



Doxorubicin hydrochloride-oleic acid conjugate loaded nanostructured lipid carriers for tumor specific drug release

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ARTICLE INFO

Article history:

Received 11 December 2015

Received in revised form 11 April 2016

Accepted 12 April 2016

Available online 13 April 2016

Keywords:

DOX

Oleic acid

NLCs

SAXS

pH-dependent drug release

ABSTRACT

The hydrophilic drug Doxorubicin hydrochloride (DOX) paired with oleic acid (OA) was successfully incorporated into nanostructured lipid carriers (NLCs) by a high-pressure homogenization (HPH) method. Drug nanovehicles with proper physico-chemical characteristics (less than 200 nm with narrow size distribution, spherical shape, layered internal organization, and negative electrical charge) were prepared and characterized by dynamic light scattering, zeta potential measurements, transmission electron microscopy, small-angle X-ray scattering and differential scanning calorimetry. The drug loading and entrapment efficiency of DOX-OA/NLCs were 4.09% and 97.80%, respectively. A pH-dependent DOX release from DOX-OA/NLCs, i.e., fast at pH 3.8 and 5.7 and sustained at pH 7.4, was obtained. A cytotoxicity assay showed that DOX-OA/NLCs had comparable cytotoxicity to pure DOX and were favorably taken up by HCT 116 cells. The intracellular distribution of DOX was also studied using a confocal laser scanning microscope. All of these results demonstrated that DOX-OA/NLCs could be a promising drug delivery system with tumor-specific DOX release for cancer treatment.

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1. Introduction

DOX, one of the oldest anthracyclines, is widely used to treat various cancers, hematological malignancies, soft tissue sarcomas, and solid tumors [1]. However, the low penetration and limited distribution of DOX in solid tumors are the main causes for its failure as a therapeutic agent [2]. Because of the low pH in the extracellular environment of a tumor, weakly basic drugs, such as Doxorubicin, are protonated and therefore display decreased cellular uptake [3]. In addition, due to its nonspecific distribution in healthy tissues, it has severe side effects, such as cytotoxicity to healthy tissues, inherent multidrug resistance, dose-limited cardiotoxicity and myelosuppression, which limit the dosage and use

in clinical applications [4–8]. Therefore, it is important to develop a novel microcarrier that can be used for targeted drug delivery to tumors and thereby improve the therapeutic index of the carried drugs [9].

One approach for increasing drug bioavailability at the tumor site is to use site-specific triggers that can release drugs in diseased tissue. Another approach is to obtain higher drug accumulation by active targeting. However, when drug carriers bind to the first line of target cells, they may obstruct the binding ligands, preventing the additional accumulation of drug carriers [10]. Endogenous variations in the local microenvironment of the disease sites (pH, enzymes, and redox-potential) and exogenous triggers (temperature, light, and magnetic field and their combinations) have been proposed to accomplish site-specific triggered drug release in tumor tissue [11–15]. Triggered release of pH-sensitive drug carriers is probably the most biocompatible method for releasing drugs directly in the cytoplasm of cells [16]. The original strategy of using

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a tumor's acidic microenvironment for triggered release [17] has not been very successful because the location of highest acidity in tumors is distant from the tumor vasculature. Because the pH value of the tumor interstitium is below 6.5, the range of possible drug carriers is limited. For example, popular liposome carriers become unstable and are disrupted before reaching their target [18–22].

One of the most promising drug delivery systems is nanostructured lipid carriers (NLCs) [23,24]. NLCs are more stable than liposomes and exhibit several additional advantages, such as increased bioavailability, enhanced chemical and physical stability of the incorporated bioactive, sustained control over release and loading capacity, and easy large-scale production [25]. Additionally, all lipids used are biocompatible and biologically safe for oral and topical administration [26–28]. The utility of NLCs is based on their potential to enhance drug delivery into tumor tissues while minimizing systemic exposure and thus enhancing drug efficacy and reducing non-specific toxicity [29].

To achieve acid-triggered release, oleic acid (OA), as a lipophilic ion pairing agent, was used to form a DOX-OA ionic conjugate (DOX-OA). The ionic pair is unstable in an acidic environment (pH < 7), such as tumor tissue, but is stable in a neutral environment (pH 7.4), such as blood [30,31]. This will help increase the accumulation of the drug in the target sites and spare normal tissues. Moreover, ionic pairing can improve the retention of DOX in the lipid matrix, thus increasing the encapsulation efficiency in NLCs [30]. Furthermore, it has been reported that OA can improve drug effectiveness by its putative involvement in perturbations in the fluidity and permeability of tumor cell membranes [29].

