Colloids and Surfaces B: Biointerfaces 170 (2018) 488–496

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Irinotecan delivery by unimolecular micelles composed of reduction-responsive star-like polymeric prodrug with high drug loading for enhanced cancer therapy

Yong-E. Gao[a,b], Shuang Bai[a,b], Xiaoxiao Shi[a,b], Meili Hou[a,b], Xiaoqian Ma[a,b], Tian Zhang[a,b], Bo Xiao[a,b], Peng Xue[a,b], Yuejun Kang[a,b,*], Zhigang Xu[a,b,*]

[a] Institute for Clean Energy and Advanced Materials, Faculty of Materials and Energy, Southwest University, Chongqing, 400715, China
[b] Chongqing Engineering Research Center for Micro-Nano Biomedical Materials and Devices, Chongqing 400715, China

ARTICLE INFO

Keywords:
Reduction-responsive
Unimolecular micelles
Drug delivery
Controlled release
Tumor therapy

ABSTRACT

Nanomedicine based polymeric prodrug have showed high impact in the inhibition of tumor growth due to its high therapeutic efficiency and improved biocompatibility. Herein, we synthesized a novel star-like amphiphilic copolymer [β-CD–P(Ir-*co*-OEGMA), denoted as CPIO] through atom transfer radical polymerization (ATRP) to deliver the hydrophilic anticancer drug irinotecan (Ir). The polymer could form monodisperse unimolecular micelles and had excellent stability in aqueous solution. Moreover, the reduction-responsive feature of the micelles facilitated controlled release of drug, thus achieving targeted therapy and reduced toxicity to healthy cells. The *in vitro* cytotoxicity assays indicated that CPIO had a notable anticancer effect against HeLa and MCF-7 tumor cells. The confocal laser scanning microscopy and flow cytometry experiments revealed that CPIO micelles could be internalized into tumor cells efficiently. Furthermore, the obtained prodrug micelles produced better efficacy compared to free Ir. Moreover, the CPIO micelles showed excellent biocompatibility *in vivo* after intravenous injection on a mouse model. This study demonstrated that CPIO carrier could provide a rational design of a stimuli-responsive polymeric prodrug for delivery of irinotecan.

