Biomedicine & Pharmacotherapy 66 (2012) 46–51

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Original article

N-octyl-O-sulfate chitosan-modified liposomes for delivery of docetaxel: Preparation, characterization, and pharmacokinetics

Guowei Qu, Xiaoli Wu, Lifang Yin *, Can Zhang *

State Key Laboratory of Natural Medicines, China Pharmaceutical University, Nanjing 210009, People's Republic of China

ARTICLE INFO

Article history:
Received 30 May 2011
Accepted 1 September 2011

Keywords:
Chitosan derivative anchored liposome
Docetaxel
Pharmacokinetics

ABSTRACT

A N-octyl-O-sulfate ch\itosan (NOSC) anchored liposome system was developed as the carrier for antitumor drug, docetaxel (DTX). The physicochemical and pharmacokinetic properties of NOSC-modified DTX liposomes (NDLs) were evaluated compared with the conventional DTX liposomes (DLs) and commercial dosage form of DTX, Taxotere®. The results showed that NDLs had DTX-loading rate of 3.41%, entrapment efficiency of 61.73%, narrow distributed particle size of 147.6 ± 1.9 nm, and high zata potential of −44.2 ± 3.9 mV. The decreased permeability of the liposome bilayer was evaluated by release behavior of calcein (CAL) from the internal phase of NOSC-modified CAL liposomes (NCLs) and enhanced stability of NDLs owed to shielding effect of sulfonic shell from adsorption by BSA. After i.v. administration at the dose of 12 mg/kg, a significant increase in the AUC, MRT, and $T_{1/2\beta}$ ($P < 0.05$) was observed in NDLs group compared with DLs and Taxotere® group. $AUC_{0-\infty}$ of NDLs was 6.14 and 1.55 times higher than Taxotere® and DLs, respectively, and $MRT_{0-\infty}$ of NDLs was 5.77 and 1.37 times higher than Taxotere® and DLs, respectively. All these results suggested that anchored liposomes could increase the stability of DTX in vitro and in vivo, as compared with conventional liposomes and Taxotere®. Therefore, NOSC as a polymeric shell to liposomes was effective to enhance the stability of liposomes containing DTX.

1. Introduction

Over the past two decades, the taxane family (Paclitaxel, Docetaxel) of anti-cancer drugs has played a significant role in the treatment of various malignancies. As an alternative to Paclitaxel (PTX), Docetaxel has shown significant antitumor activities against breast cancer, ovarian carcinoma, lung cancer, and head/neck cancer [1]. As a microtubules stabilizer, DTX binds to the β-tubulin subunit of the microtubules and promotes the assembly and stabilisation of microtubules, thus blocks cells in the M-Phase of the cell cycle and results in apoptosis [2,3].

Due to its poor water solubility, DTX is formulated using Tween 80 (polysorbate 80) and ethanol (50:50, vol/vol) and registered in the form of an injectable solution (Taxotere®). It is noteworthy that dose-limiting toxicities were observed at different degrees in the clinical research of Taxotere®, including neutropenia and peripheral neuropathy, the other side effects were quite frequent and dose-dependent including hypersensitivity reactions, fluid retention and so on [2,4,5]. There is now a general consensus that Tween 80 is a biologically and pharmacologically active compound. Indeed, previous research has showed that the administration of Tween 80 to patients presents a number of serious intrinsic adverse effects, including acute hypersensitivity reaction and peripheral neuropathy. Under some cases, Tween 80 may induce some more serious adverse effects than the drug solubilized in the formulation [6]. Oleic acid in Tween 80 has been likely implicated in the mechanism of hypersensitivity reactions observed in the therapy using Taxotere® [6,7]. Similar to Taxol®, the commercial dosage form of PTX, the presence of non-ionic surfactants in formulation may result in the unfavorable nonlinear distribution tendency which is also observed in the case of Taxotere® [8].

