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A preclinical evaluation of cytarabine prodrug nanofibers assembled from cytarabine-lauric acid conjugate toward solid tumors

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ABSTRACT

Cytarabine (Ara-C) has become cornerstones for the treatment of hatmatological malignancies for several decades; however, it still faces serious challenges in clinical applications due to its side effects such as hand foot syndrome (HFS) and stomatitis. Therefore, considerable researchers have devoted to looking for the new derivative with desirable activity and low toxicity. A new prodrug based on the conjugation of cytarabine with lauric acid (LA-Ara) was synthesized in our group, and it could self-assemble into nanofibers (NFs) in aqueous solution with high drug loading (57 wt%). The lauric acid moiety protects NH_2 group of from the enzymatic attachment and simultaneously raises the lipophilicity of Ara-C, thus obviously prolongs its plasma half-life. The oil/water partition coefficient (lg *P*) and the permeability of cell membrane of LA-Ara were obviously increased compared with Ara-C. Furthermore, the *in vitro* gastrointestinal stability results indicated the prodrug was suitable to be administrated orally. In the current study, the *in vitro* cytotoxicity and *in vivo* anti breast cancer experimental results indicate LA-Ara markedly improved antitumor activity compared with free Ara-C. The favorable safety evaluations elucidated its potentiality for oral alternative treatment to Ara-C. Importantly, LA-Ara can effectively decrease the incidence of toxic effects (HFS and stomatitis) of Ara-C, thereby exhibiting favorable skin safety profile. Overall, these results indicated the LA-Ara would be an excellent candidate for further clinical investigation and simultaneously highlight the prospects of Ara-C prodrug strategies in solid tumors therapy.

1. Introduction

Cytarabine (1-β-D-arabinofuranosylcytosine, Ara-C), a pyrimidine nucleoside analogue, has become the cornerstone of therapy for acute myeloid leukaemia (AML), acute lymphoblastic leukaemia (ALL) and other hematological malignancies for decades (Adamson 2015; Malani et al., 2017). However, its clinical application is hindered because of poor activity against solid tumors *in vivo* (Jordheim et al., 2013). This is mainly ascribed to the poor membrane permeability into the tumor cells, a limited activation via intracellular phosphorylation of Ara-C into Ara-CTP (Ara-C triphosphate, the active metabolite), or a rapid deamination and inactivation of Ara-C to Ara-U (uracil arabinoside) (Momparler 1974; Sharma et al., 2012). To circumvent these issues, nanocarriers delivery strategies of Ara-C prodrug have been pursued with biocompatible lipid conjugates in an attempt to improve the lipophilicity against solid tumors (Cosco et al., 2012; Liu et al., 2015; Liu et al., 2017). However, the progress on translating nanomedicine of cytarabine-based drugs into clinic still keeps great challenge. For example, enocitabine has limited efficacy in patients with AML, particularly in the relapse and survival rates with significant toxicity, including thrombocytopenia and cardiotoxicity (Alexander et al., 2016; Kobayashi et al., 1996). Valcytarabine (5′-L-valyl-Ara-C) targeting intestinal oligopeptide transporter 1 (PepT1) was hopeful for increasing the oral absorption. However, the bioavailability was only 4% after oral administration (Cheon et al., 2006; Sun et al., 2009).

