Journal of Molecular Liquids 251 (2018) 178–184

Contents lists available at ScienceDirect

Journal of Molecular Liquids

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

Self-assembling nanoparticles based on cytarabine prodrug for enhanced leukemia treatment

Ruiling Liu [a], Jing Zhang [a], Di Zhang [a], Kaiming Wang [b,*], Yuxia Luan [a,*]

[a] School of Pharmaceutical Science, Key Laboratory of Chemical Biology (Ministry of Education), Shandong University, Jinan 250012, China
[b] School of Biological Science and Technology, University of Jinan, 336 West Road of Nan Xinzhuang, Jinan 250022, China

ARTICLE INFO

Article history:
Received 5 November 2017
Received in revised form 13 December 2017
Accepted 15 December 2017
Available online 17 December 2017

Keywords:
Cytarabine
Self-assemble
Nanoparticles
Leukemia treatment

ABSTRACT

Cytarabine (Ara-C) is an attractive chemotherapeutic agent used for the treatment of acute myeloblastic leukemia, however, its severely drawbacks such as low lipophilicity and rapid plasma degradation limit clinical applications. Here, we synthesized a new Ara-C prodrug DTA-Ara by conjugating 2-decyltetradecanoic acid (DTA), a double-chained fatty acid with 24 carbons with Ara-C. It was the first time to see that DTA-Ara molecules could self-assemble into stable spherical nanoparticles (NPs) in aqueous solution with extremely high drug loading (63 wt%). The DTA-Ara NPs had the average sizes of approximately 130 nm and a zeta potential around −31.6 mV. Importantly, the DTA-Ara NPs were stable in deionized water or phosphate buffer solution (PBS, pH 7.4) solution for more than one week and the hemolysis rate was <10%, which indicated that it could be administrated intravenously. Moreover, the *in vitro* cytotoxicity study further manifested that the resulting prodrug showed the marked antitumor activity against human leukemia cell line K562 and HL60 cells compared to the naked drug ascribed to its improvement of the hydrophobicity and biomembrane penetrability. The strategy of delivering lipophilized nucleoside analogs using prodrug self-assembling nanoparticles demonstrate potential superiority for Ara-C and provide a new promising therapeutic schedule for leukemia.

1. Introduction

Acute myeloid leukemia (AML) is a heterogeneous malignant blood cancer, characterized by abnormal proliferation of myeloid blasts in hematopoietic stem or progenitor cells [1,2]. In AML, the incidence increases and the prognosis worsens in older adults. In 2017, there were an estimated 21,380 new cases and 10,590 deaths in the US [3]. Even though with high dose chemotherapy treatments, the five-year survival rate is <50% due to the lack of specificity of cytotoxic agents [4,5]. Therefore, the nanocarriers including hydrogels [6], micelles [7,8], nanofibers [9,10] and nanoparticles [11,12] for drug delivery system (NDDS) have been developed and applied extensively. Thereinto the strategy of covalently combining low molecular lipid materials with drugs provides a rationale for the self-assembly property of the prodrug due to the endowed hydrophilic-hydrophobic property [13]. Furthermore, these nanoassemblies possess a remarkable high drug-loading [14]. The nanoassemblies show the superior tumor accumulation ability over the parent drugs [15–19] owing to the enhanced permeation and retention (EPR) effect.

Cytarabine (cytosine arabinoside 1-β-D-Arabinofuranosylcytosine, Ara-C), a pyrimidine nucleoside analog, is one of the mainstays used in the therapy of acute myeloid leukemia and non-Hodgkin's lymphoma [20]. It showed synergistic effects against solid tumors in combination with other anticancer drugs [21,22]. *Via* intracellular phosphorylation, cytarabine is converted into cytarabine 5′-triphosphate derivative (Ara-CTP), followed by inhibition of DNA polymerase and DNA chain elongation, exerting its anti-neoplastic effect [23]. Nevertheless, there were three main drawbacks in its clinical applications, such as (i) the rapid deamination of cytarabine into an inactive 1-β-D-arabinofuranosyluracil (Ara-U) by cytidine deaminase *in vivo*; (ii) the poor membrane permeability ascribe to the hydrophilicity; (iii) the extremely short plasma half-life (10–20 min) and drug multiresistance. Thus, Ara-C has to be administrated by intravenous continuously in order to obtain the maximum therapeutic efficiency in clinical, which causes the poor tolerability to the patients. Importantly, the higher dose parent drug would lead to the toxicity to normal tissue [24,25]. To overcome these limitations, lipid conjugates are designed to enhance the efficacy of the anticancer agent. For example, gemcitabine (nucleoside analog), possessing drawbacks like Ara-C, was conjugated to squalene, which extensively improved the lipophilic character of the parent drug and overcame the gemcitabine resistance in murine leukemia cells [26,27]. In addition, considerable efforts also have been devoted to the enhancement of orally available Ara-C conjugates, where primary

