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A colon targeted drug delivery system based on alginate modificated graphene oxide for colorectal liver metastasis

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ABSTRACT

A major problem associated with colon cancer is liver metastasis. A colon-targeted drug delivery system is one way to address this problem after the resection of colorectal cancer. However, traditional drug delivery systems face many challenges, such as an inability to control the release rate, inaccurate targeting, susceptibility to the microenvironment and poor stability. Here, we report the development of a graphene oxide (GO)-based, sodium alginate (ALG) functionalized colon-targeting drug delivery system, that is loaded with 5-fluorouracil (5-FU) as the anti-cancer drug (denoted as GO-ALG/5-FU). Our results demonstrate that the as-prepared drug delivery system possesses a much lower toxicity and better colon-targeting controlled-release behaviours. We show that GO-ALG/5-FU significantly inhibited tumour growth and liver metastasis and prolonged the survival time of mice. We anticipate that our assay will help improve basic research of colon-targeted drug delivery systems and provide a new way to treat colon cancer liver metastasis.

1. Introduction

Colon cancer has become the third most commonly diagnosed cancer, and the incidence of colorectal cancer increases each year [1,2]. The primary treatment for colon cancer in clinical practice is surgical resection, but patients require portal vein chemotherapy to prevent colorectal liver metastasis [3–7]. Through chemotherapy, a high and constant drug concentration can be maintained in the portal vein plasma, with some drugs entering the peripheral blood at a relatively low concentration after the first pass in the liver, thereby reducing systemic toxicity. However, many patients have had problems with intubation failure [8,9], catheter displacement, shedding, thromboembolism, hematogenous infection, subcutaneous infection, leakage, skin necrosis or other side effects [10–13].

In recent decades, the creation and development of an oral colon-specific drug delivery system (OCDDS) have offered the possibility of implementing portal vein chemotherapy [14–16]. OCDDS provides an increased drug concentration in the colon, prolonged treatment time and reduced side effects. However, traditional colon targeted drug delivery systems face many challenges, such as the inability to control the release rate, inaccurate targeting, susceptibility to the microenvironment and poor stability, which have limited their application [17,18].

To overcome these disadvantages, we attempted to design a new alginate-graphene oxide-based colon-targeted drug delivery system. Polysaccharides exhibit good bio-adhesion, biocompatibility and swelling characteristics. The drugs that are loaded on polysaccharides are slow-release and not easy to inactivate [19,20]. Therefore, most bioadhesive colon-targeted drug delivery systems use polysaccharides as a drug carrier. Whereas alginate does not dissolve in acidic environments such as the stomach and small intestine, it does dissolve in the alkaline environment of the colon, where it adheres to the colon walls. Thus, alginate is often used as a colon-targeted drug carrier [21–24].

Graphene oxide has been widely studied for its exceptional properties, such as its high electron mobility, flexible structure and large theoretical specific surface area. Thus, it is considered an important component in the production of various functional composite materials. In particular, graphene oxide has attracted extensive attention in drug-loading fields due to its ability to load aromatic hydrophobic drugs via hydrophobic interaction and π-π stacking [25–27]. It was reported that graphene oxide can be used as cancer drug carrier due to its specific release of anti-cancer drugs [28]. Many studies on the use of graphene oxide as a drug carrier have been reported every year, including transferrin/poly(allylamine hydrochloride)-functionalized graphene oxide nanocarrier [29], biodegradable graphene oxide modified polyaptamer DNA hybrid hydrogels [30] and poly(*N*-isopropylacrylamide) covalently functionalized graphene sheets [31].

In the present study, we modified graphene oxide with sodium alginate as drug carrier. 5- fluorouracil was used as model drug for loading.

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The cytotoxicity, in vitro and in vivo drug release and a mice model were examined.

2. Experimental

The details for the materials, preparation and characterization of the GO-ALG/5-FU are described in the Supporting information.

2.1. Animal experiment

Experiments were carried out using Balb/c female mice (4–6 weeks old, 12–17 g) and Sprague-Dawley (SD) male mice (200–250 g) purchased from the animal centre of Southern Medical University (Guangdong, China). Mice were maintained in the experimental animal facilities at the Southern Medical University under controlled temperature and specific-pathogen-free conditions. Mice were fasted for 12 h before gavage, and water was freely provided. The Institutional Administration Panel for Laboratory Animal Care approved the experimental design. The university guidelines for care and use of laboratory animals were strictly followed. All animals were housed and fed in the Experimental Animal Center and were specific pathogen free.

2.2. Load of 5-fluorouracil

5-fluorouracil was used as an anticancer drug. Accurately weighed quantities of 5-FU and GO-OSA were put into a beaker with 20 mL distilled water, and then sonicated for 40 min. The products were centrifuged at 8000 r/min for 10 min. Samples were collected after freeze drying for 12 h. The 5-FU concentrations were measured using a UV spectrophotometer at 265 nm. The drug-loading efficiency (LE) and entrapment efficiency (EE) were calculated as follows:

$$\mathrm{LE}(\%) = \frac{\textit{weight of 5-FU loaded}}{\textit{weight of GO-ALG} + \textit{weight of 5-FU loaded}} \times 100$$

$$\mathrm{EE}(\%) = \frac{\textit{weight of 5-FU loaded}}{\textit{initial weight of 5-FU}} \times 100$$

The test was carried out in triplicate and the average values were reported.

2.3. In vitro drug release

In vitro drug release was carried out at pH 1.0, pH 6.8 and pH 7.4, corresponding to simulated gastric, intestinal and colonic environments, respectively. Release time in simulated gastric, intestinal and colonic environments was adjusted to 6 h, 3 h and 48 h, respectively, similar to that in the body [32]. A known quantity of GO-ALG-5-FU was placed into a dialysis membrane, immersed in corresponding buffer solutions (400 mL) and incubated at 37 °C (body temperature). The release study was carried out using a water bath shaking incubator (SHA-BA, Changzhou Aohua Instrument Co., Ltd., China) at 90 rpm. At given time intervals, 4 mL of the solution was taken and replaced with same amount of fresh buffer solution to maintain the specific volume of release medium. The released medium was diluted with distilled water and analysed at 265 nm using an UV spectrophotometer. The percentage of 5-FU released was determined as follows:

$$\text{Cumulative 5-FU release}(\%) = \frac{\textit{Cumulative amount of 5-FU released at time } t}{\textit{initial amount of 5-FU}} \times 100$$

The test was carried out in triplicate and the average values were reported.

2.4. In vitro toxicity

Cytotoxicity of samples was tested using the MTT assay, which is based on the cellular uptake of MTT and its subsequent reduction in the mitochondria of living cells to dark blue MTT formazan crystals. NIH-3T3 cells or HT-29 cells were seeded on 96-well plates ($1.5–2 \times 10^4$ cells/well) in corresponding media. Then, the NIH-3T3 cells or HT-29 cells were treated with the drug samples for 24 h. After that, MTT (5 mg/mL in PBS) was added to each well and incubated for an additional 4 h (37 °C, 5% CO_2). The cells were then lysed in dimethyl sulfoxide (150 μL/well), and the plates were allowed to stay in the incubator (37 °C, 5% CO_2) to dissolve the purple formazan crystals. The colour intensity reflecting cell viability was read at 490 nm using a Model-550 enzyme-linked immunosorbent microplate (Bio-Rad, USA), and the morphologic changes of NIH-3T3 cells or HT-29 cells were photographed using a IX-70 inverted phase contrast microscope (Olympus, Japan). All experiments were repeated four times, and the Statistical Product and Service Solutions software was used to assess statistical significance of the differences among treatment groups.

2.5. In vivo drug release

Mice were randomly separated into two groups and administrated with 5-FU (3 mg/kg^{-1}) and GO-ALG/5-FU (in which 5-FU concentration was 3 mg/kg^{-1}) respectively. Blood sampling was proceeded at the time 1, 2, 3, 4, 5, 7, 9, 12, 15, 24, 30, 36, 48 h after administration. Drug concentrations were analysed by high performance liquid chromatography (HPLC). The HPLC analyses were performed using Prominence LC-20AT (JAPAN). Chromatographic separation was achieved using a C18, 250 × 4.6 mm, 5 μm particle size, commercial column. Other experimental conditions were as follows: wavelength of measurement at 307 nm with a bandwidth of 8 mm; flow rate of 1 mL/min at 24 °C; acetonitrile: 0.5% in aqueous acetic acid = 2:98 (V/V) as the mobile phase.

2.6. In vivo toxicity

First, GO-ALG/5-FU (3 $mg \cdot kg^{-1}$) was implanted into the subcutaneous tissue of mice (back of neck). Physiological saline was used as control reagent. After 21 days, all animals were sacrificed, and the liver, heart, lung, spleen and kidney were separated, washed twice with PBS and fixed in 4% formaldehyde for histological examinations.

2.7. Intestinal adhesions

Acridine orange (AO) was purchased from Sinopharm Chemical Reagent Co. and was used as received. 10 mg acridine orange was diluted to 10 mL with distilled water, and then 10 mg GO-ALG/5-FU was added. The dispersion was sonicated for 40 min to obtain GO-ALG/5-FU/AO dispersion; this dispersion was filtered through a polycarbonate membrane (0.22 μm pore size), repeatedly washed using ultrapure water (5 × 100 mL), and dried at 60 °C for 48 h. Nude mice were treated with GO-ALG/5-FU/AO at a dose of 3 $mg \cdot kg^{-1}$, and the blank control group was treated with 0.2 mL saline. After 2, 5 and 10 h, mice were executed and the inner wall of the stomach, small intestine and colon were collected and washed twice with PBS. Fluorescence intensity was observed under a fluorescence microscope.

2.8. In vivo tumour model therapy

The Institutional Administration Panel for Laboratory Animal Care approved the experimental design. The university guidelines for care and use of laboratory animals were strictly followed. All animals were housed and fed in the Experimental Animal Center and were specific-pathogen-free.

HT-29 cells were used for colon tumour model. 0.2 mL HT-29 cells (1×10^7 cells/mL) were injected into the splenic vein of mice.