MECHANISMS OF THE CYTOTOXICITY OF CARBON NANOTUBES

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Introduction

Carbon nanotubes (CNT) cytotoxicity still remains a key topic at practical use of these unique nanostructures in biotechnologies and medicine [1]. It is clear that the mechanisms of nanotubes influence on cellular structures can include mechanical damages of cell membranes as well as an influence of nanotubes on biochemical processes in subcellular organelles such as endoplasmic reticulum, mitochondria and nucleus. To study the mechanisms of CNT cytotoxicity at the molecular and membrane levels we proposed to use the method of spin probes. This method is successfully used in a molecular biology and pharmacology for a long time. It can be judged about microviscosity and polarity of probe microenvironment, conformational changes in proteins and membranes, lipid fluidity and membrane integrity, as well as about oxidation-reduction activity in the cells and tissues by electron paramagnetic resonance (EPR) spectra of the stable nitroxyl radical (probe) being introduced into a suspension of cells or proteins [2].

An objective of this paper was to evaluate the integrity of human blood erythrocyte membranes and mitochondrial activity of hepatocytes of rat liver homogenate in the presence of CNT suspensions.

Experimental

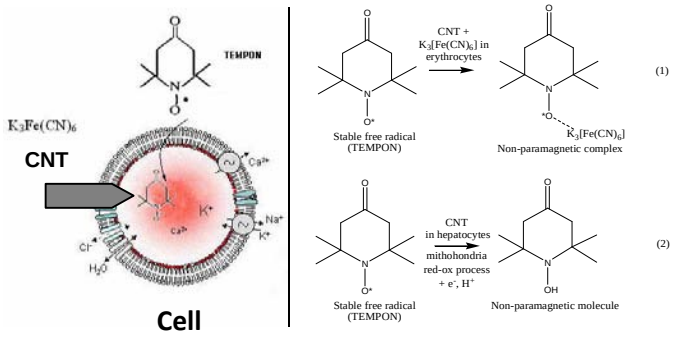
In the present work, the multi-walled high purity CNT were used. Those were produced on a pilot plant designed by Chuiko Institute of Surface Chemistry, NAS of Ukraine and Company "TMSpetsmash-Ltd" by catalytic pyrolysis of unsaturated hydrocarbons [3]. The characteristics of CNT were as follows: internal diameter, 1-2 nm; external diameter, 10-40 nm; ash content, less than 0.4%; and nanotube content, more than 95%.

The human blood erythrocytes were obtained from the erythromass, which has been washed from plasma and from the stabilization solution by centrifugation in physiological saline (pH 7.2). Rat liver homogenate was prepared by procedure described in [4].

As a paramagnetic probe there was used a water-soluble iminoxyl radical: 2, 2, 6, 6-tetramethyl-4-oxo-piperidine-1-oxyl (TEMPON).

The stable CNT suspensions were prepared as follows. To the water suspension of CNT (100 mg per 100 ml of water) we added 0.1 ml of heparin solution (5000 U/ml), which was then ultrasound treated, froze and after defrosting centrifuged at 10 000 rpm for 15 min. For each experiment the two samples of the erythrocyte suspension have been prepared, each of 0.9 ml. One sample (control) was exposed during appropriate exposure time at the appropriate temperature without CNT, and the same was done with another sample (test), but with CNT suspension. After exposure, to each sample was added 0.1 ml of the spin probe standard solution and widener (potassium ferricyanide), and then EPR spectra were registered using a radiospectrometer *Brucker ER 100 D*. Thus, a cell aging during exposure was accounted. The similar experiment has been conducted using hepatocytes suspension. Erythrocytes were in contact with CNT at 6 ^{o}C for two days, and the liver homogenate was in contact with those at 0 ^{o}C for 4 h.

Previously, a quantitative express method has been proposed and developed by Ivanov [5] for studying the integrity of isolated cells and those of tissue samples. Its principle can be seen from following schemes:



The method makes it possible the determination of the amount of native (intact) cells in the sample after some kind of influence using spin probes.

Results and Discussion

In our experiments it was demonstrated that when to erythrocyte suspension were added the CNT suspensions at concentrations of 10, 50, 100 and 200 mug/ml followed by a 10 min exposure, no change in EPR spectra was observed. This confirmed the erythrocyte integrity was intact in all the experiments. And there was an only experiment (when the concentration of CNT was 200 mug/ml), when the signal intensity of EPR has decreased by 12-15%, and that indicated membrane damages appeared in a significant quantity of erythrocytes. After 2 days the amount of damaged erythrocytes was already more than 25% (Fig. 1).

