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Research paper

A cross-linked polymeric micellar delivery system for cisplatin(IV) complex

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ABSTRACT

A polymeric cisplatin(IV) prodrug in the form of cross-linked micelles (M(Pt(IV)) was prepared by first constructing MPEG-b-PCL-b-PLL micelles and then attaching a cisplatin(IV) complex with two axial succinic moieties to the lysine residues of the carrier polymer in aqueous medium. The micelles obtained were characterized by TEM, DLS, and zeta potential measurement. Their in vitro release experiments were carried out at pH 7.4 and 5.0 or in the presence of 5 mM sodium ascorbate (NaAsc). Results showed that the micelles were sensitive to both acidic hydrolysis and mild reducing agents; in the presence of 5 mM NaAsc, cisplatin(II) was directly released and the released cisplatin(II) could chelate with nucleobases; the micelles displayed comparable cytotoxicities to cisplatin; and the micelles were much more efficiently internalized by the cells than cisplatin(II) and cisplatin(IV) counterparts. Moreover, in vivo study showed accumulation of more Pt species in the tumor site and lower systematic toxicity compared to free cisplatin(II) and cisplatin(IV). This polymeric prodrug of cisplatin is expected to be used more for future study and applications.

1. Introduction

Polymeric conjugates of conventional drugs (polymeric drug conjugates) have several advantages over their low molecular weight precursors [1–3]. Due to these advantages over the free form of a drug, the polymeric drug conjugates have led to a new era of polymeric drug delivery systems (DDSs). Despite various polymers and polymeric architectures [4–6] have been chosen for polymer–drug conjugates, polymer–drug conjugates that can self-assemble into nanomicelles are important and attractive drug delivery system for poorly water soluble drugs [7]. However, conventional non-cross-linked micelles often suffer from premature release of drugs due to disintegration of the micelle into individual polymeric unimers upon dilution in body fluids [8], although they are believed to be more stable than liposomes constructed from conventional small surfactants [9,10]. Extensive research revealed that cross-linking either the core or the shell of the drug loaded micelles can provide dramatic improvements in both pharmacokinetics and biodistribution of the encapsulated drugs [11]. A variety of cross-linking methods were established [12,13] such as usage of bi-functional reagents, free radical polymerization, photo-cross-linking, reversible (reducible) disulfides and silicon chemistry. Bi-functional reagents with —NH_2, —SH, —N_3, C═C, C≡C, etc. are very effective chemical cross-linkers [12,13]. Among them, Wooley has a lot of outstanding work on core-crosslinking or shell-crosslinking micelles for medical use [14,15]. However, till now, there are few reports to use drug molecule itself as a cross-linker to prepare core-cross-linked micelles or shell-cross-linked micelles. Cheng et al. reported a paclitaxel (PTX) derivative as a bivalent agent/cross-linker for nanomicelles via click-chemistry and showed us good examples [16,17].

Cisplatin is a first line antitumor agent and has been widely used in cancer chemotherapy [18,19]. Cisplatin and other Pt(II) drugs (carboplatin, oxaliplatin, etc.) are usually Pt(II) complexes with planar molecular structure. The chemical or biochemical activity of this planar structure not only determines their efficacy in tumor curing, but also causes severe side effects such as nephrotoxicity, peripheral neuropathy, and hearing loss [20,21]. Consequently, much attention has been paid to Pt(IV) compounds with octahedral structure. Incorporation of the two more ligands on the axial positions leads to less toxicity [22], so that they can be used in high dose. Because the Pt(IV) are reducible, they are believed to be reduced to Pt(II) counterparts within cancer cells because there are a variety of reducing agents such as mercaptan,

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glutathione, and others in cell plasma [23–25]. These reduction products are qualified antitumor agents. Therefore, the Pt(IV) complexes are generally considered as prodrugs of the corresponding Pt(II) drugs. Interestingly, Stenzel et al. reported such an cisplatin(IV) prodrug with two axial bi-functional amines [26]. This prodrug was further used as cross-linkers to react with a polymer named poly(oligo(ethyleneglycol) methyl ether methacrylate)-block-poly(styrene-co-3-isopropenyl-α,α-dimethylbenzyl isocyanate) (POEGMA-b-P(STY-co-TMI)) containing pendant isocyonates to prepare cross-linked micelles. Although this cross-linking reaction was successful, the carrier polymer chosen by Stenzel et al. is not biodegradable and can cause damage to normal tissues. Moreover, there are many —NCOs on the polymer chain. The remaining —NCO groups due to incomplete reaction could be greatly toxic. Therefore, the strategy of using bi-functional Pt(IV) drugs as cross-linker needs further improvement.

In this paper, therefore, a cisplatin(IV) prodrug with two axial succinic acid moieties as bi-functional cross-linkers was prepared and then conjugated to a biodegradable polymer MPEG-b-PCL-b-PLL reported previously by our group [27] to prepare stabilized micelles (Scheme 1). Due to the possibility for the succinic acid moieties to react with different polymer chains, the polymer–cisplatin(IV) conjugates formed were actually cross-linked micelles. The micelles demonstrated improved stability, dual drug releasing sensitivities to both acid and reducing agents such as NaAsc (sodium ascorbate), and comparable cytotoxicities to cisplatin against HeLa, MCF-7, and SKOV-3 cancer cell lines. Cellular uptake of the micelles via quantitative measurement of Pt contents in SKOV-3 cancer cells proved efficient uptake of the cross-linked micelles by the cells.

