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A 5-fluorouracil-loaded pH-responsive dendrimer nanocarrier for tumor targeting

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ABSTRACT

A novel long-circulating and pH-responsive dendrimer nanocarrier was prepared for delivering 5-fluorouracil (5-FU) to tumors through the targeting of nanoparticles to the low pH environment of tumors. The nanocarrier, poly(2-(N,N-diethylamino)ethyl methacrylate) with methoxy-poly(ethylene glycol)-poly(amidoamine) (PPD), had a core–shell structure with 4.0 G poly(amidoamine) (PAMAM) as the core and parallel poly(2-(N,N-diethylamino)ethyl methacrylate) (PDEA) chains and methoxy-poly(ethylene glycol) (mPEG) chains as the shell. The PDEA chain was pH-responsive, and the PEG chains led to long circulation in blood vessels to achieve tumor targeting. The sizes, drug encapsulation and release of PPD nanocarriers showed high pH-dependency due to the PDEA chains, as they were hydrophilic at pH 6.5 and hydrophobic at pH 7.4. The encapsulation efficiency of 5-FU in PPD nanocarriers was as high as 92.5% through the pH transition. The release of 5-FU from PPD nanocarriers was much faster at pH 6.5 than at pH 7.4. The 5-FU-loaded nanocarrier had a long half-life after intravenous administration in mice and showed high tumor targeting. This nanocarrier composite also showed enhanced anticancer effects. PPD is a promising nanocarrier of anticancer drugs with high encapsulation, tumor targeting and pH-responsive release in tumors.

1. Introduction

Dendrimers are synthetic dendritic polymers with versatile, derivatizable, well-defined, compartmentalized chemical structures (Tomalia et al., 1985). Dendrimers function as nanomedicines against tumors, bacteria and viruses. They are also used as drug carriers to achieve organ targeting and/or controlled release *in vivo* based on nanoscale functionalized surface groups (Boas and Heegaard, 2004; Gillies and Fréchet, 2005; Svenson and Tomalia, 2005). Poly(amidoamine) (PAMAM) is one typical type of dendrimer with wide biomedical applications due to its low toxicity and highly functional surface groups (Esfand and Tomalia, 2001).

One major problem in cancer chemotherapy is the severe side effects of anticancer drugs due to their random distribution in the body. However, nanoscale particles readily penetrate tumor tissues based on their enhanced permeability and retention (EPR) effects (Amiji, 2007). Tumor-targeted drug delivery can be achieved using nanocarriers based on this EPR effect (Iyer et al., 2006). As such, a variety of nanoparticulate systems are used as carriers for anticancer agents (e.g., liposomes, nanoparticles, and polymeric micelles). However, naked nanoparticles would likely be taken up by the mononuclear phagocyte system due to opsonization after intravenous (i.v.) administration (Ishida et al., 2002).

Long-circulating particles that can escape phagocytosis have the opportunity to target tumors based on the EPR effect. Long poly(ethylene glycol) (PEG) chains can form a shell on the surface of particles to prevent opsonization (Moghimi et al., 2001). However, little drug loading and weak tumor targeting of nanocarriers hinder their current development (Cho et al., 2008).

PAMAM has a very small diameter (6.5 nm diameter for 6.0 G PAMAM) and is seemingly suitable for anticancer drug delivery. However, the inner space of PAMAM is open, so entrapped drugs tend to diffuse into the surroundings if there is no strong interaction between the drugs and the interior of the dendrimers. For example, methotrexate and doxorubicin were readily released from drug-loaded poly(ethylene glycol)$_{2000}$-attached 4.0 G PAMAM dendrimers in isotonic solutions (Kojima et al., 2000). The drug loaded in dendrimer carriers is then ready for release into the circulation after i.v. administration, but not is selectively released into tumor tissues. Furthermore, covalently coupled methotrexate in