The drawbacks of Taxotere® have spurred interest in developing alternative delivery free from Tween 80. Currently, several strategies are in progress to improve therapeutic index and reduce adverse effects [9], including liposomes [10], nanoparticles [11], submicron lipid emulsion [12], micelles [13] and so on. Among various new drug carrier systems, liposomes have shown some advantages such as higher stability, good biocompatibility, non-toxicity, biodegradability, and controlled release of the encapsulated drug. Especially, the long-circulating liposomes could slowly accumulate in tumor, inflammation or infarcted sites with affected and leaky vasculature via enhanced permeability and retention effect (EPR effect) [14,15]. PEGylation on the surface of liposomes is the most popular and successful method to obtain long-circulating and biologically stable liposomes [16,17]. This approach prevents adsorption of opsonins onto liposomes and fast phagocytosis of liposomes by the reticulo-endothelial system (RES) [18], which

* Corresponding authors.
E-mail addresses: yinlifangcpu@yahoo.com.cn (L. Yin), zhangcancpu@yahoo.com.cn (C. Zhang).

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doi:10.1016/j.biopha.2011.09.010

will simultaneously increase systemic exposure while decrease blood clearance, thus lead to an alteration in the pharmacokinetic characteristics of the solubilized drug [19,20]. And another advantage achieved by PEGylation is its enhanced storage stability via preventing formation of larger vesicles between liposome particles [21].

Different types of biocompatible polymer beyond PEG and its derivatives such as chitosan can be employed to improve the efficiency of conventional liposomal systems. It is hoped that the candidates could get even better control over the stability and release behavior of the encapsulated drug [22]. Among them, chitosan, a polysaccharide derived from chitin by complete or incomplete alkaline deacetylation, is the most attractive candidate due to its biochemical activity, biocompatibility, biodegradability and low toxicity [23].

In our previous work, amphiphilic chitosan derivative, N-octyl-O-sulfate chitosan (NOSC) was prepared by octylation of amino group at C-2 position and sulfonylation at C-6 position of chitosan and evaluated as drug carrier for intravenous injection [24,25]. Furthermore, PTX and Gambogic acid were successfully solubilized in the micelles self-assembled by NOSC [26,27]. In this study, NOSC was used to modify the conventional liposomes via hydrogen bond and hydrophobic interaction between octyl moiety of NOSC and phospholipid bilayers of liposomes [28]. The flexible chitosan chains wrapped on liposome surface might enhance stability and delayed the drug released. In addition, like PEGylation, the hydrophilic sulfonic group surrounding the liposome surface rendered the liposomes stability by inhibiting the MPS uptake and therefore increases drug circulation time. Sulfonic group itself demonstrated strong electronegativity, which could significantly convert the near electrically neutral surface of soy lecithin phospholipid lipisomes to drastic negativity with high net ζ-potential. Electrostatic repulsion between NOSC-grafted bilayers might effectively enhance the physical stability of liposomes.

2. Materials and methods

2.1. Materials

Docetaxel (DTX, 99.5%) was provided by Shanghai Jinhe Biological Products Co. Ltd (Shanghai, China). Biomedical grade chitosan was purchased from the Shuanglin Biochemical Co. Ltd (Nantong, China), with deacetylation degrees of 92% and viscosity average molecular weight of 65 kDa. NOSC was synthesized using the chitosan mentioned above by our group, and the substitution of octyl degree and sulfonic degree were 0.38 and 2.56, respectively.

Soybean phosphatidylcholine (SPC, Lipoid S 100) was purchased from Lipoid (Ludwigshafen, Germany). Cholesterol (CHOL, $>$ 95%) was supplied by Shanghai Huixing Biochemical Reagent Company (Shanghai, China). Sephadex G-50 was purchased from Amersham Pharmacia Biotech (Piscataway, USA). Bovine serum albumin (BSA) was purchased from Booker Biotechnology Co. Ltd (Nanjing, China). Calcein and Mannitol were purchased from Shanghai SSS Reagent Co. Ltd (Shanghai, China). Fluorescein isothiocyanate (FITC) and triton x-100 were obtained from Sigma-Aldrich (Saint-Louis, USA). HPLC/spectra-grade reagents were used as the mobile phase in HPLC analysis, and all other reagents were analytical grade and used without further purification. Distilled and deionized water was used in all experiments.