DOX-OA ionic complexes have been loaded into lipid nanoemulsions (LNs) by the lipid film hydration method [32], which show favorable cell uptake and blood circulation characteristics. The preparation of LNs requires organic solvents and large amounts of surfactants, and the obtained LNs had a particle size above 200 nm. The enhanced permeability and retention (EPR) mechanism is expected for smaller particles (100–200 nm) [33–35].

In this report, DOX-OA/NLCs smaller than 200 nm were prepared with high entrapment efficiency, good stability and pH responsive drug release (increasing at acidic pH), which could allow active targeting to cancer cells.

2. Materials and methods

2.1. Materials

Doxorubicin hydrochloride (DOX, Beijing Huafeng Alliance Bernstein Co. Ltd., China), stearic acid (SA, Lingfeng Chemical Reagent Co. Ltd., China), monoglyceride (GMS, Aladdin Chemical Reagent Co. Ltd., China), oleic acid (OA, Aladdin Chemical Reagent Co. Ltd., China), medium-chain triglyceride (MCT, Aladdin Chemical Reagent Co. Ltd., China), Pluronic F68 (F68, Adamas Reagent Co. Ltd., China), Cremophor EL (EL, Aladdin Chemical Reagent Co. Ltd., China), trehalose (Aladdin Chemical Reagent Co. Ltd., China), and 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma, China) were used as received.

2.2. Preparation of DOX-OA

DOX-OA was prepared using a co-precipitation method [36] as described in the literature [32] with some modifications. In brief, an aqueous solution of Doxorubicin hydrochloride (5 mg/mL) was added into a centrifuge tube, and then, a sodium bicarbonate solution (50 mg/mL) was added to the tube under magnetic stirring to neutralize the charges of the cationic DOX salt. Next, an OA solution (50 mg/mL) in ethanol was added to the above mixture while stirring. After continuous stirring for 1 h, the mixture was

Table 1

Various content of lipids and surfactants for the preparation of different NLC formulations, made with double distilled water to a final volume of 100 mL.

Formulation	Lipids/mg				Surfactants/ mg		DOX-OA/mg
	GMS	SA	MCT	OA	F68	EL	
1	537	298	296	118	125	125	80
2	498	249	297	118	125	125	80
3	332	166	249	83	125	125	80
4	272	136	249	83	125	125	80
5	297	119	296	96	125	125	80

centrifuged (5000 r/min, 30 min). Finally, the red precipitate collected was DOX-OA. The red precipitate was washed three times with distilled water and then dried at 40 °C and stored at 4 °C. The postulated structure of DOX-OA is shown in Fig. 1A.

2.3. Lipophilicity of DOX-OA and DOX

To compare the lipophilicity of DOX-OA and DOX, their saturated solubilities in *n*-octanol and MCT were determined. DOX and DOX-OA were dissolved in *n*-octanol and MCT and then agitated for 48 h at 25 °C. After equilibration, the samples were centrifuged at 10000 rpm for 15 min. The supernatants were diluted in isopropanol and subsequently quantified by a UV-visible spectrophotometer (Shimadzu, Kyoto, Japan) at a wavelength of 495 nm.

2.4. Preparation of DOX-loaded nanostructured lipid carriers (DOX-OA/NLCs)

The NLCs with or without drug were prepared by the HPH method. Briefly, the lipid phase (a mixture of solid and liquid lipids) was heated until molten at 70 °C, followed by the addition of DOX-OA. At the same time, the aqueous phase was prepared by dissolving surfactants in distilled water and heating at the same temperature. Then, the hot aqueous phase was added to the molten lipid phase under stirring by a homogenizer (Turrax T25, Fluko, Germany) at 10000 rpm for 2 min. Finally, the coarse emulsion was processed through an HPH (ATS Engineering, Canada) for 6 cycles at 600 bar. The prepared nanodispersions were cooled overnight to obtain NLCs. The details of the DOX-OA/NLC formulations are shown in Table 1.

For long-term storage, DOX-OA/NLCs were freeze-dried and trehalose (3%, w/v) was chosen as a cryoprotectant to decrease the aggregation of DOX-OA/NLCs during the freeze-drying process. The DOX-OA/NLC dispersions were pre-frozen at –20 °C for 24 h. Then, the samples were freeze-dried at –50 °C for 72 h to obtain powders for further experiments by utilizing a freeze dryer (Boyikang, China).

2.5. Particle size, polydispersity index and zeta potential measurements

The mean particle size, polydispersity index (PDI) and zeta potential of DOX-OA/NLCs were measured at 25 °C using a Particle Analyzer (Delsa Nano C, Malvern Instruments, Malvern, UK). Before the measurements, the samples were diluted appropriately with distilled water. All of the measurements were performed in triplicate.

2.6. Transmission electron microscopy

The morphology of DOX-OA/NLCs was examined by transmission electron microscopy (TEM) (JEOL-1400, Jeol, Tokyo, Japan).

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