This is evidence that the damage of the erythrocyte membranes under influence of CNT is developing with time. The obtained result is in compliance with the conclusions made by authors [1], that a disintegration process of different cells has a progress within a period from some hours till some

days. It was found that even lower concentrations of CNT have a damaging effect on erythrocytes. Thus, after 2 days the CNT suspensions at concentrations of 10, 50, and 100 mug/ml had damaged 4, 10 and 16% of erythrocytes, respectively (Fig. 2).

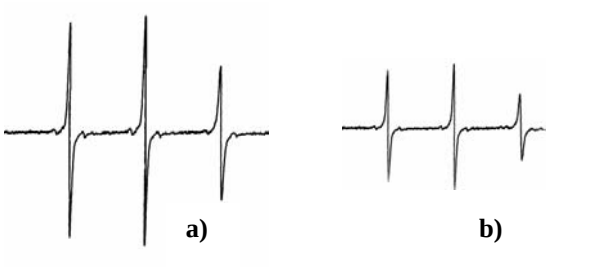


Fig. 1 EPR spectra of spin probe in cytosol of donor blood erythrocytes after a two-day incubation at 6 °C: a) control; b) in the presence of CNT suspension (~ 200 mug/ml).

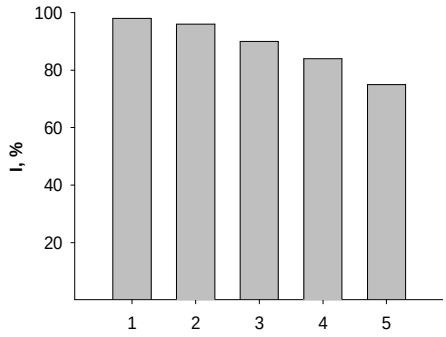


Fig. 2 An influence of incubation of donor blood erythrocytes with CNT suspensions of different concentrations on intensity of central component of paramagnetic probe EPR spectra: 1 – control; 2 – 10, 3 – 50, 4 – 100, and 5 – 200 mug/ml.

A slow dynamics of the erythrocytes under the influence of CNT seems was not tied to the mechanical nature of the erythrocyte membrane damage, the consequences of which are very rapidly developed. Obviously, the ability of CNT to manifest the semiconductor and metallic properties depending on their structure and media conditions should be taken into account [6]. We suggested that nanotubes can substantially influence the biochemical processes occurred in mitochondria, neurons, and cardiac hystiocytes etc., which are related to electron or charge transfer. In this respect, we used a modification of the spin probe method in which the activity of the electron transfer chain (respiratory chain of mitochondria), can be predetermined without mitochondria extraction from hepatocytes. A probe reduction to non-paramagnetic hydroxylamine is carried out by using a strong antioxidant, coenzyme Q_{10} of the mitochondrial respiratory chain. Hereby, the intensity of the EPR spectrum is exponentially decreased with time. By taking the logarithm of such the curves we obtain the straight lines with a slope ratio to X-axis to be in proportion to the constant of the probe reduction rate and therefore to the activity of mitochondria being inside the hepatocytes.

Fig. 3 shows the data on the influence of a CNT suspension at a concentration of 200 mug/ml on the constant of the spin probe reduction rate in the suspension of hepatocytes (mitochondrial activity). The probe reduction rate was assessed by the intensity rate fall of spectral lines of EPR probe being in the liver homogenate.

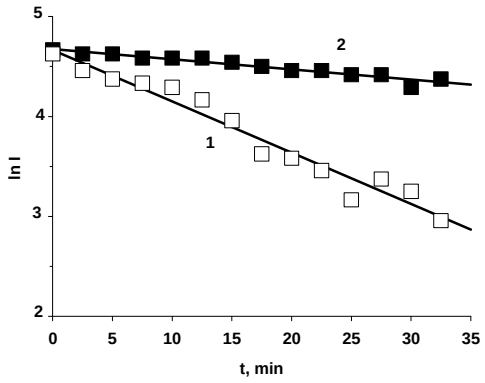


Fig. 3 A spin probe reduction in the liver homogenate after a 4 h incubation period: □ – control; ■ – in the presence of CNT suspension (~ 200 mug/ml).

Conclusions

It was demonstrated by a spin probes method that membrane damage of erythrocytes in the presence of CNT suspensions at concentrations of 10-200 mug/ml after a two-day period of exposure at 6 °C achieves 4-25%, respectively.

An exposition of liver homogenate with CNT for 4 h at 0 °C results to decreasing mitochondrial activity of hepatocytes in more than 5 times. It demonstrates an inhibition of the chain of electron transfer in mitochondria.

References

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