2.2. Animals

Sprague-Dawley (SD) rats (200–250 g, male), obtained from the Shanghai Silaike Laboratory Animal Limited Liability Company, were maintained under uniform experimental conditions (temperature 25 ± 2 °C; humidity 60 ± 5%, 12 hours dark/light cycle) for 1 week prior to experiments. All the animals were pathogen free and allowed access to food and water freely. The experiments were carried out in compliance with the Regulations for the Administration of Affairs Concerning Experimental Animals.

2.3. Preparation of DTX liposomes (DLs), NOSC-modified DTX liposomes (NDLs) and chitosan-modified DTX liposomes (CDLs) via ethanol injection method

The DLs and NDLs used in the pharmacokinetic studies were prepared by ethanol injection method, as described below. SPC, CHOL and DTX (100 mg, 7 mg and 6 mg, 100:7:6, wt/wt) were dissolved in 3.0 mL of ethanol. Then, using a 21-gauge needle, the solution was injected very slowly into 15 mL of a 55 °C phosphate buffered solution (PBS, 0.05 M, pH 7.4) under vortexing. Ethanol was removed from the solution by rotary evaporation at 40 °C to obtain the multilamellar lipid vesicle (MLV) suspension. Then the MLV suspension was treated with probe-type ultrosonic for 160 cycles (200 W), one cycle endures 1 S, and the interval was 2 S. The liposome solution was filtered with a 0.45 μm pore-sized microfiltration membrane to remove the un-encapsulated DTX from liposomes solution. 5% (wt/vol) mannitol was selected as lyophilized protective, and the dried powder of DLs was obtained using a freeze dryer system (Christ, Alpha 1-4, Germany).

NDLs were prepared using post-incubation method by anchoring NOSC on the surface of DLs. A certain amount NOSC dissolved in 1 mL water was added dropwise into 9 mL of DLs under magnetic stirring, the ratios of NOSC/SPC (wt/wt) were fixed at 1%, 3%, 5%, 7%, 10%, 12% and 15%. Then the mixture was incubated at 55 °C for 60 minutes. NDLs were obtained after filtration using a 0.45 μm pore-sized microfiltration membrane. And the lyophilized powder of NDLs was obtained using the same method mentioned above.

CDLs were also prepared using post-incubation method as a control group in the stability studies. 10% SPC weight of chitosan dissolved in 1 mL 1% acetic acid solution was added dropwise into 9 mL of DLs under magnetic stirring. After stirring for 30 minutes at room temperature, the mixture was filtrated using a 0.45 μm pore-sized microfiltration membrane. The powder of CDLs could obtain after lyophilization. All the liposome solutions and liposome powders were store at 4 °C before usage.

2.4. Preparation of calcein liposomes (CLs) NOSC-modified calcein liposomes (NCLs) via reverse evaporation method

Calcein, a fluorescent molecule, was incorporated into the conventional liposomes and NOSC-modified liposomes to investigate the effects of NOSC on the permeability of liposome bilayers. CLs and NCLs were prepared by the reverse evaporation method [29]. Briefly, 100 mg of SPC and 7 mg CHOL were dissolved in 6 mL of ether/chloroform (2:1, v/v) and mixed with 5 mL of calcein solution (5×10^{-3} mol/l in PBS) under ultrosonic to obtain a homogeneous W/O emulsion. The mixtures were placed on a vacuum rotary evaporator to remove organic solvents until the formation of O/W emulsion. After addition of subsequent 3 mL of PBS, the liposome suspension was sonicated using probe sonicator in the same intensity as above. CLs were then extruded through a 0.45 μm pore-sized microfiltration membrane and free calcein in exterior phase was separated through Sephadex G-50 column. NCLs were prepared to use the same post-incubation method as NDLs.

2.5. Characterization of liposomes

Particle sizes, size distributions and ζ-potentials of the liposomes were measured by using a Zetasizer 3000 HS instrument (Malvern Instruments, Malvern, UK). Dynamic light scattering

(DLS) was accomplished with 633 nm He-Ne lasers at 25 °C. The morphology of liposomes was studied to use transmission electron microscopy (TEM) after negative staining. Briefly, the liposomes samples were dropped onto copper grids. Then, the grids were immersed in 2% phosphomolybdic acid aqueous solution for 5 min and blotted to dry with a piece of filter paper. Finally, the grids were imaged to use a transmission electron microscope JEM-200CX (JEOL, Japan) at 200 kV.