* Corresponding authors.
E-mail addresses: bio_wangkm@ujn.edu.cn (K. Wang), yuxialuan@sdu.edu.cn (Y. Luan).

https://doi.org/10.1016/j.molliq.2017.12.086
0167-7322/

modifications at the N^4-amino and 5′-hydroxyl were made to prevent the metabolism of cytarabine to Ara-U [28,29]. A series of the N^4 fatty acyl amino acids derivatives of Ara-C (C_{14}-C_{18}) could significantly increase its lipophilicity and the synthesized prodrugs were very effective against sarcoma cell line S_{180} tumor cells [30]. In our previous work, we covalented three different length of mono-chain fatty acids, hexanoic acid (C_6), lauric acid (C_{12}) and oleic acid (C_{18}) — to 4-NH_2 of cytarabine, obtaining the amphiphilic prodrugs of cytarabine with the different hydrophobic length. Interestingly, these prodrugs could self-assemble into different morphology, such as nanospheres, nanobelts and nanofibers depending on the length of the fatty acids [31–33]. Thus it can be concluded that the kind of the fatty acid has important effect on the self-assembled morphology of the nanoassembly in the aqueous solution.

Therefore, in the present work we synthesized a double-chain fatty acid modified Ara-C prodrug by coupling 2-decyltetradecanoic acid (DTA) to 4-NH_2 of Ara-C, denoted as DTA-Ara, and studied the aggregation behavior, the morphology and the anticancer efficiency of the as-prepared prodrug. Surprisingly, the DTA-Ara prodrug could self-assemble into nanoparticles in the aqueous solution with high drug loading (about 63 wt%). Due to the EPR effect, the DTA-Ara NPs could accumulate into tumor sites, and then kill the tumor cells effectively (Scheme 1). Compared with Ara-C, this 4-NH_2 derivative has such merits, firstly, it can prolong the plasma half-life by protecting the NH_2 group of Ara-C; secondly, it can increase the membrane permeability by improving the lipophilicity of Ara-C. The prodrug was synthesized *via* the simple amidation reaction, and nuclear magnetic resonance spectra (^{1}H NMR), mass spectrometry (MS) and Fourier transform infrared spectroscopy (FTIR) techniques were used to determine the successful synthesis of the prodrug. Transmission electron microscopy (TEM), size and size distribution, zeta-potential were utilized to assess physicochemical characteristics of the assembly. The saturation solubility was decreased for 10,707 times compared with free Ara-C, indicating significantly improved lipophilicity. The stability and hemolysis assay of the formulation were evaluated *in vitro* and the results suggested DTA-Ara NPs were stable more than one week and safe to be administrated intravenously. The antitumor activities against K562 and HL60 cells *in vitro* were also conducted, indicating DTA-Ara excerted higher antitumor efficiency than Ara-C. Therefore, the self-assembled DTA-Ara NPs provide a new platform for leukemia therapy with high antitumor capability.

2. Experimental methods

2.1. Materials

Cytarabine (Ara-C) was purchased from Aladdine Industrial Corporation; 2-decyltetradecanoic acid (DTA) was provided by Shenzhen Vtolo Chemicals Co., Ltd. (Shenzhen, China); Ethyl chloroformate (Et-O-CO-Cl) was obtained from Chengdu Huaxia Reagent Chemical Co., Ltd. (Beijing, China); 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) was obtained from Sigma-Aldrich (China). *N*,*N*-dimethylformamide (DMF), triethylamine, dichloromethane and methanol were obtained from Tianjin Fuyu Fine Chemical Co., Ltd. All solvents were of analytical grade and used as received.