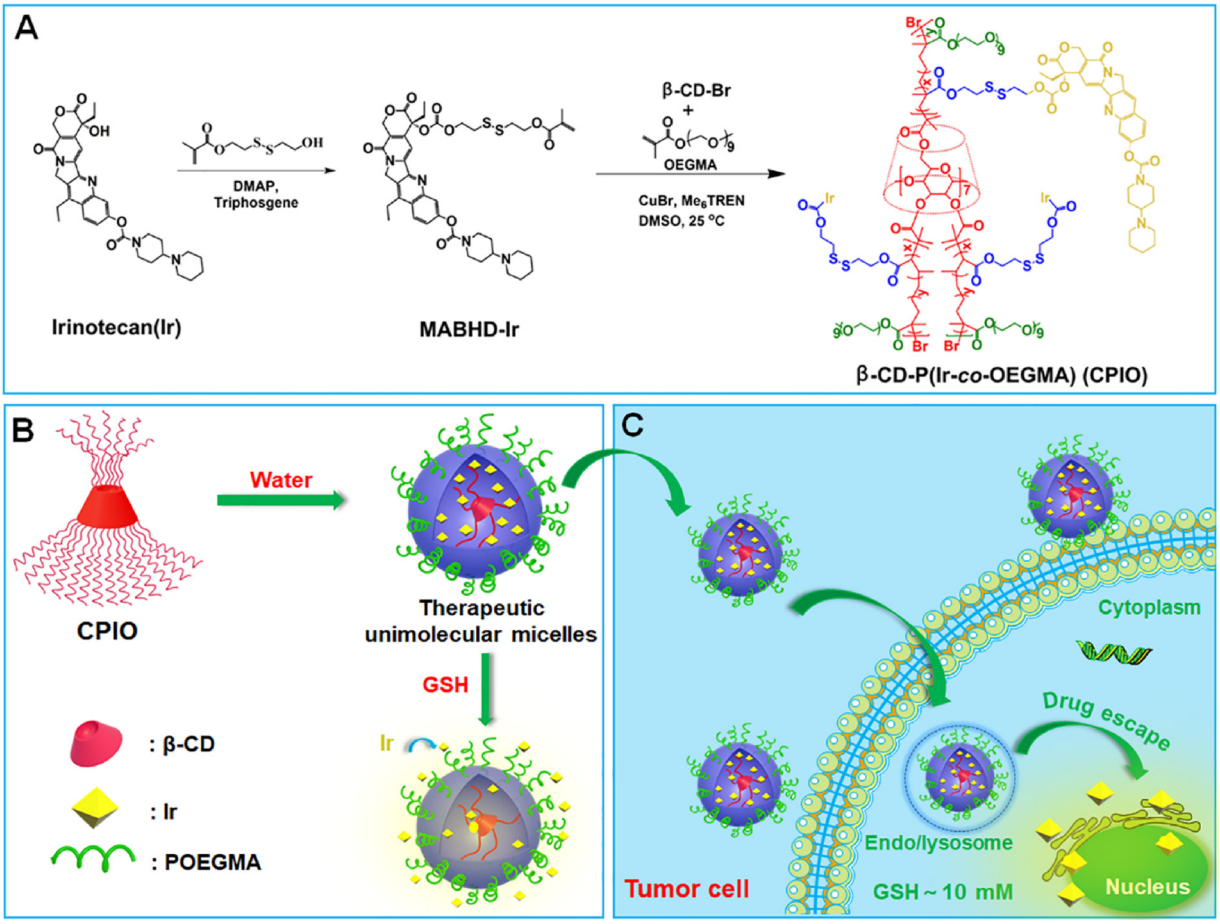
1. Introduction

As a major cause of death, cancer has become one of the most serious threats for human health worldwide [1–3]. Chemotherapy continues to be one of the mainstream modalities for cancer therapy due to the high efficiency [4].Unfortunately, conventional chemotherapy usually suffer from many limitations including lack of selectivity, poor bioavailability and severe multidrug resistance (MDR), which may induce undesirable side effects and compromise the therapeutic efficacy of antineoplastic agents [5–8]. To overcome these limitations, various nanostructured drug delivery systems including micelles [9,10], quantum dots [11,12], liposomes [13–15], drug-drug conjugates [16–18] and inorganic materials [19,20] have been developed. Applications of these nanocarriers have improved the therapeutic effect and reduced side effects that are induced by free drugs. However, many nanocarriers themselves may become toxic to human organs during degradation, metabolism and excretion [21–23]. Moreover, most of the therapeutic agents studied previously are hydrophobic drugs, such as camptothecin (CPT) [24], doxorubicin (DOX) [25] and paclitaxel (PTX) [26]. Only a very limited number of studies are focused on the delivery of hydrophilic anticancer drugs. Therefore, delivery of hydrophilic anticancer drugs using more amicable delivery platforms remains a challenge to improve the bioavailability of drugs for better therapeutic effect.

Irinotecan (Ir), also known as CPT-11, is a US Food and Drug Administration (FDA)-approved hydrophilic analogue of CPT [27]. However, Ir has severe toxicity to human organs (*e.g.*, bone marrow, liver) and suffers from large interpatient variability which have restricted its extensive applications [28–30]. Recently, several therapeutic platforms have been employed to deliver hydrophilic anticancer agents. For example, Huang and co-workers constructed a novel self-delivery system for anticancer drugs by conjugating hydrophobic chlorambucil with hydrophilic Ir through ester-containing linkers [31]. This system can achieve controlled drug release and high drug loading rate. However, the self-assembled nanostructures tend to aggregate at above critical micelle concentration, which led to serious stability problems. As reported previously, star-like polymers could form unimolecular micelles that had excellent stability and integrity under the