2.6. Permeability of liposomes

The permeability of bilayer was evaluated by released behaviors of calcein from the internal phase of CLs and NCLs prepared above [30]. Briefly, 5 mL of the CLs or NCLs was placed into the dialysis tube (Molecule Weight Cut-Off 10,000, Sigma-Aldrich Co., USA) and dialyzed against 100 mL PBS in a shaker (60 rpm, 37 ± 0.5 °C). At 0, 2, 6, 10, 15, 20, 24, 36 and 48 h, 2 mL of the release media was collected and then the fresh release media were added. The release amount of CAL was determined by fluorescence spectrophotometry as described earlier. The accumulative release percentage of CAL (% CAL release) was calculated according to the following equation:

$$\%\ \text{CAL release} = (D1/D2) * \%,$$

where D1 is the amount of drug released from the beginning to the scheduled time, and D2 is the total amount of drug in liposome suspension.

2.7. Stability studies of liposomes

The physical stability of liposomes was studied by monitoring the changes of particle sizes over time. A measured amount of DLs, CDLs and NDLs were dissolved in 15 mL PBS with or without 1.5 wt% BSA to obtain a DTX concentration of 0.25 mg/mL. The liposome solutions were incubated at 37 ± 0.5 °C and the experiments were carried out for 4 h. At scheduled time intervals, the particle size of each liposome solution was measured three times and recorded.

2.8. Pharmacokinetic studies of Taxotere®, DLs and NDLs in rats

The pharmacokinetic behavior of NDLs was evaluated by the determination of the DTX content in rat plasma, Taxotere® and DLs were studied as control. The lyophilized powder of NDLs and DLs were reconstituted with 5% glucose injection and sterilized through 0.45 μm pore-sized micropore films before intravenous injection. Taxotere® was used in accordance with directions. 15 SD rats (200–250 g) were used and randomly divided into three groups (n = 5). Then the solutions were injected into the tail vein of rats at a dose of 12 mg/kg. At 2, 5, 10, 20, 30, 60, 120, and 240 minutes after injection, blood samples (0.5 mL) of Taxotere® group were collected from the plexus venous in eye ground. While the blood samples (0.5 mL) of DLs and NDLs groups were collected at 2, 5, 10, 20, 30, 60, 120, 240, 480 and 720 minutes after injection using the same method as above. 0.15 mL plasma was obtained by centrifugation at 4000 rpm for 10 min, 3 mL acetonitrile was added before vortex for 3 min. After centrifugation at 10,000 rpm for 10 min, 20 μL of clear supernatant was injected into HPLC system directly and the DTX concentration in rat plasma was calculated by standard curve. Drug and Statistics for Windows (DAS ver2.0) was utilized to analyze the pharmacokinetic parameters of the area under the plasma concentration–time curve ($AUC_{0-\infty}$), the apparent volume of distribution (V_c), total body clearance (CL), distribution half-life ($t_{1/2\alpha}$), elimination half-life ($t_{1/2\beta}$) and mean residence time (MRT) of DTX for each formulation.

2.9. HPLC analysis of DTX

The DTX concentrations were analyzed by a reverse-phase HPLC sytem (Agilent 1200 series, Agilent Technologies, USA), and chromatographic separation was achieved using a Lichrospher C18 column (4.6 × 250 mm, Hanbon, China) at 25 °C. The mobile phase was a mixture of methanol and water (80:20 v/v). The samples were delivered at a flow rate of 1.0 mL/min and detected at a wavelength of 229 nm. The in vitro and in vivo standard curves were set up and satisfactory linearities were obtained.

The DTX content in the liposomes was determined by measuring the DTX concentration after destroying the liposome structure with addition of an appropriate amount of mobile phase. A certain volume of DTX-loaded liposome solution was diluted 50 times with mobile phase, and 20 μL of dilution was injected into the HPLC system. Otherwise, the DTX levels in blood samples were determined with pretreatment. The DTX-loading rate in liposomes was calculated by the following equation:

$$\text{DTX-loading rate} = C * V/(X_{\text{LIPOSOME}}) * 100\%,$$
$$\text{Entrapment efficiency} = C * V/(W_{\text{TDX}}) * 100\%.$$

where C, V, W_{LIPOSOME} and W_{DTX} represented the DTX concentration of liposome solution, the volume of liposome solution, the weight of liposomes after freeze-drying and the weight of DTX feeded, respectively.

