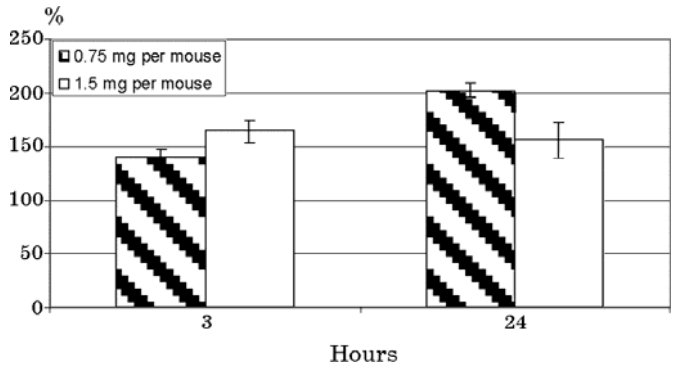

Fig. 2. Level of free radicals production in hepatocytes after injection of the CNTs suspension.

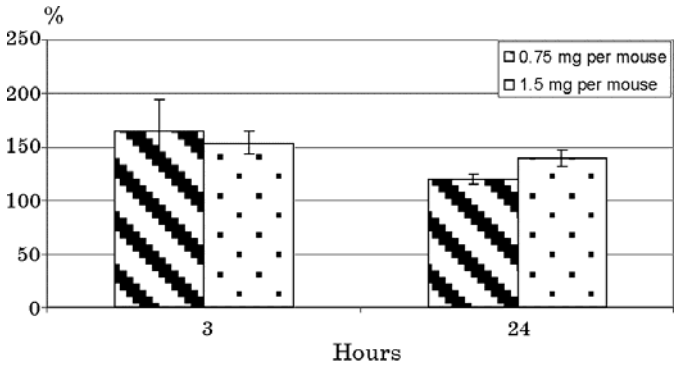

Fig. 3. Level of free radicals production in EAC cells after injection of the CNTs suspension.

damaged EAC cells were not detected in visual field (Fig. 4), unlike to hepatocytes (Fig. 5).

Our research has shown that it is hard to tell whether CNTs penetrate in cell membranes. It was speculated in the works [5–7] that CNTs penetrate through the cell membranes and thus affect on their physiological cycles. This hypothesis is quite controversial, because the probability of membrane damaging from the CNTs satisfies their aspect number ($\cong 1000$). In addition, it was shown that CNTs in water suspensions are presented as agglomerates in the form of entangled tubes (Fig. 1) with sizes ranging from 200 to 300 nm. Attraction of CNTs to definite receptors on the cell surface may be more probable because they have different reactive groups (see Table). Complete blockage of receptors or covalent binding to the active centres on the membrane may explain this CNTs effect on cells viability. This ability of CNTs to interfere in the physiology of cells opens up new prospects for

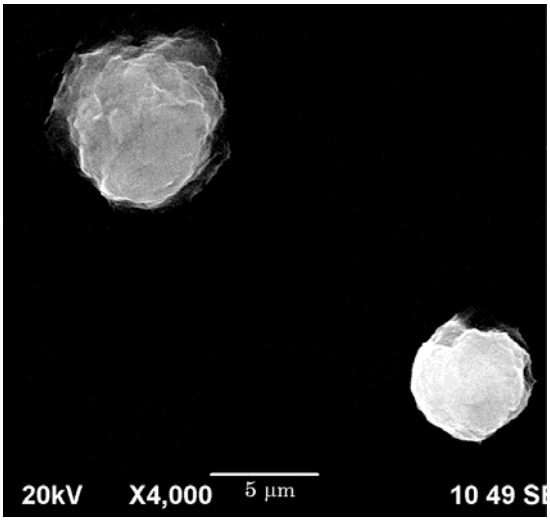

Fig. 4. EAC cells after exposure with CNTs with concentration of 0.75 mg per mouse (SEM data).

their using for targeted drug delivery.

5. CONCLUSION

This study shows that direct contact of CNTs with cells leads to increasing of free radicals production and to injury of cells membrane, particularly in the case of EAC cells. In any case, CNTs influence on

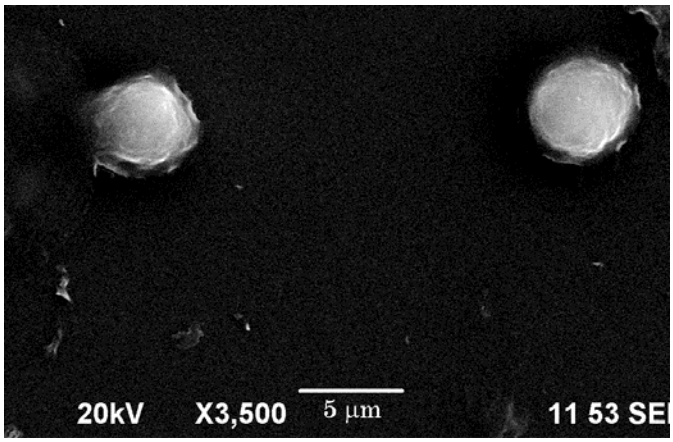

Fig. 5. Hepatocytes after exposure with CNTs at concentration of 1.5 mg per mouse (SEM data).

cells physiology in the manner depending on their nature, and it is necessary to provide further investigations.

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