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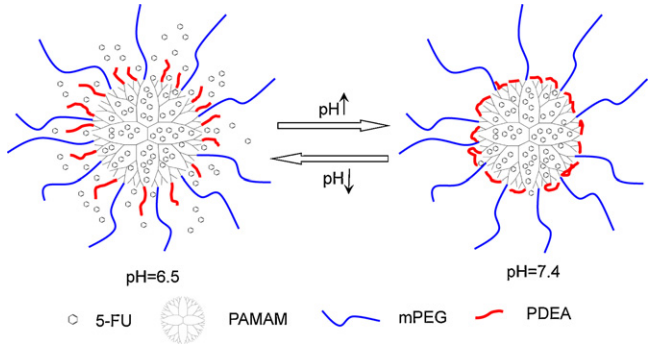

Fig. 1. Illustration of pH effect on the structure of PPD nanocarriers and their encapsulation and release of drugs. In the weakly acidic environments (pH 4.0 or 6.5) of buffered solutions and tumor tissues, the PDEA chains of PPD were hydrophilic and extended, so the drugs were free to enter or exit the nanostructure. In the neutral or weakly basic environments (pH 7.4 or 8.0) and in circulation, the PDEA chains of PPD were hydrophobic and contracted, so the drugs were tightly enclosed in the inner space.

dendrimer conjugates was stable, and the release of drug was slow (Patri et al., 2005). Stimulus-responsive dendrimers may address the above problem (Kojima, 2010). These stimulus factors include pH (Hui et al., 2005), temperature (Kono et al., 2007), and photosensitivity (Nishiyama et al., 2009).

Poly(2-(N,N-diethylamino)ethyl methacrylate) (PDEA) is a pH-sensitive polymer containing tertiary amine groups. PDEA is hydrophobic in neutral or basic medium, but hydrophilic in acidic medium. Taking advantage of this property, some pH-responsive copolymers or nanoparticles were prepared through PDEA conjugation (Guo et al., 2009; Oishi and Nagasaki, 2010). Most tumor tissues have a lower pH environment (less than 7.0), so pH-responsive drug release is useful for anticancer treatment (Lee et al., 2008).

PDEA can be chemically attached to other polymers or nanoparticles to form pH-responsive copolymers or nanoparticles. The PDEA-attached carriers are then used to load anticancer agents or genes for cancer treatment for selective release in tumor tissues (Guo et al., 2009; Oishi and Nagasaki, 2010; Xu et al., 2006; Zhang et al., 2009).

A novel PAMAM derivative, poly(2-(N,N-diethylamino)ethyl methacrylate) with methoxy-poly(ethylene glycol)-poly(amido amine) (PPD) was synthesized in this study as a nanocarrier for 5-fluorouracil (5-FU), an anticancer agent, with high encapsulation efficiency, tumor targeting and rapid release in tumor tissues. In the acidic environment of a tumor, the hydrophilic PDEA chains facilitate rapid entry and release of drug molecules. In the neutral/basic medium of the blood, the hydrophobic PDEA chains trap drugs tightly within the PPD core (Fig. 1).

2. Materials and methods

2.1. Materials

5-FU was obtained from Shandong Boyuan Chemical Co. Ltd. (China). Methoxy-poly(ethylene glycol)$_{750}$ (mPEG$_{750}$) and 2-(N,N-diethylamino)ethyl methacrylate (DEA) were from Acros. Organic solvents were of analytical grade, and other chemicals were of reagent grade. A murine hepatoma H_{22} cancer cell line was a gift from Prof. Shoujun Yuan (Beijing Institute of Radiation Medicine, BIRM). Purified water was prepared with a Heal Force Super NW Water System (Shanghai Canrex Analytic Instrument Co. Ltd., China) and was always used unless otherwise indicated. Infrared (IR) spectrum and ^{1}H nuclear magnetic resonance (NMR) (400 MHz) spectrum were recorded on a Bio-Rad FTS-65A infrared ray spectrometer and a JNM-ECA-400 NMR spectrometer, respectively.