2.10. Statistical analysis

Statistical analysis was performed by Student's t-test for two groups, and one-way ANOVA for multiple groups. All results were expressed as the mean ± standard deviation (mean ± SD) unless noted exceptionally, a probability (P) of less than 0.05 is considered statistically significant.

3. Results

3.1. Preparation and characterization of liposomes (DLs, NDLs, CDLs, CLs and NCLs)

An ethanol injection method was utilized to prepare DLs and NDLs. The formulation for the preparation of DLs was optimized by central composite design (Design expert 7.0) and DTX-loading rate, entrapment efficiency, particle size and polydispersity index of DLs and NDLs were measured. The drug-loading rate and entrapment efficiency were 3.98 ± 0.15% and 72.64 ± 2.19%, while the predicted values obtained from formulation Optimization Model were 4.01% and 74.59%, respectively, which brought a bias (%) less than 3%. The different NOSC/SPC weight ratios were investigated to prepare NDLs. The effects of NOSC/SPC weight ratio on the size and zeta potential of NDLs were shown in Fig. 1. As shown in Fig. 1, the DTX contents in NDLs decreased to a constant value around 85% with modification of NOSC (NOSC/SPC ratios (wt/wt) ranged from 1 to 15%). Correspondingly, the drug-loading rate and entrapment efficiency would fall to about 3.41% and 61.73%. As for the explanation of the negative effect on drug entrapment, it possibly accounted for that octyl moiety of NOSC could insert the phospholipid bilayer thus occupied some space which was thought to host the hydrophobic drug and ultimately decreased the drug-loading rate. In the following stability studies and pharmacokinetic studies, NDLs with 10% NOSC/SPC ratio (wt/wt) was used. The liposome size and polydispersity of DLs were 138.1 ± 2.1 nm and 0.13 ± 0.02, and those of NDLs were 147.6 ± 1.9 nm and 0.15 ± 0.02, respectively, which were all measured by dynamic light scattering.

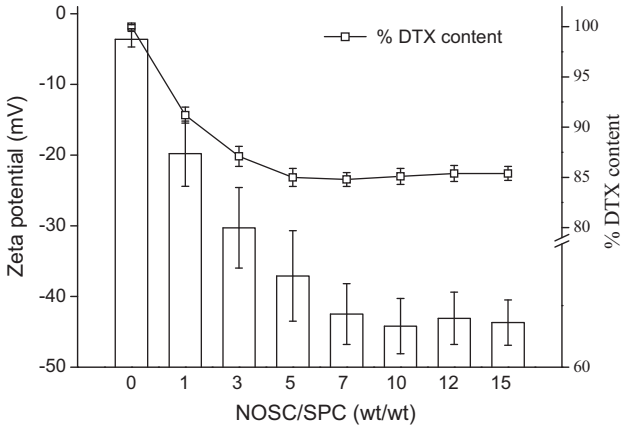

Fig. 1. Evaluation changes of the zeta potential and percentage DTX content of NDLs over different NOSC/SPC ratio (wt/wt) ($n = 3$).

These sligh increases in particle size was also demonstrated in the morphology observations of liposomes using TEM. The spherical structures or nearly orbicular in shape with particle diameter ranging from 100 to 200 nm were observed in Fig. 2.

3.2. Permeability of liposomes

NCLs released about 30% of its initial drug content over the period, while release of CAL from CLs was nearly 50%, as shown in Fig. 3. A remarkable retarded release of CAL from NDLs was observed compared with that from CLs. As referred above, octyl moiety of NOSC could insert the phospholipid bilayer and decrease the fluidity of bilayers, thus decrease the permeability of the bilayers and ultimately enhanced the stability of the whole liposome structure.

