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Dual-responsive polymer–drug nanoparticles for drug delivery

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ABSTRACT

A well-defined, dual temperature- and pH-responsive drug carrier was synthesized through the radical copolymerization of methacrylic acid, *N*-isopropylacrylamide, and an *N*-(methacryloyl)glycylglycine 4-nitrophenyl ester. When the anticancer agent gemcitabine or antibiotic sulfamethoxazole was conjugated with a polymer and heated beyond its low critical solution temperature (40 °C), a dual temperature- and pH-induced phase transition was observed. This temperature was considered ideal for activating drug aggregation under hyperthermic and acidic conditions. The structure and properties of polymer drugs were investigated using nuclear magnetic resonance, Fourier transform infrared spectrometry, ultraviolet–visible absorption, transmission electron microscopy, and gel permeation chromatography. At a critical micelle concentration of 1 mg/mL, both polymer drugs formed micellar structures with diameters ranging from 50 nm to 150 nm, based on TEM image. These micelles exhibited higher pharmacological efficacy than the free drug alone did, and the cytotoxicity at the target site was substantially enhanced compared with that of the polymer–drug conjugate formed under normal physiological conditions.

1. Introduction

Stimuli-responsive polymers have recently attracted a considerable amount of attention with regard to their biomedical applications such as drug delivery [1–5], cell culture surfaces [6], and targeted therapy [7–10]. Polymers that respond to various stimuli, such as pH, temperature, light, oxidation–reduction, magnetic fields, and enzymes, have been developed, and the applications for anticancer drug delivery and diagnoses are among the most studied topics. In the stimuli-responsive delivery system, the anticancer agent can be released by an appropriate stimulus that facilitates accumulation to the desired pathological area, thereby maximizing the drug efficacy, and enables the modulation of the pharmacokinetics of drug release. When the drug is localized at specific sites, the stimuli-responsive polymers reduce adverse side effects and improve antitumor activity. Several types of polymers have been evaluated in clinical trials or clinically used as nanomedicines.

To improve the targeting specificity and therapeutic efficacy of polymer drugs, considerable research effort has been invested in modifying the structures of carriers to enable them to respond to multiple stimuli, such as temperature, pH, oxidation–reduction, photons, electrons, enzymes, and magnetic fields [11–14]. For example, temperature and pH dual-responsive polymers have been designed to improve drug release caused by the deformation and precipitation of core/shell nanoparticles in acidic tumor microenvironments [15,16]. Chen [17] recently reported that a dual-responsive block copolymer containing poly(ε-caprolactone) and amino-acid-functionalized poly(triethylene glycol) exhibited greater antitumor activity and higher release efficacy in acidic environments than did free doxorubicin in xenograft tumor models. Other researchers have constructed temperature and pH dual-responsive nanoparticles by copolymerizing *N*-isopropylacrylamide with acrylic acid, methacrylic acid (MAA), and *N*,*N*-dimethylarylamide [18–21], and have indicated that the loading capacity and release kinetics of drugs can be modulated by varying the structural and physicochemical properties of the constituent block copolymers. According to a review of these studies, most of the anticancer drugs have been physically incorporated into micelles or hydrogels, a few drugs have been tailored to bond covalently to polymer carriers, and no report on the chemical conjugation of gemcitabine (GEM) in dual-responsive nanoparticles system has been published.

GEM exhibits substantial anticancer activity and is used as the first-line therapy for various solid tumors, including ovarian, breast, nonsmall-cell lung, and pancreatic carcinomas, as well as transitional cell carcinoma of the urothelium [22–24]. However,

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the efficacy of this drug is limited because of its short plasma half-life and low water solubility and, therefore, high drug doses are administered for therapeutic activity, which causes substantial adverse effects during chemotherapy [25]. Conjugating GEM directly onto a multifunctional polymeric carrier is expected to provide an increased number of therapeutic advantages, such as plasma stability, reduced toxicity, and a prolonged blood circulation time. In addition, by using a local stimulus characteristic of the pathological site, additional selectivity and drug accumulation can be achieved if these functional moieties are incorporated into the drug carrier. Several methods for reducing the negative effects of free GEM by conjugating it onto liposomes [26–29] or water-soluble polymers [30–36] have been investigated. When these methods are applied, liposome carriers generally incur a low drug payload, and considerable renal loss of GEM occurs because of its inherent physical entrapment property. Polymer-based platforms, carrying either no stimulus or only one stimulus, are superior to free GEM in both plasma stability and antitumor efficacy. In consideration of the increasing need for targeted therapy involving nanomedicine, a novel polymer–drug conjugate exhibiting multifunctional activity must be developed.

