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Enhanced intercellular release of anticancer drug by using nano-sized catanionic vesicles of doxorubicin hydrochloride and gemini surfactants

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ABSTRACT

In this study, we provide an alternative formulation to develop gemini surfactant (GSs)–drug nano-sized catanionic vesicles with multi stimuli-responsiveness, which have great potential applications in the fields of controlled release and drug delivery in chemotherapy. The main aim of this study was to investigate how catanionic vesicles could influence the encapsulation and release of the anticancer drug Doxorubicin (DOX). The DOX was self-associated in an aqueous medium according to reported studies still controversial. We present here the simple and convenient methods, surface tension, conductance and circular dichroism (CD) studies to give direct evidence that DOX was self-associated at the critical concentration about 0.841 mmol L^{-1}. The influence of micelle based on DOX-loaded (DLs) and mixed micelle ($C^*(\alpha_{DOX} + \alpha_{GSs})$) catanionic vesicles (DGCs) showed the highly synergistic interaction. The physicochemical properties, molecular docking, drug uptake and release of DOX in in-vitro for DL and DGC were explored. The substantial amounts of DOX could successfully encapsulate into the catanionic vesicles. The important point of DGC vesicles was they could reduce toxicity and revealed better therapeutic effects when the DOX was in high concentration. Moreover, the cell viability of DL-12 vesicles was 44%, and DL-16 vesicles was 70% at 18 μmol L^{-1}, while at 20.0 μmol L^{-1} it was 44% in DGC-12 as α_{DOX} was 0.6 and 56% in DGC-16 as α_{DOX} was 0.9. The in-vitro cytotoxicity and fluorescence microscopic images of DL and DGC vesicles on MCF-7 cell lines showed the improved nuclear localization of DOX uptakes. These findings showed the high importance of catanionic vesicles to enhance the free DOX entrance mainly via endocytosis for breast cancer cell.

1. Introduction

Though human has struggled with cancer in the past five decades, the incidence of cancer is still on the rise. Due to the ageing of the population and the possible role of risk factors, it is expected that the worldwide cancer deaths continue to increase. At present, the main treatment methods of cancer are surgical treatment, chemotherapy, radiotherapy, hormonotherapy, gene therapy and immunotherapy. These methods are often used together, and chemotherapy is the most widely used method. The key of chemotherapy is to improve efficacy and reduce the toxicity of drugs. Nano-sized drug carriers can improve efficacy and reduce the toxicity of drugs by increasing the permeability of biomembrane and improving bioavailability. It is of significance to study the nano-sized drug carriers for chemotherapy. Gemini surfactant is inclined to form aggregates at a lower concentration, which could be a good candidate for drug carriers [1–4]. It is well-known that DOX is an anticancer drug widely used in clinical practice, therefore, taking advantage of gemini surfactant to carry DOX would be an effective way to improve efficacy and reduce the toxicity of drugs. However, DOX-gemini surfactant catanionic vesicles used as nano-sized drug carriers are few reported.

Mixed micelles formed by the self-aggregation of surfactants, lipids or block copolymers in aqueous solution [5,6], are a kind of nano-sized drug carriers. Bhattacharjee et al. [7] developed and characterized the mixed micelles of Tween-80 and sodium deoxycholate and evaluated their potential in the delivery of DOX, a cationic anticancer drug. In their work, they suggested that salt-induced growth of the mixed micelles caused by both the electrostatic interaction of the anionic bile salts and steric repulsion of the ethylene oxide groups in nonionic components at different compositions were affected by the presence of electrolytes. The anionic bile salt could be successfully bound to the cationic drug DOX. The studies in various cancer cell lines in-vitro cytotoxicity revealed that DOX-loaded micelles have greater in-vitro anticancer activity as compared to DOX solution. Therefore, the nano-sized drug carriers for DOX may offer the right solutions to overcome the problems related to cytotoxicity and resistance [8–18]. Moreover, the nano-sized drug carriers would be more effective when the DOX shows the aggregation behaviour. Several studies for anthracycline drug on concentration-dependent absorbance have been interpreted by proton NMR and

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circular dichroism spectra regarding simple dimerization model [19–22]. These anthracycline drugs are well-known to aggregate beyond the dimer insignificantly, although Barthalemy-Clavey et al. [20] did suggest that further aggregation might occur at drug concentrations ≥5 mM.

Vesicles are of technological interest for applications ranging from drug delivery and controlled release to bio-separations and sensing [20–23]. Many of these application relies upon the ability of vesicles to entrap desired chemicals in their interior and after that release these chemicals into the external medium in a controlled manner. Recently, a new class of self-assembled amphiphilic aggregates referred to catanionic systems with rich supramolecular architectures (worm-like micelles, vesicles, lamellar structure) has been developed for drug delivery [24–29]. In catanionic systems, the amphiphilic drug molecules with the charged hydrophilic head group and hydrophobic chain have potential to form the drug participating catanionic vesicles with an oppositely charged surfactant. The drug molecules participate in its self-formulation and the loaded content could largely increase over the surfactant constructed catanionic vesicles. The cytotoxicity of this system could greatly decrease due to the smaller the content of carrier material. Moreover, the drug-participating catanionic vesicles can further effectively load other hydrophilic drugs inside the aqueous cell or hydrophobic drugs in the hydrophobic bilayer to obtain a multifunctional drug delivery system [30,31]. Therefore, the kind of drug-participating catanionic vesicles can solve the current problems due to their several advantages over the pure surfactant-constructed catanionic vesicles.