3.3. Stability studies of liposomes

The particle sizes of DLs, CDLs and NDLs incubated with 1.5 wt% BSA in PBS were measured over time, and control groups were undertaken incubation in PBS alone without BSA. The curves of particle sizes over time were obtained (Fig. 2), which illustrated that there was no obvious alteration of particle size of control group through the whole incubation piriod of 4 h, while the the samples demonstrated the entirely distinct fates. In the first 10 min, the particle size of CDLs group reached about 400 nm, approximately three times as the initial value. The second leap of CDLs particle size to about 800 nm was shown around 60 min and

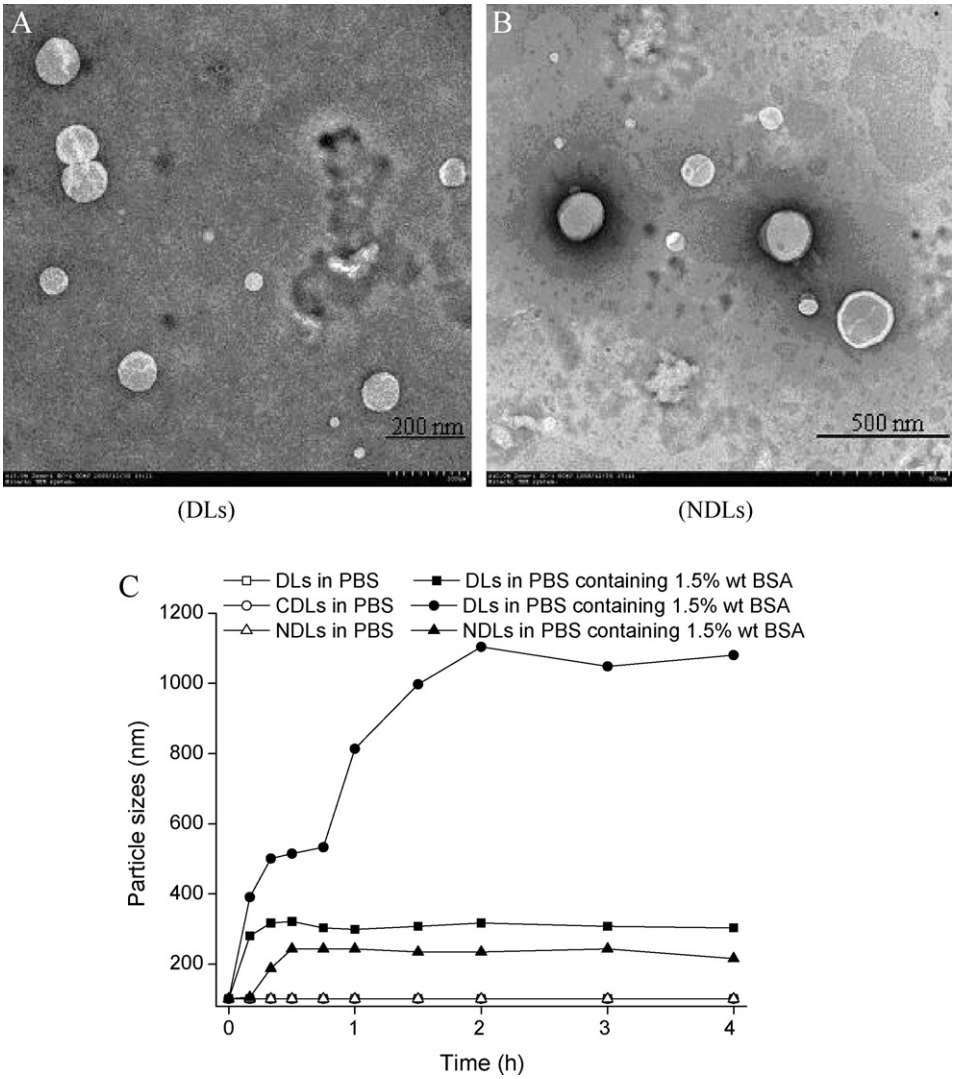

Fig. 2. Transmission electron microscopies of DLs (A) and NDLs (B) in PBS (pH 7.4), respectively, and particle sizes of DLs, CDLs, and NDLs on time of incubation (C) in PBS (pH 7.4) or PBS (pH 7.4) with 1.5 wt% BSA at 37 ± 0.5 °C. All values are the mean of three measurements.

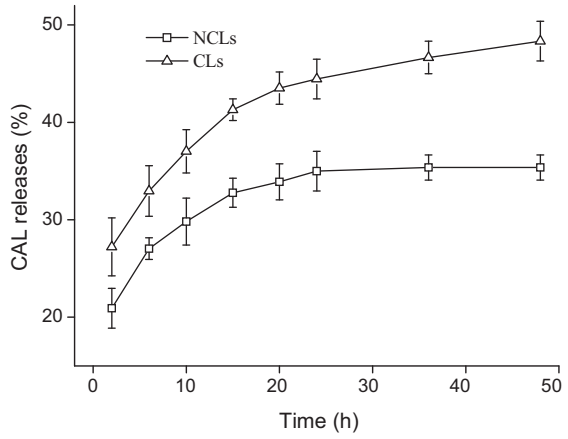

Fig. 3. Dynamic release profiles of CAL from CLs and NCLs in PBS (pH 7.4) ($n = 3$).

Table 1
The main pharmacokinetics parameters of DTX in the three formulations after i.v. administration at the dosage of 12 mg/kg (mean ± SD, $n = 5$).

Parameters	Taxotere®	DLs	NDLs
$AUC_{0-\infty}$ (μg min/mL)	131.59 ± 32.681	522.548 ± 85.336[a]	808 ± 91.175[b]
MRT (min)	37.998 ± 8.268	159.965 ± 25.53[a]	219.453 ± 4.597[b]
V_C (L/kg)	0.296 ± 0.229	1.043 ± 0.161[a]	1.033 ± 0.029
Cl (L/min)	0.081 ± 0.02	0.02 ± 0.006[a]	0.01 ± 0.001
$T_{1/2\alpha}$ (min)	0.874 ± 0.509	2.682 ± 0.33[a]	3.384 ± 1.245
$T_{1/2\beta}$ (min)	2.326 ± 0.878	17.506 ± 2.427[a]	29.867 ± 6.106[b]

$AUC_{0-\infty}$: the area under the plasma concentration–time curve from time 0 to time infinity; MRT: mean residence time from time 0 to time infinity; V_C: apparent volume of distribution; Cl: total body clearance; $T_{1/2\alpha}$: apparent plasma half-life ($T_{1/2}$) of distribution phase (α-phase); $T_{1/2\beta}$: apparent plasma half-life ($T_{1/2}$) of elimination phase (β-phase).
[a] $P < 0.05$ versus Taxotere®.
[b] $P < 0.05$ versus DLs.

large particle could be seen by the unaided eye after 90 min. 1.5 wt% BSA in PBS charged zeta potential around −7 mV, which dramatically adsorbed on the positive charged surface of CDLs via electrostatic action, thus resulted in the growth of the particle size and aggregation between particles bridged by BSA.

DLs and NDLs had the similar trend in their size change, while NDLs seemed to show a more good stability. The particle size increments were observed only in the first 10 min for DLs and NDLs groups, and the NDLs which held higher nagative zeta potentials shown better stability than the other two groups.

3.4. Pharmacokinetic studies of Taxotere®, DLs and NDLs in rats

As shown in Fig. 4, after the intravenous injection of the formulations, rapid eliminations of the drug from the blood circulation were observed during the initial phase in all three groups. Especially for Taxotere® group, the DTX concentration at 5 min after injection was about 50% of initial concentration. After a slower decrease, the docetaxel concentration in the plasma was below the lowest determination line after 240 min. The pharmacokinetic behavior of DTX was significantly altered after encapsulation in liposomes. The DTX in liposomes was more slowly removed from the circulation than it in Taxotere®. DLs exhibited concentrations lower than NDLs at the same time point, but noticeably higher than those in Taxotere® group. The DTX plasma concentrations over times fitted to three-compartment model analyzed by compartmental model, and the main pharmacokinetic parameters are listed in Table 1.