* Corresponding authors at: Institute for Clean Energy and Advanced Materials, Faculty of Materials and Energy, Southwest University, Chongqing, 400715, China.
E-mail addresses: yjkang@swu.edu.cn (Y. Kang), zgxu@swu.edu.cn (Z. Xu).

https://doi.org/10.1016/j.colsurfb.2018.06.054
Received 6 March 2018; Received in revised form 20 June 2018; Accepted 25 June 2018
Available online 25 June 2018



Scheme 1. Schematic diagram of the star-like reduction -responsive CPIO polymer. (A) Synthesis and chemical structure of the polymer; (B) Formation and degradation of the CPIO micelles in water; (C) The cellular internalization and controlled drug release of the CPIO micelles.

variation of external conditions [32,33]. In addition, owing to the good biocompatibility and biodegradability, β-cyclodextrin (β-CD) with 21 hydroxyl groups has been used as the core [34] to fabricate star-like copolymers through reversible addition fragmentation chain-transfer (RAFT) [35] polymerization and atom transfer radical polymerization (ATRP) [36]. Furthermore, tumor cells can create physiological microenvironment that are distinct to normal tissues, such as higher intracellular concentration of glutathione (GSH) [37–39]. Taking advantage of this unique property, drug delivery systems containing disulfide bonds could rapidly release the therapeutic agents intracellularly through cleavage of the disulfide bonds while remain very stable in the extracellular environment [40–43], thereby realizing stimuli-triggered release of the carried drugs. It is a rational design of a drug-delivery platform taking advantages of both star-like polymers and the reduction–responsive release of hydrophilic Ir, which could be a novel and promising treatment for tumor therapy.

In the present work, we prepared a star-like amphiphilic copolymer (CPIO) through atom transfer radical polymerization (ATRP) to deliver the hydrophilic anticancer drug Ir (Scheme 1). The star-like amphiphilic copolymer was able to form monodisperse and stable unimolecular micelles in aqueous solution. The reduction-responsive property of the polymeric micelles contributed to the controlled release of drug and the reduced toxicity to healthy tissues. Additionally, microscopic and flow cytometry analyses revealed the excellent cellular uptake efficiency of the prodrug micelles and enhanced efficacy on tumor cells. The unique advantages of this drug self-delivery system may provide a new and efficient strategy for future cancer chemotherapy.

2. Experimental

2.1. Reagents and materials

All chemical reagents were obtained from Sigma-Aldrich (USA) except otherwise specified. Irinotecan (Ir) was acquired from Dalian Meilun Biotechnology Co. Ltd (Dalian, China) and Nile red was purchased from Aladdin Co. China. 2-Hydroxyethyl disulfide (BHD), β-cyclodextrin (β-CD, 99.7%), 2-bromoisobutyryl bromide (BIBB, 98%), triphosgene (BTC, 99%), *N, N, N, N, N*-pentamethyldiethylenetriamine (MPDETA, 99%), 4-(dimethylamino) pyridine (DMAP) and Glutathione (GSH) were used as received. The oligo-(ethylene glycol methylether methacrylate) (OEGMA) was passed through a column of activated basic alumina to remove inhibitors before using and copper(I) bromide (CuBr, 98.0%) was purified according to our previous report [43]. Organic solvents including tetrahydrofuran (THF) and Dimethyl sulfoxide (DMSO) were supplied by Adamas-beta® (China). Other chemicals were used directly without further purification. Penicillin/streptomycin mixture, fetal bovine serum (FBS), Dulbecco's modified eagle medium (DMEM), TrypLE™ Express Enzyme, phosphate buffered saline (PBS), cell viability reagent (PrestoBlue) and Alexa Fluor 633 phalloidin were purchased from Life Technologies (China).

2.2. Characterizations

^{1}H NMR spectra and ^{13}C NMR spectra were recorded by a Bruker AVIII600 NMR (Rheinstetten, Germany) using $CDCl_3$ or DMSO-d_6 as a reference solvent. The synthesis of prodrug MABHD-Ir was further characterized using a mass spectrometer (Bruker Dalton). The number-