However, the self-assembly properties of DOX and mixed catanionic vesicles of the DOX/gemini surfactant are relatively unexplored as catanionic systems. The main aim of current work is to construct the nano-sized DOX/GSs catanionic vesicles (DOX–GSs) and investigate their cytotoxicity and anticancer activity in cell lines. Also, the self-association behaviour of cationic drug DOX in the aqueous medium is detected. The *N*,*N*′-dialkyl-*N*,*N*′-diacetate ethylenediamine amino acid GSs is selected to form vesicles as the drug carriers. DOX used as an amphiphilic molecule can form aggregates, showing that hydrophobic or electrostatic interactions in the present system are sufficiently strong to ensure the thermodynamic stability of the complex. Our results suggested that the mechanism of binding for DOX-GSs might explore the new approach for the most suitable drug delivery systems [32]. Moreover, the loaded DOX could release effectively in a tumor-simulated microenvironment with weakly acidic medium and the high mole ratio of DOX–GSs. Notably, cytotoxicity experiments indicated that DOX–GSs vesicles could significantly enhance the anticancer efficiency of DOX on breast cancer cell (MCF-7). Cellular uptake measurements revealed that the DOX–GSs mixed nano-sized vesicles, entering cancer cells mainly through endocytosis, could result in a remarkable drug accumulation, especially in tumor cells, indicating a good potential for cancer treatment.

2. Experimental

2.1. Materials

DOX was purchased from Tokyo Chemical Industry Co. Ltd (Tokyo, Japan). Dulbecco's modified Eagle medium, fetal bovine serum and penicillin-streptomycin solution were purchased from GE Healthcare Life sciences (Logan, Utah). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide was purchased from KeyGEN BioTECH (Nanjing, China). Sodium hydrogen phosphate, monobasic potassium phosphate, potassium chloride and sodium chloride were purchased from Tianjin Bodi Chemical Co., Ltd (Tianjin, China). Millipore water was used throughout the study.

The used pH-sensitive amino acid GSs have the chemical structure Ace(n)-2-Ace(m), where GS-12 was n = 10 and m = 12, and GS-16 was n = 10 and m = 16. They were synthesized and characterized using previously reported methods [33–35]. The structures of GSs was shown in Fig. S1.

2.2. Experimental methods

2.2.1. Preparation of catanionic vesicles

The stock anionic GSs (5.0 mmol L^{-1}) and cationic DOX (5.0 mmol L^{-1}) were firstly dissolved in buffer individually. Two different types of vesicles were prepared for this works. 1) DL-12 and DL-16 vesicles were prepared by simple mixing of 0.1 mmol L^{-1} of DOX into 0.5 mmol L^{-1} GSs. 2) DGC-12 and DGC-16 vesicles were prepared at different molar ratios (C*($\alpha_{DOX} + \alpha_{GSs}$), α_{DOX} = 0.0, 0.1, 0.3, 0.4, 0.5, 0.6, 0.7, 0.9) at a total concentration (C) of mixture 0.5 mmol L^{-1}, and C was 5.0 mmol L^{-1} when α_{DOX} was 1.0, shown in Table 1. The different mole ratios of GSs were α_{GSs} = 1.0, 0.9, 0.7, 0.6, 0.5, 0.4, 0.3, 0.1. All samples of catanionic vesicles were prepared in 0.1 M phosphate buffer saline (PBS, pH 7.4) for determined different parameters. Phosphate buffer (pH 5.8) was used to comparative in-vitro drug release study with pH 7.4.

The other experimental techniques were explained in the electronic supplementary information (ESI) Section 1.0.

3. Results and discussion

3.1. Self-association of DOX

Dimers and higher order oligomers of DOX would aggregate in aqueous solution. These aggregates were highly sensitive in the presence of additives like buffer and excipients [18,36,37]. Based on the method reported by Barthalemy-Clavey et al. [20] and Anand et al. [22] that CD spectra could be used for measuring the extent of anthracycline drugs dimerization, we calculated the CD spectra of DOX monomers and dimers to allow for a direct comparison with experimental results. Fig. 1 (A) shows the CD spectra of DOX at different concentrations in the wavelength range 200 to 600 nm. At low concentration, DOX was mainly in monomeric form, its spectrum was composed of two negative bands with maxima at 300 and 560 ± 5 nm, and four positive bands with maxima at 268, 350, 435 and 500 ± 5 nm. Increasing the concentration of DOX led to important changes in its CD spectra. The intensities of the negative band decreased at 300 nm from a concentration 0.2 to 0.4 mmol L^{-1} and increased from a concentration 0.45 to 2.0 mmol L^{-1} of DOX. The negative bands at 560 nm shifted to higher wavelength 588 nm at 0.2 to 2.0 mmol L^{-1}.

The intensities of a positive band at 350 nm increased at a concentration from 0.20 to 0.75 mmol L^{-1} and decreased at a concentration from 1.5 to 2.0 mmol L^{-1}. It is very interesting that with increasing concentration of DOX, the four positive bands were found in CD spectra as shown in Fig. 1(A). Moreover, the positive band at 500 to 563 nm shifted to a higher wavelength while that at 435 to 410 nm shifted to a lower

Table 1
The DL and DGC vesicles formulation with concentration.

DL vesicles		DGC vesicles formation with varying mole fraction ($\alpha_{DOX} + \alpha_{GSs}$) at concentration (C) of the mixture. (C*($\alpha_{DOX} + \alpha_{GSs}$)		
DOX (mM)	GSs (mM)	C (mM)	α_{DOX}	α_{GSs}
0.1	0.5	0.5	0.0	1.0
		0.5	0.1	0.9
		0.5	0.2	0.8
		0.5	0.3	0.7
		0.5	0.4	0.6
		0.5	0.5	0.5
		0.5	0.6	0.4
		0.5	0.7	0.3
		0.5	0.8	0.2
		0.5	0.9	0.1
		1.0	1.0	0.0