2.2. Animals

Female Kunming mice from the Laboratory Animal Center of BIRM were used. All animal handling and surgical procedures were strictly conducted according to the Guiding Principles for the Use of Laboratory Animals. This study was approved by the Animal Care Committee of the Beijing Institute of Radiation Medicine. Mice were sacrificed to obtain tissues. Mice tissue homogenates used in the tissue distribution experiment were prepared in tissue/water (1:1, w/w). All studies were conducted in accordance with the Declaration of Helsinki.

2.3. Synthesis of PPD

The synthesis of PPD consisted of three steps. First, 4.0 G PAMAM dendrimers were synthesized using a stepwise, divergent strategy reported in the literature (Boas et al., 2006; Tomalia et al., 1985). The second step involved the combination of mPEG and PAMAM to obtain mPEG–PAMAM. Finally, mPEG–PAMAM was coupled to PDEA using atom transfer radical polymerization (ATRP) to obtain mPEG–PAMAM–PDEA (i.e., PPD). The detailed synthetic process is described as follows, and the route is shown in Fig. 2.

The 4.0 G PAMAM was synthesized with ethylene diamine as the initial core using Michael alkylation with methyl acrylate to yield a tertiary amine as the branching point. This was followed by aminolysis of the methyl ester (Boas et al., 2006; Newkome et al., 2001; Tomalia et al., 1985, 1986). There were 64 terminal amine groups found on each 4.0 G PAMAM molecule. The structure of 4.0 G PAMAM was consistent with other reports (Tomalia et al., 1985). IR (KBr) ν_{max}: 3429.4, 3078.1, 2930.2, 2850.8, 1636.5, 1549.7, 1125.7, 1197.0, and 581.0 cm^{-1}. ^{1}H NMR (DMSO-d_6) δ_H: 7.62 (CONH), 3.23, 2.70, 2.29, 2.27, 2.24, 2.13, (the above δ_H attributed to the ethylene hydrogen), 1.82 (terminal NH_2) ppm.

The above PAMAM (2.8 g, 0.2 mmol) was dissolved in formic acid and then mixed with mPEG750 (0.0225 g, 0.03 mmol). Excess formaldehyde was added to the above solution and the reaction was stirred for 24 h. The solution was then filtered, and the filtrate was placed in a dialysis bag (cut MW, 7000 Da, Union Carbide Co., USA) against water for 48 h, refreshing the water every 4 h. The solution in the bag was distilled under vacuum to remove most of the water, and the resulting product was then freeze-dried to obtain a gel of mPEG–PAMAM. IR (KBr) ν_{max}: 3433.6, 2920.7, 2148.7, 1634.1, 1558.7, 1463.7, 1350.7, 1293.4, 1249.9, 1101.3, 949.0, and 586.4 cm^{-1}.

The above mPEG–PAMAM (2 g, 0.1 mmol) was dissolved in 0.1 M NaOH with stirring for 12 h to obtain a slightly white, viscous solution. Excess chloroacetyl chloride (3 ml or more) was slowly added dropwise into the solution followed by agitation at room temperature for 24 h. The solution was dialyzed against water for 72 h as above, distilled under vacuum to remove water, and then freeze-dried to obtain a gel of mPEG–PAMAM–Cl that was stored under vacuum.

The mPEG–PAMAM–Cl gel (2.2 g, 0.1 mmol) was dissolved in methanol/water (1:1, v/v, 20 ml) under nitrogen. DEA (1.9 g, 1 mmol), cuprous bromide (CuBr, 0.144 g, 0.1 mmol), and bipyridine (bpy, 0.313 g, 0.2 mmol) were then added, and the resulting mixture was stirred at 50 °C for 12 h. A blue suspension was obtained and was subjected to dialysis against water for 72 h as above. The suspension was then distilled under vacuum to produce a light blue, viscous solid that was purified on an Al_2O_3 chromatographic column using methanol as the elution solvent. The recovered solution was distilled to obtain PPD as a white powder. IR (KBr) ν_{max}: