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Octreotide-modified and pH-triggering polymeric micelles loaded with doxorubicin for tumor targeting delivery

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ABSTRACT

A multifunctional mixed micelle was assembled for drug targeting delivery by combining two newly synthesized amphiphilic polymers, which were octreotide-polyethylene glycol-monostearate (OPMS) and N-octyl-N-succinyl-O-carboxymethyl chitosan (OSCC), respectively. The mixed micelle was designed to be characterized with long circulation, somatostatin receptors (SSTR)-mediated endocytosis and pH sensitivity. A series of assembling proportions of OPMS and OSCC was tested to reveal the effect of compositions on the functions. The particle size, zeta potential, drug loading and critical micelle concentration were examined. The dialysis test indicated a pH-triggering release behavior of the doxorubicin-loaded mixed micelle (DLMM), and faster release in acidic media (pH 4.0–6.0) in response to the protonation of carboxyl group. In addition, the PEG segments could efficiently protect the mixed micelle from plasma protein adsorption *in vitro*, and the DLMM composed of 20% OPMS and 80% OSCC provided the longest residence time after intravenous injection in rats *in vivo*. Due to SSTR mediated endocytosis, the significantly higher uptake of DLMM was observed in the tumor cells (SMMC-7721), compared with that in the normal cells (CHO) without SSTR expression. All the results suggested that the mixed micelle with multifunctional characteristics could be used as an effective approach for tumor treatment.

1. Introduction

Cancer has been one of the most dreaded diseases and a major threat to human life. Chemotherapy remains the primary modality for cancer treatment, but not always successful. One of the most important factors dictating the success of chemotherapy is the dose control such that the beneficial anticancer activity is balanced with the toxicity in normal organs and tissues (Campbell, 2006; Oh et al., 2009), because many anticancer drugs are simply designed to destroy indiscriminately tumor cells or normal cells. In this respect, it is important to maximize the efficacy of the drugs to tumor and minimize the toxicity to normal tissue, which is undeniably relevant to the development of cancer-selective drug delivery systems.

It is very important that the drug carriers can be selectively controlled for the passive distribution in tumor to achieve effective and safe cancer chemotherapy (Bae et al., 2007; Kano et al., 2007; Minchinton and Tannock, 2006). However, the conventional drug carriers are plagued by opsonin, so their circulation time in systemic bloodstream is seriously shortened. The capture of macrophages of the reticuloendothelial system (RES) also results in the rapid elimination of drug carriers (Jung et al., 2010). In addition, conventional drug carriers are structurally unstable due to the adsorption of plasma proteins during their circulation (Patel and Moghimi, 1998). To enhance the stability of drug carriers by inhibiting the adsorption, attachment of polyethylene glycol (PEG) to the surface of the carriers has been developed extensively (Yin et al., 2008), so that the drug carriers can target the leaky vessels and poorly developed lymphatic drainages in tumor, and then accumulate in tumor tissue by means of enhanced permeability and retention (EPR) effect (Hong et al., 2010). Nevertheless, recent studies have revealed that tumor-targeting drug delivery can not be completely achieved only by the EPR effect because drug carriers often encounter difficulties in accessing cancer cells in the deeper place of the tumor tissue or encounter difficulties in interacting with the targeted cells (Kamb, 2005; Minchinton and Tannock, 2006). It is well known that solid tumors are often characterized by specific receptors or antigens on cell surfaces (Daniels et al., 2005; Parker et al., 2005). Receptors or antigens provide special uptake routes for nutrients and signals from the surrounding environment, which are essential for active growth of tumor cells (Parker et al., 2005).

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From these aspects, active targeting can be achieved by installing ligands to the polymers or polymer assemblies, so that they can interact with the receptors overexpressed on tumor cell membranes (Alexis et al., 2008; Lavasanifar et al., 2002). The active targeting can also be achieved by using stimuli-sensitive polymers, especially pH sensitive polymers (Qiu and Park, 2001), because the solid tumors manifest themselves by a decrease in pH. The intracellular environment sensitive carriers, which can release the loaded drug through sensing pH decreases in the acidic endocytic compartments such as endosomes (pH 5.0–6.0) and lysosomes (pH 4.0–5.0), have been reported (Bae et al., 2005a,b). It is obvious that targeting drug delivery would be achieved in tumor tissue and cells if the carriers can be assembled with different functions together, such as pegylation, pH-sensitivity and receptors mediation.