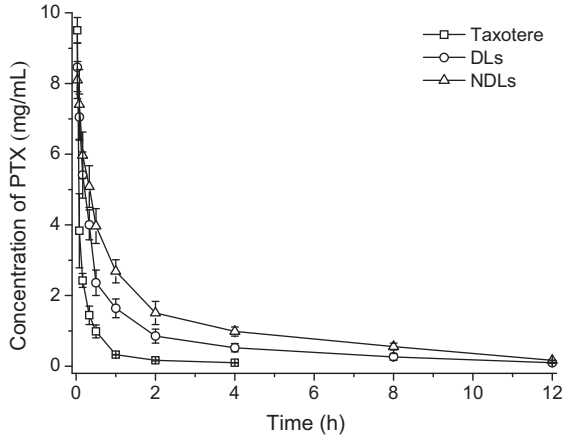

Fig. 4. PTX plasma levels-time courses in rat after i.v. administration of Taxotere®, DLs, and NDLs at the dose of 12 mg/kg. Each point represents the mean ± SD ($n = 5$).

4. Discussion and conclusion

Steric protection of conventional liposomes can be successfully achieved by grafting their surface with amphiphilic and/or flexible polymers. In the present studies, the modification of DTX liposomes with NOSC was confirmed by details of TEM, DLS and the zeta potential of liposomes.

DLs and NDLs micelles with smaller particle size (< 200 nm) were obtained and might avoid recognition of RES [31], and it is beneficial to tumor location by EPR effect within this size range (100–200 nm) [32]. Compared with the DLs (zeta potential −3.6 ± 1.1 mV), CALs had significantly higher negative zeta potentials, which also indicated that sulfonic residues conjugates successfully coated on the surface of the DLs to form the anionic shielding shells. The zeta potentials of NDLs evidently decreased with the NOSC/SPC ratio (wt/wt) increasing from 0 to 10% but slightly changed when the NOSC/SPC ratio (wt/wt) continued increasing from 10 to 15%, which indicated that NOSC-modified on the liposome surface reached a saturation state. Therefore, 10% was selected to be the optimal NOSC/SPC ratio (wt/wt) to prepare CALs. Furthermore, the high negative surface potential and sulfonic hydrophilic shell of NDLs were advantageous to down-regulate the opsonization, thus reduced the phagocytosis by RES and prolonged the blood circulation time of particles [31]. Long circulation was the prerequisite to utilize the EPR effect for the accumulation of pharmaceuticals in the areas with leaky vasculature [14]. In the other hand, a high net potential might contribute to the enhanced physical stability of liposomes by reducing the rate of aggregation and fusion [33]. Therefore, it could be promisingly deduced that NDLs had better stability than DLs due to their high zeta potential.

Serum proteins, erythrocytes and other blood cells could bind to the liposomes in systemic circulation leading to an alteration in its physicochemical properties such as particle size and electrical charge [34], which would strangle inherent advantage of the drug delivery system and fail to transport the pharmaceuticals to the special sites, followed by weakened potential in targeted delivery of drugs through the systemic circulation [35]. To a certain degree, the modification of NOSC on DLs might decrease the adsorbtion of blood components and enhance the stability of the system, which were tentatively confirmed in the stability studies of liposomes (Fig. 2).

In the pharmacokinetic study, the introduction of NOSC increases the circulation time of liposomes after intravenous

administration. Encapsulation of DTX in liposomes significantly decreased the Cl ($P < 0.05$). A significant increase in the AUC, MRT, $T_{1/2\alpha}$ and $T_{1/2\beta}$ ($P < 0.05$) were observed in DLs group compared with Taxotere®. Furthermore, a significant increase in the AUC, MRT, and $T_{1/2\beta}$ ($P < 0.05$) were observed in NDLs group compared with DLs group, which were attributed to the protection of the sulfonic hydrophilic shell against the rapid MPS uptake of liposomes and delayed drug release from stabilized liposomes bilayers anchoring octyl moiety, as demonstrated in the permeability and stability studies of CLs and NCLs. With regard to the two probable interpretations mentioned above, the second one more likely made greater contribution on the prolonged circulation time as also verified in other researches [36].

Therefore, the present studies revealed that the NOSC modification might provide an efficient approach to enhance the stability of liposomes containing DTX, and the further investigations in vivo have been designed to confirm the more advantages of NDLs as the carrier of DTX over the conventional DTX liposomes.

Disclosure of interest

The authors declare that they have no conflicts of interest concerning this article.

Acknowledgment

This study was financially supported by National Natural Science Foundation of China (grant No. 81072589), 111 Project from the Ministry of Education of China and the State Administration of Foreign Expert Affairs of China (No. 111-2-07).

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