Journal of Drug Delivery Science and Technology 35 (2016) 252–259

Contents lists available at ScienceDirect

Journal of Drug Delivery Science and Technology

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

Improving the anticancer activity of α-hederin by physically encapsulating it with targeted micelles assembled from amphiphilic block copolymers

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ARTICLE INFO

Article history:
Received 1 May 2016
Received in revised form
2 August 2016
Accepted 2 August 2016
Available online 4 August 2016

Keywords:
Alfa-hederin
RGD
Polymeric micelles
Anticancer therapy

ABSTRACT

Alfa-hederin (Hed) is regarded as a promising drug in cancer therapy especially in drug-resistant cancers. However, its reduced anticancer activity caused by protein absorption limits its wide clinical applications. The purpose of this study was to develop diblock copolymer-based micelles as an integrated platform for targeted delivery of Hed to overcome its intrinsic drawbacks. Amphiphilic diblock copolymers, poly(-epsilon-caprolactone)-*b*-poly(oligo(ethylene glycol) monomethyl ether methacrylate-*co*-RGD) (PCL-*b*-P(OEGMA-*co*-RGD)) consisting of hydrophobic PCL, hydrophilic POEGMA and targeting peptide (RGD) were fabricated via the combination of ring-opening polymerization (ROP), atom transfer radical polymerization (ATRP), and polymer post-functionalization. Hed-containing targeting micellar nanoparticles, Hed-NP-RGD, were obtained via the co-micellization of PCL-*b*-P(OEGMA-*co*-RGD) and Hed, showing hydrodynamic diameter 108 ± 1 nm and zeta potential −4.10 ± 0.15 mV. In vitro experiments demonstrated that Hed-NP-RGD possessed superior anti-proliferative and cellular apoptosis in comparison with that of free Hed possibly due to the assumption that the presence of polymeric micelles protected Hed molecules from absorption by proteins and tumor-targeting effect.

1. Introduction

Alfa-hederin (Hed, also known as kalopanaxsaponin A or sapinoside A), first isolated from the leaves of Hedera helix in 1912, is a monodesmosidic triterpenoid compound [1]. Among its various biological activities (such as hemolytic, anti-spasmodic, anti-inflammatory, and anti-leishmanial) its application in cancer therapy attracts more and more attention in recent years [2–10]. Hed can effectively bypass many drug-resistant pathways, exhibiting promising applications in the combat of conventional drug-resistant tumors [9,11]. It was reported that Hed might exert its antitumor activity via promoting apoptosis, vacuolization of the cytoplasm and/or membrane alterations [7,12–14].

Danloy et al. reported that Hed could effectively inhibit the proliferation of mouse B16 melanoma cells and non-cancerous mouse 3T3 fibroblasts in serum-free media at a concentration less than 5 mg/L [12]. However, its anticancer effect decreased significantly in the presence of serum possibly due to the absorption of Hed molecules by serum proteins [12]. Swamy et al. found that Hed exhibited a dose- and time-dependent increase in apoptosis of murine leukemia P388 cells via rapidly depletion of intracellular GSH, protein thiol and reactive oxygen species [13]. Rooney et al. further reported the anticancer effect of Hed against four human cancer cells lines (A549, HEp-2, HT-29, and MIA PaCa-2) [5]. Tian et al. then showed that the proliferation of both conventional HepG2 and drug-resistant HepG2 cells can be effectively inhibited by Hed via apoptosis mechanism [11]. Bun et al. reported the potentiation effect of Hed to 5-fluorouracil [15]. It was demonstrated that Hed enhanced the 5-fluorouracil cytotoxicity for up to 3.3-fold.

However, it should be noted that the poor water solubility, short circulation time, hemolysis effect, and low bioavailability restrict its

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http://dx.doi.org/10.1016/j.jddst.2016.08.001
1773-2247/

further clinical applications [16]. Organic solvents such as dimethyl sulfoxide (DMSO) are often employed to facilitate its administration. Unfortunately, DMSO might lead to safety concerns due to the risk of vasoconstriction neurological toxicity, liver damage, and kidney damage [17,18]. Besides, it was reported that the anticancer performance of Hed could be significantly decreased in the presence of proteins presumably due to protein absorption [12]. However, proteins are abundantly distributed both inside and outside cells around the body, which will substantially restrict the amount of active Hed molecules reaching cancer cells.

To address the intrinsic limitations associated with small molecular anticancer drugs various successful strategies based on nanotechnology have been developed, including physically encapsulating drugs into or covalently linking drugs onto polymeric assemblies (e.g., micelles, nanogels, and vesicles) or polymer chains [19,20]. Thus, we hypothesized that encapsulating Hed by polymeric micelles could effectively improve the anticancer performance of Hed. In addition, by stopping the direct exposure of Hed molecules to proteins should effectively reduce the activity loss caused by protein absorption.

In the current work, we utilized polymeric micelles as an integrated platform for the cancer cell-targeted delivery of Hed to overcome its intrinsic drawbacks such as poor water solubility and reduced efficacy caused by protein absorption (Scheme 1). Amphiphilic diblock copolymers, PCL-*b*- P(OEGMA-*co*-RGD), consisting of hydrophobic PCL and hydrophilic POEGMA covalently attached with RGD were fabricated via the combination of ROP, ATRP, and post-functionalization (Scheme 2). Micellar nanoparticles assembled from PCL-*b*-P(OEGMA-*co*-RGD) possesses hydrophobic PCL core for loading Hed and hydrophilic outer corona of POEGMA functionalized with RGD for stabilizing nanoparticles and targeted drug delivery. ^{1}H nuclear magnetic resonance (NMR), dynamic laser light scattering (LLS), and gel permeation chromatography (GPC) were employed to characterize the polymers and polymeric nanoparticles. In vitro drug release and cytotoxicity measurements of free Hed and Hed-loaded micellar nanoparticles were conducted to probe the feasibilities and capabilities of this new type of micelle-based Hed delivery system. Apoptosis and cell cycle experiments were also performed to gain insights into the mechanism of its anticancer performance.