Nanoscaled drug delivery systems (nano-DDS) for anticancer drug

Abbreviations: Ara-C, cytarabine; LA, lauric acid; LA-Ara, conjugation of cytarabine with lauric acid; LA-Ara NFs, LA-Ara nanofibers; AML, acute myeloid leukaemia; ALL, acute lymphoblastic leukaemia; lg *P*, oil/water partition coefficient; Ara-CTP, cytarbine triphosphate; Ara-U, uracil arabinoside; Valcytarabine, 5′-L-valyl-Ara-C; PepT1, intestinal oligopeptide transporter 1; nano-DDS, nanoscaled drug delivery systems; CP-4055, elacytarabine; PPE, Palmar Plantar Erythrodysesthesia; HFS, hand foot syndrome; MTT, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; DMSO, dimethyl sulphoxide; MTD, maximum tolerated dose; NS, normal saline; H&E, hematoxylin and eosin; IC_{50}, half-maximal inhibitory concentration; TIR, tumor inhibition ratio; ALT, aminotransferase; AST, serum aspartate aminotransferase; Crea, creatine; BUN, blood urea nitrogen; WBC, white blood cells; RBC, red blood cells; PLT, blood platelet; HB, hemoglobin; ABCB, ATP-binding cassette sub-family B

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delivery have experienced tremendous development in the past decades. Among others, prodrug-based nano-DDS, which integrates the prodrug strategy and nanotechnology into one single nano-platform, have demonstrated distinct drug delivery advantages in terms of high drug loading, enhanced stability and reduced side effects (Miller et al., 2017; Xu et al., 2018; Zhang et al., 2015; Zhou et al., 2017). The lipophilic 5′-elaidic acid conjugated to cytarabine (elacytarabine, CP-4055), was designed to facilitate cellular accumulation, increasing the retention of cytarabine in leukemic cells and bypassing drug resistance (Roboz et al., 2014; Sun et al., 2017). Unfortunately, the outcomes of clinical trials of CP-4055 fell short of expectations, probably due to the unsatisfactory non-hematologic toxicities including Palmar Plantar Erythrodysesthesia (PPE) and mucositis (Karol et al., 2017; Keane et al., 2013; Wang et al., 2015b). PPE (named as “hand and foot syndrome” (HFS)) is the erythema, dysesthesias, swelling of palms and soles sometimes accompanied by blisters, which also commonly occurs with cytarabine (Sharma and Baghmar, 2013). The cytotoxicity-induced epithelium damage and high risk of infection predispose patients lead to severe mucositis (Sharma et al., 2005).

The concept of hydrophobic fatty acid conjugated to hydrophilic anticancer drugs provided a rational design for the amphiphilic Ara-C prodrug, endowing the prodrugs with self-assembly property and possessing a remarkable high drug-loading (Han et al., 2017; Wang et al., 2015a; Zhang et al., 2017). Our earlier efforts of designing an active prodrug of cytarabine by conjugation of cytarabine with lauric acid (LA) named LA-Ara, led to enhanced biological activities toward leukemia (none solid tumor). After oral administration, LA-Ara absorbed largely intact across the gastrointestinal tract and deliver LA-Ara to systemic circulation. Once inside the cells, LA-Ara was hydrolyzed intracellularly by carboxylesterases to release free Ara-C which is subsequently phosphorylated to the active Ara-CTP, exerting its antineoplastic activity (Chhikara et al., 2010; Wickremsinhe et al., 2013). The prodrug could self-assemble into nanofibers (NFs) in the aqueous solution. The LA-Ara has the merits such as prolonged the plasma half-life by protected NH_2 group of Ara-C and improved membrane permeability (8.2-fold than Ara-C) by the increased lipophilicity. Importantly, the LA-Ara NFs realized the feasibility of Ara-C for oral administration (Liu et al., 2016). The pharmacokinetic studies *in vivo* demonstrate that the oral bioavailability of LA-Ara NFs dramatically increased (32.8-fold than Ara-C).

Recently developed nucleoside analogues are expanding application in wide cancer chemotherapy, leading to our renewed interest in the use of such Ara-C derivatives against solid tumors. Herein, we evaluate the cytotoxicity *in vitro* against 4T1 and MCF-7 cells and assess preclinical antitumor efficacy of LA-Ara NFs on 4T1 breast cancer BALB/c mice model *in vivo*. The results show LA-Ara NFs presented notably more effective therapy than that of free Ara-C for the solid tumors. The safety evaluation indicated that LA-Ara NFs could be safely administered at repetitive doses without untoward toxicity such as hepatorenal dysfunction and blood routine abnormality. Notably, the limitations of Ara-C involving HFS and stomatitis could be overcome by LA-Ara NFs. Therefore, we claim that the LA-Ara NFs might offer a new and efficient platform in the treatment of solid tumors in clinical setting, thereby decreasing the incidence of toxic effects and broadening the applications of Ara-C in oncology.

