Carbohydrate Polymers 121 (2015) 132–139

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Synthesis and *in vitro* evaluation of a hyaluronic acid–quantum dots–melphalan conjugate

Haixing Xu[a,1], Jingbo He[b,1], Yu Zhang[a], Lihong Fan[c], Yaqiong Zhao[a], Tengfei Xu[a], Zhuang Nie[a], Xiangnan Li[a], Zhijun Huang[a], Bo Lu[a], Peihu Xu[a,*]

[a] *Department of Pharmaceutical Engineering, School of Chemistry, Chemical Engineering and Life Sciences, Wuhan University of Technology, Wuhan 430070 PR China*
[b] *The affiliated Baoan Hospital of Southern Medical University, Shenzhen 518101 PR China*
[c] *Department of Chemical Engineering, School of Chemistry, Chemical Engineering and Life Sciences, Wuhan University of Technology, Wuhan 430070 PR China*

ARTICLE INFO

Article history:
Received 9 November 2014
Received in revised form
15 December 2014
Accepted 24 December 2014
Available online 3 January 2015

Keywords:
Hyaluronic acid
Quantum dots
Melphalan
Drug delivery system

ABSTRACT

Polymer-drug conjugates have played an important role in improving tumor cell targeting and the selectivity of anticancer drugs. In this study, quantum dots and melphalan were attached to the backbone of hyaluronic acid to synthesize a polymer–drug conjugate. The physicochemical properties of the conjugate were characterized by FT-IR, XRD, ^{1}H NMR, UV-Vis spectra and DLS. The *in vitro* drug release profiles and cell evaluation were investigated. The results showed that the conjugate was synthesized and self-assembled into nanoparticles with a diameter of 115 ± 2.3 nm. The conjugate had a pH-sensitive drug controlled release property. It was an ideal receptor-mediated delivery system and can be internalized into the human breast cancer cell. It had a better inhibition effect on human breast cancer cell and a poorer inhibition effect on normal breast cell than melphalan. These results supported that the conjugate would be a promising candidate for cancer therapy.

1. Introduction

The increasing worldwide prevalence of cancer has made the cancer therapy one of the most intensively investigated targets in recent years. Great efforts have been made to improve therapeutic options (Goodarzi, Varshochian, Kamalinia, & Atyabi, 2013). The most considerable challenges facing effective cancer therapy are the systemic toxicity of cytotoxic drugs due to their nonspecific property, their lack of tumor localizing, and an even distribution throughout the whole body. Besides, short half-lives of anticancer drugs in blood circulation and their undesirable pharmacokinetic behavior are among other drawbacks which are present in the way of cancer treatment. All these negative effects have limited the clinical application of most of the anticancer drugs. Hence, there has been an eager quest to develop safe and efficient drug carriers which can deliver anticancer drugs exclusively to the targeting site without provoking adverse reactions in cancer therapy. Consequently, the conjugation of low molecular weight (MW) anticancer drugs to polymer carriers was commenced to form a polymer–drug conjugate which generally enhances the distribution of anticancer drug molecule (Shen, Li, Tu, & Zhu, 2009). The main advantages of polymer–drug conjugates include (Khandare & Minko, 2006; Majoros, Thomas, Mehta, & Baker, 2005; Moghimi, Hunter, & Murry, 2005; Patri, Majoros, & Baker, 2002): (1) an increase in water solubility of low soluble or insoluble drugs, and therefore, enhancement of the drug bioavailability; (2) protection of the drugs from deactivation and preservation of their activities during circulation; (3) a reduction in antigenic activity of the drug leading to a less pronounced immunological body response; and (4) most importantly, the ability to provide active targeting of the drug specifically to the site of its action.