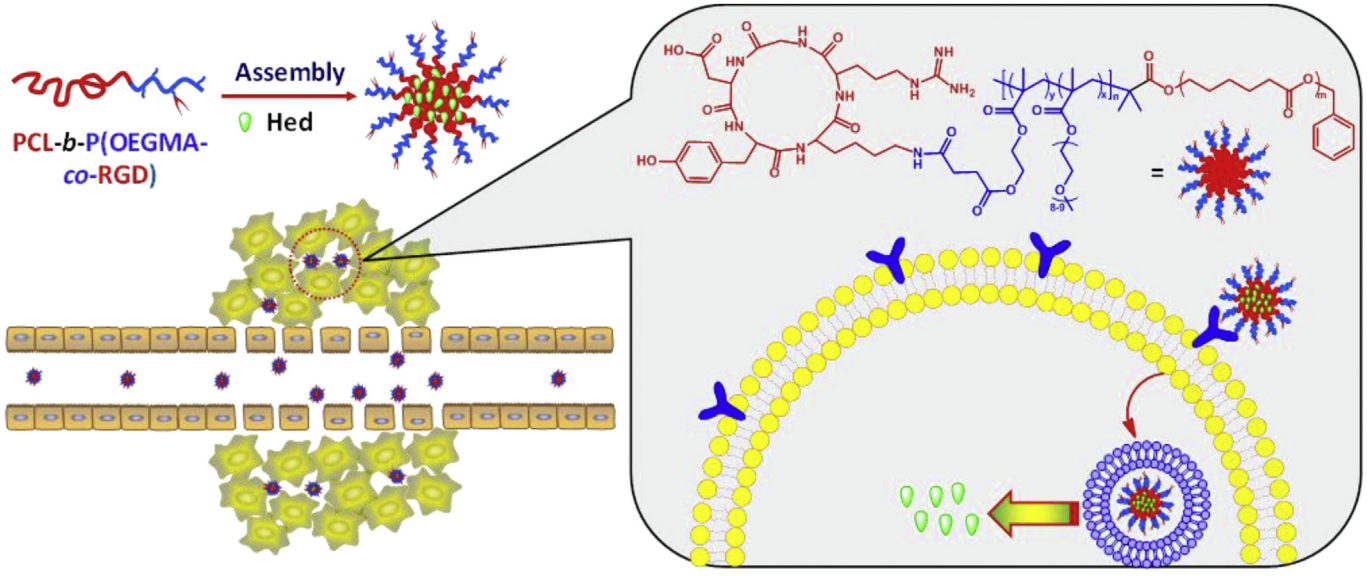
2. Materials and methods

2.1. Materials

Oligo(ethylene glycol) monomethyl ether methacrylate (OEGMA, M_n = 500 g/mol, mean degree of polymerization, DP, is 8–9) purchased from Aladdin (Shanghai, China) was passed through a neutral alumina column to remove the inhibitor and then stored at −20 °C prior to use. Benzyl alcohol (Aladdin) and epsilon-caprolactone (CL, 99%, Acros) were dried over calcium hydride (CaH_2) and distilled at reduced pressure before use. 2-Bromoisobutyryl bromide (98%), *N,N,N′,N″,N″*-pentamethyldiethylenetriamine (PMDETA, 98%), stannous(II) octanoate ($Sn(Oct)_2$, 95%), and copper(I) bromide (CuBr, 98%) were purchased from Sigma-Aldrich (St Louis, MO, USA) and used as received. Cyclic RGD (cRGD, shortened as RGD in the subsequent sections) was purchased from Chinese Peptide Company (Hangzhou, China) and used as received. Hed (98%) was purchased from Nanjing Spring & Autumn Biological Engineering Co. Ltd., (Nanjing, China) and used as received. Annexin V FITC/PI apoptosis detection kit (BD Biosciences, San Jose, CA, USA), cell cycle detection kit (Beyotime Biotech, Shanghai, China), and 4% paraformaldehyde solution (Beyotime Biotech) were used as received. Fetal bovine serum (FBS), penicillin, streptomycin, and RPMI 1640 were purchased from Thermo Fisher Scientific and used as received. *N,N′*-Dicyclohexylcarbodiimide (DCC), 4-dimethylaminopyridine (DMAP), *N*-hydroxysuccinimide (NHS, 99%), and all other reagents were purchased from Sinopharm Chemical Reagent Co. Ltd., and used as received. Triethylamine (TEA) and dichloromethane (CH_2Cl_2) were dried over CaH_2 and distilled just prior to use. Toluene were dried by refluxing over sodium shavings and distilled prior to use. Water was deionized with a Milli-Q SP reagent water system (Millipore) to a specific resistivity of 18.4 MΩ cm. Butanedioic acid mono 2-(methacryloyloxy)ethyl ester (2-succinyloxyethyl methacrylate, SEMA) was synthesized according to literature procedures [21].

2.2. Sample synthesis

Synthetic scheme employed for the preparation of well-defined amphiphilic block copolymers, PCL-*b*-P(OEGMA-*co*-RGD),



Scheme 1. Schematic illustration for the fabrication of tumor-targeted polymeric micelles via the assembly of amphiphilic block copolymers, PCL-*b*-P(OEGMA-*co*-RGD), in aqueous solution. The micelles consist of PCL as the hydrophobic core and a hydrophilic outer corona of POEGMA covalently labeled with RGD for targeted drug delivery.