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RESEARCH PAPER

The cytotoxicity of water-soluble carbon nanotubes on human embryonic kidney and liver cancer cells

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Abstract: The cytotoxicity of polyethylene glycol-modified carbon nanotubes (PEG-CNTs) on human embryonic kidney cells (293T) and hepatoma cells ($HepG_2$) was studied in vitro. The effect of the concentration of PEG-CNTs on the cell activity and survival rate was detected by a colorimetric assay method after exposure for 24, 48 and 72 h. The dead $HepG_2$ cells obtained by incubation in solutions with different concentrations of PEG-CNTs for 24 h can be stained by a dye, propidium iodide, and the cell mortality was determined by flow cytometry. Results show that the toxicity of PEG-CNTs with a good water solubility towards 293T and $HepG_2$ cells increases with increasing PEG-CNT concentration. When the concentration of the PEG-CNTs is less than 100 μg/mL, the toxicity of the PEG-CNTs is Grade Ⅰ(non-toxic) and when it is greater than 100 μg/mL but less than 200 μg/mL it is Grade Ⅱ(mildly toxic), according to the ISO2109932-5 cytotoxicity standard. The toxicity grade does not change with increased time.

Key Words: Water-soluble carbon nanotubes；Human embryonic kidney cells；Hepatoma cell；Cytotoxicity

1 Introduction

At present, carbon nanomaterials with a high stability and good biocompatibility have had extensive application prospects in biomedical fields including biosensors, drug and vaccine delivery system, composite bone materials, tumor targeted therapy and novel biological materials[1-2]. Carbon nanotubes (CNTs) have become a hotspot of study in drug carrier because of their hollow structure and large specific surface area allowing the accommodation of specific biomolecules and drug molecules, and excellent cell penetrability [3-4]. However, they produce a strong immunogenicity in the body because of their insolubility in physiological buffer, causing great limitations in the application of CNTs to a large extent [5].

Polyethylene glycol (PEG) with a superior biocompatibility was used to modify CNTs to prepare water-soluble CNTs (PEG-CNTs), which can improve the biocompatibility, solubility and drug delivery of CNTs[6-8]. As a drug carrier, some characteristics, including non-toxic, non-antigenic and excretion ability, are necessary besides good biocompatibility. Our previous study[9] shows that the functionalized CNTs introduced into living rabbit were mainly distributed in lung, heart, liver, spleen and kidney, and they had not deposited in the body because they were gradually excreted through urinary system with the passing of time. But the study also confirms that CNTs had a lightly longer retention time in liver and kidney than in other organs.

Based on this phenomenon, this paper aims at evaluating the toxicity of PEG-CNTs in tumor cells and normal cells in order to verify that PEG-CNTs can be used as a drug carrier to inhibit tumor cells without the damage of PEG-CNTs themselves towards tumor cells. Therefore, an in vitro experiment was conducted, where hepatoma cells ($HepG_2$) were chosen as the experimental groups and normal cells of human embryonic kidney cells (293T) as the control groups. Functionalized PEG-CNTs with different concentrations were introduced into the two classes of cells. The relative survival rates of 293T cells and $HepG_2$ cells were measured with a water-soluble tetrazolium salt (MTT assay), which can indirectly reflect the influence on the cell survival rate. The actual survival of cells was measured by a flow cytometer

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(FCM) after 24 h of continuous exposure. Finally, the in vitro cytotoxicity of PEG-CNTs in 293T cells and $HepG_2$ cells was studied. This research article gives a strong support for CNTs as a drug carrier in biomedical field.

2 Experimental

2.1 Materials

CNTs, with an inner diameter of 8-15 nm, length of 0.5-2 μm, specific surface area of 233 m^2/g and purity of 95 wt%, were purchased from Chengdu Organic Chemistry Co. Ltd., Chinese Academy of Sciences. Human embryonic kidney cells (293T) were purchased from Shanxi Medical University Parasites Laboratory. Human liver cancer cells (HepG2) were purchased from the Cell Center of the Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences & Peking Union Medical College. DMEM/High Glucose (1×) medium was purchased from Thermo Fisher Scientific Chemical Co., Ltd. Fetal bovine serum (FBS) was purchased from Hangzhou Sijiqing Co. Trypsin-EDTA digestion solution, green streptomycin mixture (100×), polyethylene glycol 800, thionyl chloride, chloroform, dimethyl formamide (DMF) and dimethyl sulfoxide (DMSO) were purchased from Beijing Solarbio Technology Co., Ltd.. (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (MTT) was purchased from Sigma, USA. Propidium iodide (PI) staining kit (containing 10×BufferA) was purchased from KGI. Phosphate buffer (PBS, 1x) was prepared in laboratory.