Active targeting polymer–drug delivery systems have been investigated in recent years. These delivery systems recognize their targets by localization in tumors *via* conjugation to a chemical moiety with an affinity for an overexpressed/unique tumor cell marker (*i.e.* CD44 receptor, folic acid receptor, monoclonal antibody, *etc.*), or by triggering the release of anticancer drug from an environment-responsive carrier using a local stimulus (*i.e.* enzyme, pH, temperature, *etc.*) (Carole, Guy, Corinne, & Thierry, 2012). Among them polysaccharide-based systems have gained increasing attention due to their promising physicochemical and biological

* Corresponding author. Tel.: +86 02787749300.
E-mail address: xhx040328@whut.edu.cn (P. Xu).
[1] These authors contributed equally.

http://dx.doi.org/10.1016/j.carbpol.2014.12.057
0144-8617/

characteristics and simplicity of chemical reactions required for specific modifications (Carole, Guy, Corinne, & Thierry, 2011). Hyaluronic acid (HA) is a naturally-derived linear non-sulfated glycosaminoglycan polysaccharide, and a major constituent of the natural extracellular matrix (Laura, Marco, & Luisa, 2014). HA is naturally degraded in the organisms by a complex enzymatic mechanism involving hyaluronidase and HA cell internalization by CD44 cell surface receptors (Aruffo, Stamenkovic, Melnick, Underhill, & Seed, 1990; Jedrzejas & Stern, 2005). The hyaluronidase in tumor site is an acid-sensitive activity profile enzyme (Girish & Kemparaju, 2007). Recently, the use of HA as a drug carrier and a ligand to form conjugate has attracted much attention in active targeting drug delivery systems because of its ability to specifically bind to various cancer cells that overexpress CD44 receptors (Akima et al., 1996; Taetz et al., 2009; Sonia, Ana, & Carmen, 2011). The drugs are released once the covalent bonds are cleaved by intracellular hyaluronidase in the organisms, ideally at the specific target sites (Luo & Prestwich, 1999; Luo, Ziebell, & Prestwich, 2000; Pouyani & Prestwich, 1994; Xin, Wang, & Xiang, 2010).

Quantum dots (QDs) have been widely used owing to their interesting photophysical properties (Hridyesh, Rohit, & Dutta, 2013). These attributes make QDs superior fluorescent probes to organic fluorescent dyes and proteins for *in vitro* and *in vivo* biomedical imaging (Hauck, Anderson, Fischer, Newbigging, & Chan, 2010; Li, Duan, & Jing, 2011; Wang & Chen, 2011; Zhao, Liu, & Li, 2010). During the past years, CdTe and CdSe nanocrystals have become the most prominent QDs in area of life science, which have been widely used in cellular labeling and *in vivo* long-term fluorescence imaging (Hridyesh et al., 2013).

Melphalan (MEL), also known as l-phenylalanine mustard, is a potent anticancer drug and has been extensively utilized as a chemotherapeutic agent (Baracu, Balaban, & Wilman, 1990; Hansson, Lewensohn, Ringborg, & Nilsson, 1987). However, MEL is highly reactive, which can easily react with other cellular components including proteins and nucleic acids, resulting in the loss of therapeutic activity and the provocation of many undesired side effects such as bone marrow toxicity and genotoxicity (Li et al., 2014; Lu et al., 2013; Maze et al., 1996; Suzukake, Vistica, & Vistica, 1983). Moreover, it is water insoluble which leads to a very low bioavailability (Fermeglia, Ferrone, Lodi, & Pricl, 2003) In this study, a HA–QDs–MEL conjugate was developed to self-assemble nanoparticles for breast tumor treatment (Fig. 1). As expected, it has a promising active targeting ability to breast tumor and improved bioavailability. The results demonstrated that the conjugate had an ideal *in vitro* drug release, cytotoxicity properties, and cellular uptake characteristics, suggesting its potential as a future anticancer drug.

2. Materials and methods

2.1. Materials

Melphalan and hyaluronic acid (MW = 1,000,000) were purchased from Sigma–Aldrich. $NaHB_4$, Tellurium powder, $CdCl_2$ and L-cysteine were purchased from Sinopharm Chemical Reagent Co., Ltd., Shanghai, China. All other chemicals used were of analytical reagent grade. Double-deionized water was used throughout the experiment.