In this paper, we report the first sulfamethoxazole (SMX) and GEM-conjugated polymer drug featuring both pH and temperature sensors for drug delivery systems. The responsiveness and self-assembling behavior of these polymers were investigated, and cytotoxicity studies against *Escherichia coli* were conducted in vitro to assess the response of these polymers to both pH and temperature.

2. Experimental

2.1. Materials

N-isopropylacrylamide (NIPAm; Tokyo Chemical Industry Co., Ltd., Japan) was recrystallized from n-hexane. MAA (Showa, Tokyo, Japan) was purified through distillation and stored in N_2 prior to use. An *N*-methacryloylglycylglycine 4-nitrophenyl ester (MA-GG-ONp) was synthesized according to a protocol described in the literature through the reaction of methacryloyl chloride with glycylglycine followed by esterification with *p*-nitrophenol in the presence of *N*,*N*′-dicyclohexylcarbodiimide (DCC) [37]. Glycylglycine was purchased from Alfa Aesar (New Jersey, USA). *p*-Nitrophenol, DCC, and SMX were purchased from Fluka. GEM·HCl (Gemzar) was purchased from Eli Lilly and Company (USA). Luria–Bertani (LB) broth was obtained from Lab M Limited. *E. coli* (DH5α) was purchased from GeneMark (Taichung, Taiwan).

2.2. Instrumentation and measurements

^{1}H spectra were recorded using a 300-MHz Varian–Mercury$^+$300 spectrometer by employing a deuterated solvent. Fourier transform infrared spectra were measured using a Shimadzu 8400 spectrophotometer. UV–VIS absorbance spectra were recorded with Agilent 8453 spectrophotometer using quartz cuvettes of 10 mm path length. Sample concentrations were adjusted between 0.1 and 0.5 mg/mL so that spectra could be recorded with an appropriate optical density. Gel permeation chromatography (GPC) was performed using a dimethylformamide (DMF) eluent containing 0.01 mol L^{-1} lithium bromide at 80 °C and a flow rate of 0.8 mL/min^{-1}. Prior to GPC analysis, the polymers were modified by methylating the carboxylic acid groups by using trimethylsilyldiazomethane in DMF [38,39]. Transmission electron microscopy (TEM) measurements were conducted using a JEOL JEM-2100 electron microscope operated at an acceleration voltage of 100 kV. The polymer solutions (2 mg/mL) were dropped onto carbon-coated copper grids and then allowed to dry in air at room temperature before observation. Dynamic light scattering (Zetasizer Nano ZS90, Malvern, England) was used to confirm the particle size distribution of polymer drugs at 4 mW He—Ne laser. Fluorescence measurements were performed using a HITACHI F-4500 FL spectrophotometer. The pH- and temperature-sensitive behaviors of the polymers were characterized by using the JASCO V-630 spectrophotometer to perform cloud point measurements at 1 mg/mL. The pH of the test solutions was controlled by adding tris(hydroxymethyl)aminomethane or phosphoric acid.

2.3. Synthesis

2.3.1. Synthesis of Poly(MAA-co-NIPAm -co-MA-GG-ONp) (Terpolymer)

A solution of MAA (0.08 g, 0.93 mmol) in anhydrous acetone (5 mL) was added to a mixture of 0.2798 g of NIPAm (2.47 mmol), 0.2556 g of MA-GG-ONp (0.796 mmol), and 0.04 g of AIBN (0.243 mmol) in nitrogen through a cannula. The solution was heated in nitrogen at 75 °C under ultraviolet (UV) irradiation (mercury lamp, 100 W) for 24 h. After polymerization, the polymer was filtered and purified by dissolving it in a minimal amount of methanol and reprecipitated in a 10-fold excess of an Et_2O solution. The polymer was collected using filtration, washed with Et_2O, and dried in a vacuum overnight (0.486 g, yield: 79%). ^{1}H nuclear magnetic resonance (NMR) (dimethylsulfoxide (DMSO)-d_6) δ: 0.53–1.33 (CH_3— of polymer backbone and —CH(CH_3)$_2$ of NIPAm), 1.57–2.28 (methylene and methine protons of polymer backbone), 3.79 (—NHC*H*(CH_3)$_2$ of NIPAm and —NHCH_2CONH— of MA-GG-ONp), 4.19 (—NHCH_2COOAr of MA-GG-ONp), 7.41 (d, 2H, ONp-*H*, *J* = 6.9 Hz), 8.29 (d, 2H, ONp-*H*, *J* = 6.9 Hz), 8.57 (—N*H* of MA-GG-ONp). ^{13}C NMR (DMSO-d_6) δ: 15.85–19.13, 22.99, 43.68–446.18, 52.12, 116.22, 126.72, 139.51, 164.29, 171.16, 176.44, 178.92. IR (KBr) ν (cm^{-1}): 1168 (C—O), 1206 (C—O), 1349 (N═O), 1543 (N—H), 1642 (C═O), 1715 (C═O), 2993, 2936 (C—H), 3326 (N—H), 3472 (O—H).

2.3.2. Synthesis of Poly(MAA-co-NIPAm-co-MA-GG-GEM)(Poly-GEM)

The free gemcitabine (0.034 g, 0.112 mmol) and terpolymer (0.4 g) were dissolved in 3.0 mL of anhydrous DMF and heated to 90 °C in nitrogen for 24 h. The solvent was removed in a high vacuum, dissolved in a minimal amount of methanol, and reprecipitated in a 10-fold excess of an Et_2O solution. The polymer was washed with Et_2O and dried in a vacuum overnight (0.381 g, yield: 94.5%). ^{1}H NMR (DMSO-d_6) δ: 0.53–1.33 (CH_3— of polymer backbone and —CH(CH_3)$_2$ of NIPAm group), 1.57–2.28 (methylene and methine protons of polymer backbone), 3.61–3.97 (—CH_2OH of GEM, —NHC*H*(CH_3)$_2$ of NIPAm, —OC*H*CH$_2$OH of GEM and —NHCH_2CO— of MA-GG-GEM), 4.11–4.39 (—CF_2C*H*OH and —CH$_2$OHC*H*— of GEM), 5.19 (—CO*NH*-GEM of MA-GG-GEM), 5.75 (d, CC*H*═CHN of GEM, *J* = 9.0 Hz), 6.19–6.22 (—NC*H*CF_2 of GEM), 7.09–7.8 (—CON*H*CH— of NIPAm), 7.67 (d, CCH═C*H*N of GEM, *J* = 9.0 Hz), 8.10–8.57 (—N*H* of MA-GG-GEM), 12.1 (—COO*H* of MAA). ^{13}C NMR (DMSO-d_6) δ: 16.40–19.46, 22.99, 42.26, 43.94–45.92, 51.93, 59.34, 64.36, 68.73, 70.07, 71.77, 80.76, 94.93, 140.01, 155.06, 155.65, 164.29, 170.53, 174.04, 176.89, and 178.42. IR (KBr) ν (cm^{-1}): 1172 (C—O), 1715 (C═O), 1543 (N—H), 1642 (C═O), 1720 (C═O), 2977, 2933 (C—H), 3326 (N—H), 3472 (O—H).

2.3.3. Synthesis of Poly(MAA-co-NIPAm-co-MA-GG-SMX)(Poly-SMX)

The aforementioned procedure was used to prepare the poly-SMX; however, the quantity of SMX was 0.029 g. The product was washed with Et_2O and dried in a vacuum overnight. (0.345 g, yield: 86.3%). ^{1}H NMR (DMSO-d_6) δ: 0.53–1.33 (CH_3— of polymer backbone and —CH(CH_3)$_2$ of NIPAm), 1.57–2.28 (methylene and methane protons of polymer backbone), 2.25 (CH_3—of oxazole of