In this paper, we designed and assembled such a multifunctional mixed micelle as the targeting carrier, which was composed of two newly developed amphiphilic polymers, octreotide-polyethylene glycol-monostearate (OPMS) and N-octyl-N-succinyl-O-carboxymethyl chitosan (OSCC). Doxorubicin (DOX) was used as model anticancer drug and was loaded in the nanocarrier (Fig. 1). The mixed micelle had a hydrophilic PEG outer shell and octreotide was used as the targeting ligand. Octreotide is a synthetic somatostatin analog with low molecular weight (MW 1019.28) and has high affinity for somatostatin receptors (SSTRs), which are overexpressed on the surface of cells membrane of some tumors (Su et al., 2008; Surujpaul et al., 2008). Furthermore, the mixed micelle can rapidly collapse and release more drug when pH changes occur in tumor cell microenvironment from endocytosis to endosomes and finally to lysosomes, in which the proton concentration is 100-times higher (pH 4.0–6.0) than that in normal physiological condition (pH 7.4). This mixed micelle involves not only a stealth carrier to protect passively from the recognition of normal physiological system, but also has the active targeting characteristics by means of the SSTR-mediated endocytosis and pH triggering release in tumor cells.

In this study, novel polymers of OSCC and OPMS were synthesized and characterized. DOX-loaded mixed micelle (DLMM) was prepared and the properties of DLMM, such as encapsulation efficiency, loading content, particle size, zeta potential, morphology and *in vitro* release behaviors were investigated. The pharmacokinetics of DLMM were investigated in rats after intravenous injection and compared with free DOX and DOX-loaded OSCC micelle (DLOM). Finally, the cellular uptake *in vitro* was examined by using tumor cells and normal cells.

2. Materials and methods

2.1. Materials

Chitosan was provided by the Nantong Shuanglin Biochemical Ltd., China, with deacetylation degrees of 90.5% and viscosity average molecular weight of 50,000 D. Doxorubicin was purchased from RPG Life Sciences Company (India). Pyrene (>99%) and polyethylene glycol (100) monostearate (PGMS) were purchased from Sigma (Milwaukee, WI, USA). Other reagents were of analytical grade and used without further purification. Double distilled water was used in this study.

2.2. Animals

Sprague–Dawley (SD) rats were obtained from the Shanghai Silaike Laboratory Animal Limited Liability Company. All the animals were pathogen free and allowed to access food and water freely before the tests. All the animal experiments were in accordance with the guide for the care and use of laboratory animals regulated by China Pharmaceutical University.

2.3. Synthesis of N-octyl-N-succinyl-O-carboxymethyl chitosan (OSCC)

Firstly, O-carboxymethyl chitosan (CMCS) was prepared. In brief, chitosan (10.0 g, 0.06 mol) was alkalized in 100 mL of 60% NaOH ethanol/water (1/1, v/v) solution at −18 °C for 8 h, followed by reacting with chloroacetic acid (25 g, 0.26 mol) in ethanol solution (300 mL) at 45 °C for 4 h. The precipitate in the reaction mixture was filtered and washed with a solution of ethanol/water (3/1, v/v). The white powder of CMCS was obtained after oven drying.

N-octyl-O-carboxymethyl chitosan (OCCS) was prepared by introducing the octyl group to NH_2 on C_2 of glucosamine unit in CMCS. One milli liter octaldehyde was added in the suspension of CMCS (6 g) in 50 mL of methanol, while stirring at 30 °C. After 6 h of reaction, 5 g of KBH_4 was added to the reaction mixture. The resulting mixture was stirred at 30 °C for 24 h and neutralized using 1 M NaOH solution. After precipitation with methanol, OCCS

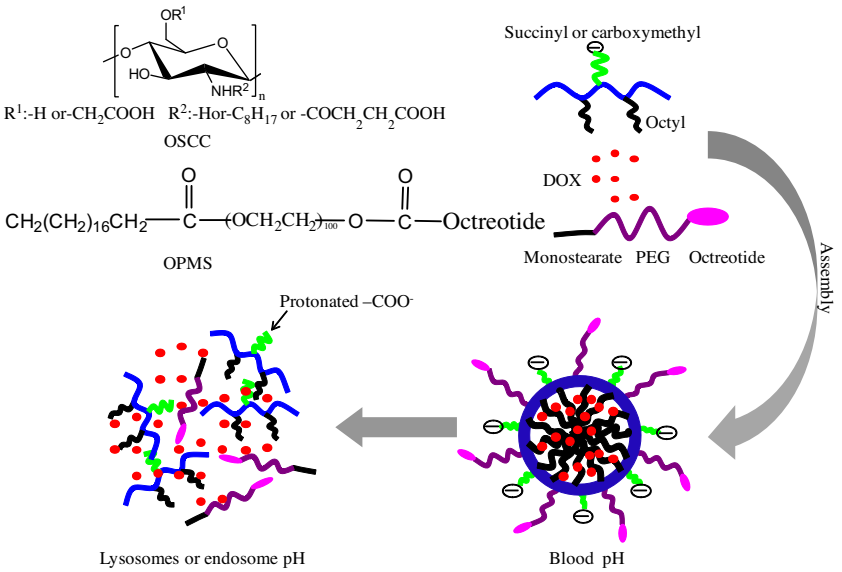

Fig. 1. Schematic diagram of the multifunctional mixed micelles.