2.2. Synthesis of HA–QDs–MEL conjugate

37.8 mg of $NaHB_4$ and 63.8 mg of tellurium powder (2:1, molar ratio) were dissolved in 6 mL of deionized water and stirred at room temperature in darkness with N_2-saturated to prepare NaHTe solution until the color of the solution became to black. Then 114.2 mg of $CdCl_2$ and 90.87 mg of L-cysteine (1:1.5, molar ratio) were dissolved in 40 mL of deionized water in a 100 mL three-neck flask and the solution was alkalized to pH = 8.0 with 0.5 mol/L aqueous NaOH solution. Subsequently a certain amount of fresh NaHTe solution was added and saturated by N_2 for 30 min then 1 mL of Na_2S in water (7.5 mmol/L) was added and the mixture was refluxed to afford L-Cys-CdTe/CdS QDs solution.

100 mg of HA was dissolved in 100 mL of PBS (pH = 6.8) with 200 mg of EDC and 30 mg of NHS. After 30 min, 5 mL of L-Cys-CdTe/CdS QDs solution was added. After another 6 h, the solution was dialyzed (MW cut-off = 3000–5000 Da) and lyophilized, providing HA–QDs powder.

648 mg of Benzyl alcohol (BA) and 36 mg of $SOCl_2$ were mixed with vigorous stirring in ice bath, and 64.4 mg of MEL was added for esterification. Then the reaction mixture was concentrated and the residue was recrystallized from CH_3OH and ether to give a white powder (BA-MEL). The above HA–QDs were then dissolved in 50 mL of PBS with EDC and NHS. After 4 h, 60 mg of BA-MEL was added into the reaction mixture and stirred for 24 h. Then the reaction solution was dialyzed (MW cutoff = 3000–5000 Da) in double-distilled water and lyophilized. The HA–QDs–MEL conjugate was obtained upon removal of the methyl ester group by hydrogenolysis over palladium-charcoal.

2.3. Characterization

The Fourier transform-infrared (FT-IR) spectra were obtained and analyzed by a Flourier transform-infrared spectrometer (Nexus, Thermo Nicolet, USA) and the proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on an Inova 600 spectrometer (Varian, USA). HA–QDs–MEL was also characterized by ultraviolet–visible (UV–vis) spectroscopy (UV-2600, Shimadzu, Japan). The average hydrodynamic diameters and Zeta potential of HA–QDs–MEL nanoparticles (1 mg/mL) were measured by dynamic light scattering (DLS) using a Zeta sizer (Nano-ZS SDSB-10032; Malvern, UK). The experiment/test was carried out at 25 °C, and repeated three times.

2.4. In vitro drug release study

The drug release experiments were carried out at 37 ± 0.5 °C. Briefly, 5 mg of HA–QDs–MEL was dissolved in 50 mL of phosphate buffer solution (PBS) with 150 U/mL hyaluronidase and dialyzed in dialysis bags (MW cut-off = 12,000 Da) (Shan et al., 2012). Three buffer solutions with pH = 7.4, pH = 7.0, and pH = 5.8 were employed to simulate the pH values of microenvironments in blood vessels and tumor tissues, respectively. The dialysis bags were sealed at both ends with clips and placed into the medium with the same pH values of those in dialysis bags. At appropriate time intervals, aliquots of 1 mL medium were withdrawn and fresh equal volumes of PBS were added to the medium. Samples were analyzed by a Waters liquid chromatographic system (Waters 1525, USA) with a UV detector (SPD-10A, Japan) operated at 260 nm. A Waters ODS C18 column (4.6 × 150 mm, 5 μm particle size) was used at 30 °C. The mobile phase was a mixture of methanol, deionized water, and acetic acid (49:49:2, v/v/v). The injection volume was 10 μL, and the flow rate was 1.0 mL/min. The accumulative release rate % (Q%) was calculated by the following the formula,

$$Q\% = \frac{V_0 C_n + V(C_0 + C_1 + C_2 + \cdots + C_{n-1})}{m_0} \times 100\% \quad (1)$$

where V_0 stands for the total volume of the release medium, C_n (mg/L) is the concentration of the sample withdrawn at the interval of T_n, V is the volume of the sample withdrawn at the interval of T_i, C_i (mg/L) is the concentration of the sample withdrawn at the interval