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The covalent and non-covalent conjugation of graphene oxide with hydroxycamptothecin in hyperthermia for its anticancer activity

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ABSTRACT

Many studies have focused on the possibility of applying graphene oxide as an effective drug delivery system, but also as an effective tool for a thermally-responsive system. Thus, the main aim of the study was to evaluate the cellular response of the MCF-7 cell line to covalently and non-covalently conjugated graphene oxide with hydroxycamptothecin (GO-HCPT) and the effect of an 8-h rotating magnetic field (RMF) exposure. The objective of this novel approach is to use graphene oxide platform not only as a drug delivery system, but also as an effective tool for a thermally-responsive system. The present study analyzed the effect of nanocomposites and RMF on the activity of MCF-7 cells and revealed that mitochondrial metabolism was significantly reduced in the cells incubated under all tested RMF parameters. In all tested variants of c-GO-HCPT concentrations and RMF inductions, cell viability was reduced by approximately 10–50% (data from WST-1 assay) for cells incubated with nanocomposite for 24 h and 70–90% for cells incubated with nanocomposite for 48 h. The observed LDH leakage was higher for cells exposed to c-GO-HCPT for longer incubation time. In the case of nc-GO-HCPT, the relative viability was reduced by 10–30%, respectively to the sample concentration and magnetic induction value for 48-h incubation. The data obtained in the NR assays after 24 h confirmed slightly higher cytotoxic co-effect of nc-GO-HCPT with cellular metabolism reduced by 90–98%. The concentration of nanomaterials also influenced mitochondrial metabolism. Both tested nanomaterials, c-GO-HCPT and nc-GO-HCPT, and RMF affected effectively the metabolism and viability of the cells, but not in a dose-dependent manner. A small difference in cellular response was observed in the presence of non-covalent and covalent GO-HCPT, with a slightly higher cytotoxicity effect of nc-GO-HCPT, visible 24 h after the experiment. These findings could have potential clinical application, including targeted tumor chemotherapy and thermal ablation.

1. Introduction

Graphene has become one of the hottest topics in the scientific community since its isolation and characterization by Novoselov and Geimin (2004) [1]. This two-dimensional sp^2 hybrid of carbon atoms with a honeycomb structure is an extraordinary substance with unique mechanical, thermal, optical and electronic properties that has found applications in nanotechnology, for example as nanocomposites, sensors, batteries, supercapacitors, nanoelectronics and in nanobiomedicine [2,3]. An excellent thermal conductivity of graphene and its derivatives is one of those exceptional properties that makes these materials attractive for industry and biomedicine. Thermal conductivity of carbon materials spans an extraordinary large range, e.g., ~0.01 Wm^{-1} K^{-1} in an amorphous carbon or more than 2000 Wm^{-1} K^{-1} at room temperature in a diamond or graphene [4]. This astonishing

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characteristic of heat conduction in carbon materials is dependent on phonons. A strong covalent sp^2 binding has an effect on the effective heat transfer [4]. Balandin et al. [5] and Ghosh et al. [6] in recent studies on the thermal properties of graphene described the thermal conductivity value of about 5000 Wm^{-1} K^{-1} obtained for a suspended graphene monolayer. Another unique property of graphene is its structure with a large specific surface area that makes this material an ideal carrier of many various molecules, e.g., drugs, fluorescent dyes, photosensitizers, ferromagnets, etc. [7,8]. Graphene oxide (GO) – graphene derivative – shares most of the above-mentioned unique properties of graphene and is a potential candidate for wide industrial and biological applications. For example, many recent studies have focused on the application of GO as a drug delivery system or suitable for a thermally-responsive system [9,10]. However, little is known about the thermal properties of graphene oxide. Mu and co-workers [11] claim that thermal conductivity of graphene oxide is lower than in monolayer graphene and the exact value strongly depends on the content of oxygen-functional groups. This theoretical calculations are in agreement with experimental results of Mahanta et al. [12].

Camptothecin I (CPT) is a quinoline alkaloid discovered in 1966 by M. E. Wall and M. C. Wani in the systematic screening of Chinese medicinal herbs for anticancer agents [13]. Camptothecin is a metabolite isolated from the Chinese plant –*Camptotheca acuminata* (family *Nyssaceae*), which was subjected to pharmacological, molecular and pharmacogenomic analyses using methods of cell and molecular biology [13,14]. It was found that camptothecin inhibited an enzyme – DNA topoisomerase I (topo I) [15]. CPT was also shown to have potent anticancer (antileukemic) and antitumor activity. Due to its low solubility and adverse drug reaction, natural camptothecin has been replaced by numerous synthetic derivates of camptothecin with reduced toxicity and improved anticancer activity. Two camptothecin analogues – topotecan and irinotecan– have been approved by the FDA for cancer treatment. Several other analogues, e.g., silatecan or lurtotecan are in various stages of preclinical and clinical trials [16]. Because of the strong therapeutic effect of camptothecin on cancer cells, it was used to prepare a small-size graphene oxide supramolecular assembly [17].