2.2. Synthesis of DTA-Ara prodrug

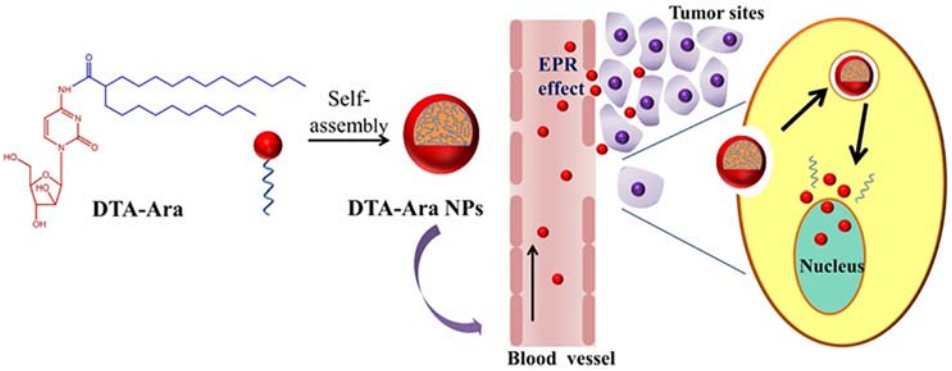
DTA-Ara was synthesized *via* a simple amidation reaction between Ara-C and carbonyl residues of DTA [34,35] and the synthesis route was depicted in Fig. 1. Typially, triethylamine (95.0 mg, 0.940 mmol) and 2-decyltetradecanoic acid (346.5 mg, 0.940 mmol) were dissolved in anhydrous DMF (5 mL). Ethyl chloroformate (102.0 mg, 0.940 mmol) in anhydrous DMF (3 mL) was then added drop-wise into the triethylamine and 2-decyltetradecanoic acid mixture solution under nitrogen. The reaction was kept in an ice bath for 20 min, followed by addition of anhydrous DMF (5 mL) containing cytarabine (246.6 mg, 1.014 mmol). After being stirred for 72 h under nitrogen at room temperature (RT), the reaction solution was filtered to remove the white precipitate. After rotary evaporation, crude product was obtained. The crude product was then purified on a silica gel column with an eluting solvent of methanol/dichloromethane (1:70, v/v). The eluted fraction was monitored by thin-layer chromatography (TLC), and the fractions containing the product were evaporated at 30 °C to obtain the pure compound.

2.3. Characterizations of DTA-Ara prodrug

The structure of the resulting product was determined by ^{1}H NMR (Bruker Avance 400 MHz) with DMSO d_6 as the solvent, electrospray ionization mass spectrometry (ESI/MS, AB SCIEX API 4000) and fourier transform infrared spectroscopy (FTIR, Nicolet 6700).

2.4. Saturation solubility of DTA-Ara prodrug

Saturation solubility of DTA-Ara was determined in various media by the shake flask method [36]. In brief, an excess of DTA-Ara was placed in vials followed by the addition of distilled water and PBS buffer (pH 7.4) with or without 0.5% Tween-80, separately. These mixtures were stirred in a shaker water bath at 100 rpm under the temperature of 37.0 ± 0.5 °C. Samples were incubated for 72 h, and then centrifuged at 13,148 ×*g* for 30 min with Zonkia HC-2062 high speed centrifuge (5 mL × 10, Anhui USTC Zonkia Scientific Instruments Co., Ltd.). The supernatant was analyzed by high performance liquid chromatography (HPLC, Agilent 1200) at 247 nm using methanol/water/acetic acid (90:10:0.1%) as the mobile phase. In addition, the saturation solubility of Ara-C in aqueous solution was studied as described above, and determined by HPLC at 272 nm with PBS (pH 7.4)/methanol (95:5) as the mobile phase.



Scheme 1. Schematic illustration of the preparation, self-delivery and intracellular release of DTA-Ara nanoparticles for cancer therapy.