2. Materials and methods

2.1. Materials

Ara-C was purchased from Aladdin Industrial Corporation. LA was provided by J & K Technology Co., Ltd. 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) was obtained from Beijing Solarbio Technology Co., Ltd.

2.2. Preparation of prodrug nanofibers

The synthesis and characterization of LA-Ara prodrug were described in details in our previous study (Liu et al., 2016). LA-Ara NFs were prepared by a nanoprecipitation method as reported in our previous work (Liu et al., 2016).

2.3. Cell culture

Murine breast cancer cells 4T1 and human breast cancer cells MCF-7 were kindly donated by Department of Pharmacology, School of Pharmaceutical Sciences, Shandong University. The two cells were cultured in RPMI-1640 medium with 10% fetal bovine serum, penicillin (100 units·mL^{-1}) and streptomycin (100 μg·mL^{-1}), under a humidified atmosphere of 5% CO_2. The culture medium was replaced once every two days.

2.4. In vitro cytotoxicity assay of LA-Ara prodrug

The cytotoxicity of LA-Ara against two kind cells was determined using the MTT assay. Briefly, 4T1 and MCF-7 cells were plated in 96-well plates (5000 cells well^{-1}) and incubated at 37 °C for 24 h to allow cell attachment. Different concentrations of Ara-C or LA-Ara were added to the cells before further incubation for 24 or 48 h. Subsequently, 30 μL of MTT solution (5 mg·mL^{-1}) was added to each well and incubated for 4 h at 37 °C, allowing viable cells to reduce the yellow tetrazolium salt into dark blue formazan crystals. The medium was removed carefully and then 100 μL of dimethyl sulphoxide (DMSO) was added. The absorbance at 490 nm was measured using a microplate reader (ELIASA, Perkin Elmer). The cells without treatment served as positive control and the cell inhibition rates were calculated based on the relative absorbance of the samples to the control untreated cells. The calculation of the half-maximal inhibitory concentration (IC_{50}) values and the statistical analysis were performed using GraphPad Prism 5.

2.5. Tumor-bearing animal model

Female BALB/c mice (6–8 weeks old, 18–22 g) were obtained from the Shandong University laboratory animal center (Jinan, China). All the animal experiments in this work were carried out according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals approved by the Institutional Animal Ethical Care Committee (IAEC) of Shandong University.

For preparation of tumor-bearing mice, suspended 4T1 cells (0.2 mL) were injected into the right anterior armpit region of BALB/c mice (female, 18–22 g) at a density of 1×10^7 cells mL^{-1} per mouse (Kanapathipillai et al., 2012; Papa et al., 2017; Sugumaran et al., 2018). Animals were housed in a 12 h light and 12 h dark cycle with free access to food and water.

2.6. Maximum tolerated dose (MTD)

Six week female Kunming mice (n = 5) were administered intravenously with the free Ara-C in 200 μL of normal saline (NS) at concentrations of 20, 40 mg·kg^{-1} or LA-Ara NFs by gavage at concentrations of 15, 20, 25, 30 and 40 mg·kg^{-1}. Mice were monitored once two days for weights and signs of distress daily (anorexia, labored breathing, unresponsive and survival). At day 22 post-injection, blood was collected and frozen at −20 °C, and then mice were euthanized. We monitored the white blood cells (WBC) counts in the Pathology Laboratory (Servicebio technology co., LTD, Wuhan, China).

2.7. In vivo therapeutic efficacy

When tumor volume reached approximately 100 mm^3, the mice