Hyperthermia means generation of heat, and with respect to cancer therapy, this term is used to describe a novel approach to anticancer treatment based on the generation of heat at the tumor site [18]. Higher temperature at the tumor site results in cancer cell apoptosis. This alternative therapy usually uses magnetic nanoparticles (MNPs) loaded with antitumor drug, implanted selectively to the tumor cells, and an external alternating current (AC) magnetic field that converts the magnetic energy into thermal energy [18,19]. This type of alternative therapy has a great application as a non-invasive technique that creates localized heat that kills tumor cells without affecting the neighboring healthy cells.

The purpose of our study was to investigate the effect of graphene oxide (GO) covalently and non-covalently bonded to hydroxycamptothecin (HCPT) in human breast adenocarcinoma cell line, MCF-7, exposed to the rotating magnetic field (RMF). Due to the widely discussed thermal properties of graphene-based materials, cells treated with GO-HCPT were additionally tested under specific conditions of the magnetic field (at different magnetic induction values ranging from 1.23 to 10.06 mT). This novel approach may provide a possibility to use graphene oxide platform not only as a drug delivery system, but also as an effective tool for thermally-responsive system. Determination of the potential coeffect of GO bonded to anticancer drug, and RMF, on the cellular metabolism of cancer cells can be useful for further improvements and evaluation of anticancer therapies.

2. Materials and methods

Natural graphite flakes (-325 mesh), HCPT, DMAP, EDC and NHS were purchased from Sigma Aldrich, $KMnO_4$, H_2SO_4, H_3PO_4, H_2O_2 and HCl were received from Chempur.

2.1. Synthesis of graphene oxide

Graphene oxide (GO) was synthesized using a modified Hummers method, according to Ref. [20]. Briefly, 1.0 g of graphite and 6.0 g of $KMnO_4$ were placed together in a round-bottom flask. Then a mixture of two concentrated acids: 120 ml of H_2SO_4 and 15 ml of H_3PO_4 was slowly poured. The reaction mixture was heated to 50 °C and stirred with magnetic stirrer for 12 h. The mixture was cooled down to room temperature and then 1 ml of H_2O_2 (30%) was slowly added. The mixture was purified with sequential washing and centrifugation with water, HCl aqueous solution (1:3) and ethanol to remove metal ions and to reach neutral pH. The obtained product was left for vacuum drying for 12 h at 60 °C.

2.2. Synthesis of HCPT-graphene oxide nanocomposites

We synthesized HCPT-graphene oxide nanocomposites using two routes. The first one involved the physical adsorption of hydroxycamptothecin on the surface of graphene oxide through π-π interactions. Briefly, 7.0 mg of graphene oxide was ultrasonicated in water (70 ml). Then it was mixed with 5.0 mg of HCPT dispersed in 2 ml of dimethyl sulfoxide (DMSO) and stirred for 24 h in the dark. The obtained product was thoroughly washed with dimethylformamide (DMF). The sample was named nc-GO-HCPT. The second route was based on the chemical bonding of modified HCPT to GO. Here, hydroxycamptothecin was treated with 4-dimethylaminopyridine (DMAP) and succinic anhydride according to the method described in Ref. [21]. The prepared d-HCPT was dispersed in DMF with N-hydroxysuccinimide (NHS) and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC) to activate carboxyl groups. GO was dispersed in DMF, then ethylenediamine (EDA) was added and the mixture was stirred for 1 h to produce the diamine-functionalized graphene oxide. To remove free substrates, the solution was centrifuged and GO was washed thoroughly with ethanol. After re-dispersion of GO in DMF, the prepared solutions of modified GO and HCPT were mixed together and stirred in the dark at room temperature for 12 h. DMF was used in order to purify the obtained nanocomposite. The sample was named c-GO-HCPT.

2.3. Characterization of the synthesized nanomaterials

High-resolution transmission electron microscopy (HRTEM) (FEI Tecnai F30, Frequency Electronics Inc.) was employed to examine the morphology of the samples. Atomic force microscopy (Nanoscope V Multimode 8, Bruker AXS, Mannheim, Germany) was used to examine the thickness of graphene oxide flakes and nanocomposite. Raman spectrum was acquired on the in Via Raman Microscope (Renishaw PLC, New Mills Wotton-under-Edge, Gloucestershire, UK) at an excitation wavelength of 785 nm. IR absorption spectra were measured on a Nicolet 6700 FTIR spectrometer (Thermo Nicolet Corp.). Additionally, the dynamic response of the medium with nanocarriers and the specific absorption rate ($SAR_{specific}$) value of the were calculated in the context of ablation process.

2.4. Stability of nc-GO-HCPT and c-GO-HCPT dispersions

The stability of the dispersions of the prepared materials (c-GO-HCPT and nc-GO-HCPT) in solution of phosphate buffer saline (PBS,