2.2 CNTs modified by PEG

First, carboxyl functional groups were introduced onto CNTs by a mixed acid treatment. CNTs (0.2 g) were added to 2.5 mol/L concentrated HNO_3 under sonification for 24 h, then reacted in the mixed acid consisting of concentrated HNO_3 and H_2SO_4 (*v*/*v*=3:1) for 4 h. After the reaction, black powder deposited in the lower layer after standing. The supernatant was removed, and the black powder were thoroughly rinsed with deionized water, filtered and dried under vacuum for 8 h to obtain CNTs containing –COOH, which is denoted as CNTs-COOH.

Then, the surface of CNTs-COOH was grafted with hydrophilic groups of polyethylene glycol with the following methods. CNTs-COOH (200 mg) was added to a 50 mL round bottom flask, and then a mixture of sulfoxide chloride (15 mL) and 3 drops of DMF was added. The reaction system was heated at 60 °C with reflux for 10 h. After the completion of reaction, the system was cooled to room temperature, and the mixtures were filtered by a 0.48 μm filter membrane, followed by removal of sulfoxide chloride residues through chloroform washing, and drying under vacuum at room temperature to obtain acyl chlorinated CNTs. The mixture of 15 mL of chloroform, 0.1 mL of polyethylene glycol 800 and 3 drops of DMF were added to the acyl chlorinated CNTs in a 50 mL round bottom flask, followed by heating and reflux at 50 °C for 10 h. After the completion of the reaction, the reaction system was cooled to room temperature, and then the mixture was filtered by a filter membrane, washed with chloroform and dried under vacuum at room temperature to obtain PEG-CNTs.

2.3 In vitro cytotoxicity of PEG-CNTs against 293T cells and $HepG_2$ cells

MTT assay was performed to determine the relative cell count and cell viability of 293T cells and $HepG_2$ cells against different concentrations of PEG-CNTs, which can indirectly reflect the influence of PEG-CNTs on the cell survival rate. In addition, the actual survival of cells was measured by a FCM detection instrument after continuous exposure to PEG-CNTs. The in vitro cytotoxicity of PEG-CNTs against 293T cells and $HepG_2$ cells was explored.

2.3.1 Preparation of PEG-CNTs solutions and reagent

PEG-CNTs (0.01 g) were dispersed into 10 mL of PBS to prepare 1 mg/mL stock solution, which was autoclaved at 121°C for 30 min and then sealed. The solution was ultrasonically dispersed for 30 minutes before using. The experiment was divided into an experimental group and a control group. The solution was treated to obtain 6.25, 12.5, 25, 50, 100 and 200 μg/mL PEG-CNTs solutions with DMEM/High Glucose (1×) medium.

The concentration of MTT was 5 mg/mL in MTT method. MTT (0.5 g) was dissolved in 100 mL of PBS, filtered with a 0.22 μm filter membrane to remove the bacteria, and then placed at 4 °C under the dark condition (keeping out of the sun in the process of preparation and preservation).

Preparation of 1× Buffer A: 10×Buffer A was diluted 10 folds with double distilled water before using.

2.3.2 Cell culture

293T cells and $HepG_2$ cells were grown in a DMEM/High Glucose (1×) medium with a 10% (volume) fetal bovine serum inactivated at 56 °C, 100 U/mL penicillin and 100 μg/mL streptomycin at 37 °C in 5% CO_2 atmosphere. The cells were digested using trypsin-EDTA every 3 days according to the growth condition, until they reached the logarithmic growth phase.

2.3.3 Cytotoxicity detection

(1) The relative survival rate of cells determined by MTT assay

MTT colorimetric method is adopted to detect the cell survival and growth, of which the detection principle is that the succinate dehydrogenase in living cell mitochondrias can reduce exogenous MTT to water-insoluble blue-violet crystal forma (Formazan) depositing in the living cells, but no such reaction occurs in dead cells. DMSO can dissolve formazan in cells, and the absorbance value of formazan can be measured at 490 nm wavelength by an enzyme-linked immunometric meter to indirectly reflect the number of living cells. In a certain number range of cells, the crystal number formed by MTT assay is in proportion to the number of cells, which is one of the most commonly used method of cytotoxicity