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Combination of chemotherapy and photodynamic therapy using graphene oxide as drug delivery system

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ABSTRACT

Previous research indicated that graphene oxide (GO) can be used to deliver photosensitive anticancer drug, Hypocrellin A (HA), in photodynamic therapy (PDT). However, the anticancer activity of HA was obviously decreased after been loaded on GO. To solve this problem, a chemotherapy drug, 7-ethyl-10-hydroxycamptothecin (SN-38), was co-loaded on the HA loaded GO (HA/SN-38/GO) as a multimodal carrier for the synergistic combination of PDT and chemotherapy for cancer. *In vitro* results showed that the combination therapy exhibited a synergistic antiproliferative effect compared with PDT and chemotherapy alone. Therefore, HA/SN-38/GO delivery system has the potential to offer dual therapies for the synergistic combination of PDT and chemotherapy for the treatment of cancer.

1. Introduction

GO with an extremely high specific surface area can interact with various biomolecules for applications in biosensor, drug delivery and gene transfection [1–3] because of well water solubility, easy preparation, good biocompatibility, long-term biodistribution and abundant hydrophilic groups, including hydroxyl, carboxyl and epoxy groups, on its surface or edge [4–7]. Utilizing their intrinsic high near-infrared (NIR) absorbance, GO have also been used as photothermal agents for *in vivo* cancer treatment with encouraging therapeutic outcomes [8]. In addition, GO-based nanocomposites that exhibit interesting optical and magnetic properties can be utilized as contrast agents for various biological imaging modalities including fluorescence imaging, photoacoustic imaging and magnetic resonance imaging [9,10]. Especially, GO can load aromatic drug with high efficiency via simple noncovalent method. The large π conjugated structure of GO can form π–π stacking interaction with the quinone portion of aromatic drug as well as the hydrophobic effect between them. Besides, the —OH and —COOH groups on GO sheet it can form a strong hydrogen-bonding interaction with groups may form hydrogen bonds in aromatic drug [11,12,1,13–15,10,16].

Our previous research indicated that GO can be used as a delivery system of HA for *in vitro* PDT application. PDT is an FDA approved modality for the local treatment of a variety of oncological, cardiovascular, dermatological, and ophthalmic diseases [17]. PDT utilizes reactive oxygen species (ROS), such as singlet oxygen ($^1O_2$), free radicals, or peroxides, to produced from light sensitive photosensitizer (PS) molecules, under suitable irradiation conditions, to induce cytotoxicity. Compared with chemotherapy or radiotherapy, PDT shows relatively minimal side effects and improved tumor specific killing [18–21]. HA has been isolated from the nature fungus sacs of *hypocrella bambusae*. HA has been proposed as potential second-generation PS for PDT because of its high $^1O_2$ quantum yields, low aggregation tendency, and rapid metabolism *in vivo*. Thus, they presented strong photodynamic activity against many tumor cell lines. Compared with Photofrin, HA is a single substance and not prone to aggregation. A more intriguing merit of HA lies in its much less delayed skin photosensitivity. [22,23] However, HA is hydrophobic, and therefore, preparation of pharmaceutical formulations for parenteral administration is highly hampered. Our previous research indicated that HA can be effectively loaded on the surface of GO and the result complex can be well dispersed in aqueous solutions. However, its anticancer

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activity was obviously decreased after been loaded on GO [14]. So, finding effective ways to solve this problem is urgent.

Recently, reports indicated that combination treatment of chemotherapy and PDT can effectively improve anticancer activity [24–32]. Chemotherapy is the treatment of cancer with cytotoxic anti-neoplastic drugs as part of a standardized regimen. Resistance is a major cause of treatment failure in chemotherapy drugs. Tumor cells utilize multiple mechanisms to reduce the accumulation of the these drug at its intracellular site of action. Overexpression of P-glycoprotein (P-gp), a drug efflux transporter, is an important determinant of tumor drug resistance. Recent studies indicate that PDT process may also be able to inhibit P-gp mediated drug efflux [33]. Thus, combination treatment of chemotherapy and PDT may overcome tumor drug resistance and increase anticancer activity [25,34]. A major issue for traditional combine therapy with chemotherapy and PDT is the decreased therapeutic efficacy and increased toxicity associated with the nonspecific accumulation of therapeutic agents in non-target tissues [26]. Furthermore, many of the commonly used chemotherapy drugs and PS are hydrophobic in nature. Therefore, preparation of pharmaceutical formulations for parenteral administration is highly hampered [1,35].