2. Experimental part

2.1. Materials

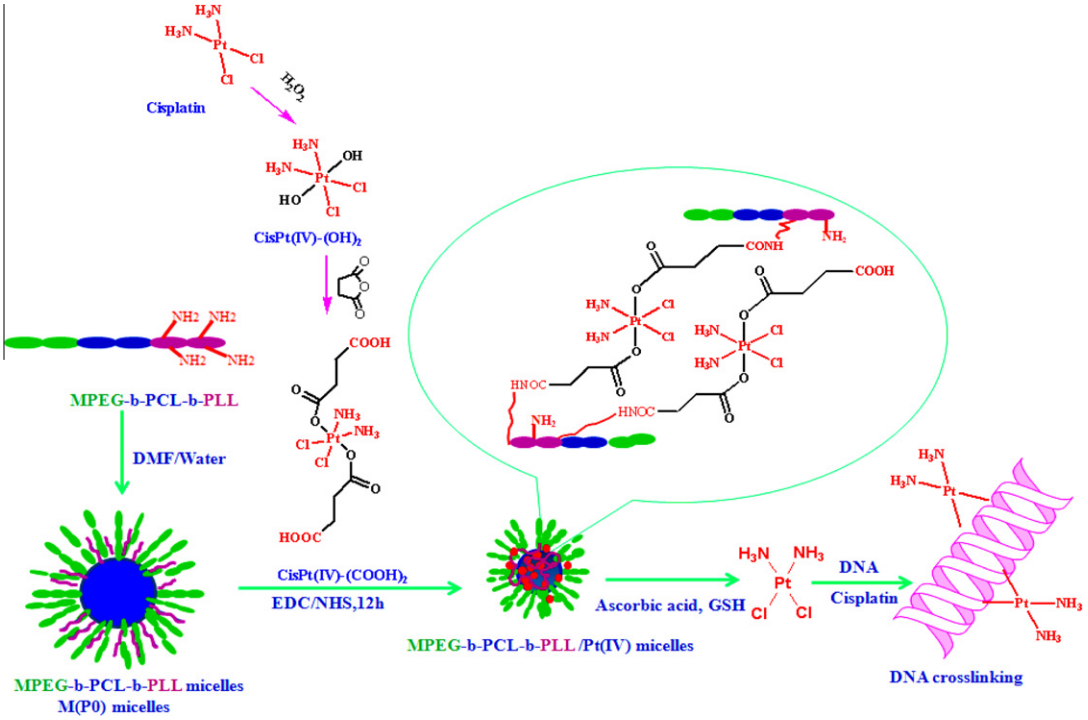
Monomethoxyl poly(ethylene glycol)-block-poly(ε-caprolactone)-block-poly(L-lysine) was synthesized as previously described [27]. And its structure could be written as $MPEG_{114}$-b-PCL_{20}-b-PLL_{10}, where the subscript numbers denote degrees of polymerization of the blocks. N-hydroxysuccinimide (NHS), 1-ethyl-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC·HCl), and succinic anhydride were purchased from Sigma–Aldrich, China. Cisplatin (purity 99%) was bought from Shandong Boyuan Pharmaceutical Co. Ltd., China. To distinguish from the cisplatin(IV) prodrug synthesized in which Pt atom is in +4 valence, the commercial cisplatin is also written as cisplatin(II) to indicate the +2 valence of the central Pt atom. All other chemicals and solvents were obtained commercially and used without further purification.

2.2. General measurements

^{1}H NMR spectra were measured by a Unity-300 MHz NMR spectrometer (Bruker, Germany) at room temperature. Fourier transform infrared (FT-IR) spectra were recorded on a Bruker Vertex 70 spectrometer. Mass spectroscopy (ESI-MS) measurements were performed on a Quattro Premier XE system (Waters) equipped with an electrospray interface (ESI). Matrix-assisted laser-desorption ionization and time-of-flight mass spectroscopy (MALDI-TOF-MS, Waters, USA) was used to study the chelation of Pt species with 5′-GMP. Inductively coupled plasma optical emission spectrometer (ICP-OES, iCAP 6300, Thermoscientific, USA) was used to determine the total platinum contents in the polymer–Pt(IV) conjugate and samples obtained outside of the dialysis bags in drug release experiments. Inductively coupled plasma mass spectrometer (ICP-MS, Xseries II, Thermoscientific, USA) was used for quantitative determination of trace levels of platinum in cells. Size and size distribution of micelles were determined by DLS with a vertically polarized He—Ne laser (DAWN EOS, Wyatt Technology, USA).

2.3. Synthesis of c,c,t-[$Pt(NH_3)_2Cl_2(OH)_2$] (cisPt(IV)—$(OH)_2$)

CisPt(IV)—$(OH)_2$ (Scheme 1) was synthesized according to Ref. [27].



Scheme 1. Preparation of MPEG-b-PCL-b-PLL/cisPt(IV) micelles and the proposed pathway of action. (For color interpretation in this figure the reader is referred to see the web version of this article.)