Therefore, for delivery of chemotherapy drugs and PS simultaneously, special formulations are required to make their aqueous dispersion, using nanostructure-based delivery systems [28,30,31]. Most of these systems can well disperse in aqueous environment and upon systemic administration, such delivery systems increased passive accumulation at the tumor site of its action by the "enhanced permeability and retention effect" [24,35,36].

So, we proposed that aromatic chemotherapy drugs and HA can be loaded on GO, simultaneously, and used in combination treatment of chemotherapy and PDT for cancer. Besides, in this combined system, each drug (HA and chemotherapy drug) can be loaded at its optimal treating dose, which can avoid the intolerable side effects.

To demonstrated this concept, here, chemotherapy drug SN-38 [37,38] and HA were co-loaded on the surface of GO (HA/SN-38/GO) by a simple noncovalent method (Fig. 1). It was hypothesized that combination of these drugs on GO may improve their dispersed ability in aqueous solution and produce synergistic effects and has higher efficiency than each agent alone. Researches indicated that SN-38 and HA can effectively load on GO and well dispersed in aqueous system for a long time. Besides, we investigated the efficacy of combination PDT and chemotherapy *in vitro*, and demonstrated that combining PDT with chemotherapy by invoking multiple anticancer mechanisms can greatly increase their anticancer activity comparing with any single treatment alone.

2. Material and methods

2.1. Chemicals

Graphite powder (99.9995%, 325 mesh) was purchased Alfa Aesar. 9,10-Anthracenedipropionic acid was purchased from Sigma. 3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyl tetrazoliumbromide (MTT) were purchased from Amosco. Dulbecco's minimum essential medium (DMEM) and fetal bovine serum (FBS) were purchased from Gibico. Hoechst 33342 was purchased from Beyotime.

2.2. Preparation of GO

Aqueous dispersion GO was prepared by the modified Hummer's method. Graphite flakes were oxidized using strong oxidants under vigorous stirring. In a typical experiment, 2 g of graphite and 1 g of $NaNO_3$ were put into a flask at 0 °C. Then, 50 mL concentrated H_2SO_4 was added to the mixture and the mixture was stirred for 30 min at 4 °C. Then, 7 g $KMnO_4$ was steply added to the reaction system and the reaction temperature was kept below 20 °C. Then, the temperature was lifted to 35 °C and stirred for 2 h. After that, 100 mL Millipore™ water was slowly added into above solution, and the reaction temperature was increased to 70 °C rapidly. Finally, 7 mL of H_2O_2 (30%) and 55 mL of Millipore™ water were added into above solution, resulting in the formation of bright yellow suspension. The sample was washed using 3% hydrochloric acid in Millipore™ water with further washing by ultracentrifugation, vortexing and sonication. The details of the synthesis process have been discussed in details elsewhere [39,40].

2.3. Drug loading

30 μL GO (10 mg/mL) was dispersed in 10 mL Millipore™ water and sonicated for 4 h to break the big GO sheet to nanosheet. Then, 10 μL HA (15 mM in dimethyl sulfoxide, DMSO), 30 μL SN-38 (5 mM in DMSO) was added in above system and slow stir for 24 h in dark (the ratio of HA and SN-38 were optimized according to our preliminary *in vitro* anticancer experiments). Then the sample was ultracentrifuged at 16,000 rpm for 1 h and washed by Millipore™ water for 3 times. The SN-38 and HA concentration in the upper layer was measured using their standard concentration curve. Free SN-38 or HA control was prepared by dissolving SN-38 (5 mM in DMSO) or HA (15 mM in DMSO) in Millipore™ water, directly. HA loaded GO (HA/GO) and SN-38 loaded GO (SN-38/GO) were prepared by above method by only adding one drug in GO suspension.

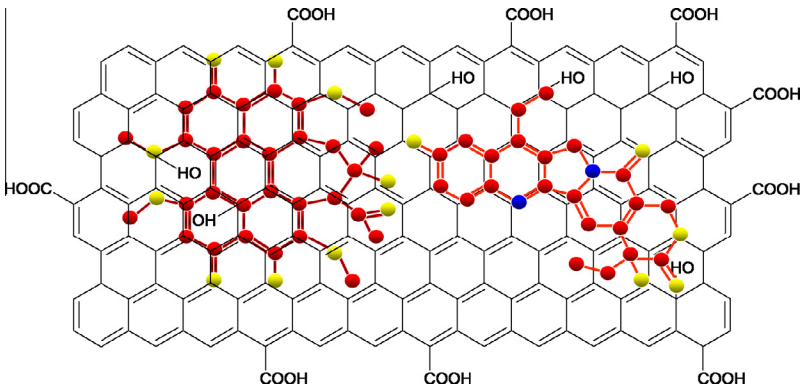

Fig. 1. Schematic representation of the SN-38 and HA co-loaded GO.