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Inhibition of intrinsic coagulation improves safety and tumor-targeted drug delivery of cationic solid lipid nanoparticles

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ABSTRACT

Cationic solid lipid nanoparticles (cSLNs) are promising nanoparticles for controlled drug delivery. Increasing surface charge and/or reducing PEG density enhance cellular uptake of cSLNs *in vitro*, but for unknown reasons fail to improve drug delivery *in vivo*. Herein, we show that cSLNs present a risk for systemic platelet activation and aggregation *in vivo*, and this toxic effect can be significantly augmented by increasing the surface charge and reducing the PEG density. Furthermore, thrombotic toxicity significantly reduces blood circulation time and *in vivo* cellular uptake of cSLNs. Mechanistic studies revealed that the intrinsic coagulation pathway is responsible for cSLN-induced platelet activation. Importantly, pretreatment of the recipient mice with heparin, a clinically-approved intrinsic coagulation inhibitor, was highly effective in preventing toxicity, prolonging the circulation time of cSLNs, and improving cSLN-based antitumor drug delivery and therapeutic efficacy in tumor-bearing mice. This study offers a useful strategy for improving both the safety and efficacy of cSLN-based anticancer therapies.

1. Introduction

There is an increasing interest in the use of nanostructured systems for targeted drug delivery [1–3]. Among nanostructured systems, cationic solid lipid nanoparticles (cSLNs) are considered more attractive as nanocarriers for the delivery of therapeutic agents [4–7]. The blood half-life and biodistribution of cSLNs, the two major factors determining the efficacy of targeted drug delivery, are affected mostly by the magnitude of surface charge and the density of surface-coated poly (ethylene glycol) (PEG) [8–10]. Under *in vitro* conditions, positive charge and low PEG density have been shown to facilitate nanoparticle-based drug-delivery by promoting cellular uptake, distribution in subcellular compartments, and permeability in multicellular spheroids of the nanoparticles [11–13]. However, under *in vivo* conditions, increased positive charge enhances serum protein adsorption on nanoparticles, resulting in shorter circulation half-life [14–16]. PEGylation, coating the particle surface with PEG, is a breakthrough technology for improving the biocompatibility of nanocarriers [17–19]. The steric stabilization provided by PEG polymers confers a relative “invisibility” to cSLNs and, thus, reduces their chance of being recognized by the reticuloendothelial system *in vivo* [20] and enhancing their circulation half-life [19,20]. However, PEGylation limits the cellular uptake of cSLNs by cancer cells, which is a major drawback in anticancer therapy [19,21]. Therefore, understanding the combinatory or synergistic effects of the surface positive charge and PEG density on the blood circulation time and biodistribution of cSLNs is critical.

Toxicity is another issue to be considered when designing cSLNs as drug carriers for cancer therapies. Surface charge and PEG density are important factors in determining the toxicity of cSLNs.

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Although the effects of these two parameters on the ability of cSLNs to adsorb plasma proteins have been extensively investigated, the interaction of cSLNs with the major blood cellular components, including red blood cells (RBCs) and platelets, has been overlooked. Furthermore, it remains unclear whether or not the interactions between cSLNs and blood cellular components may significantly affect the blood circulation time and biodistribution of cSLNs. In this study, we prepared a series of PEGylated lipid-associated cSLNs with varying positive surface charges and PEG densities. Using these cSLNs, we demonstrate that high surface charge and low PEG density act together to induce systemic platelet activation and aggregation through the intrinsic coagulation pathway. Importantly, pretreatment of the recipient mice with heparin, an intrinsic coagulation pathway inhibitor, was highly effective in preventing toxicity, while enhancing the circulation time and therapeutic efficacy of cSLNs.

2. Materials and methods

2.1. Chemicals, antibodies and cells

Poly (D, L-lactic-co-glycolide) (PLGA, MW 7000–17,000) was purchased from sigma-aldrich (St. Louis, MO, USA). mPEG-DSPE (MW 5000) was purchased from Laysan Bio (Arab, AL, USA). 1, 2-dioleoyl-3-trimethylammonium-propane (DOTAP) was obtained from Avanti Polar Lipids (Alabaster, AL, USA). Dulbecco's modified eagle medium (DMEM), phosphate buffer saline (PBS), Penicillin/streptomycin, and L-glutamine were all obtained from Invitrogen (Carlsbad, CA, USA). Fetal bovine serum (FBS), 1,1-dioctadecyl-3,3,3,3-tetramethylindotricarbocyanine iodide (DiR), and 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindodicarbocyanine, 4-chlorobenzenesulfonate salt (DiD) were purchased from Thermo Fisher Scientific (Waltham, MA, USA). Sodium heparin was obtained from Shanghai NO.1 biochemical & pharmaceutical Co. Ltd (Shanghai, China; 12,500 IU/2 ml, lot number H31022051). Fluorescence-labeled rat anti-mouse CD41 and anti-mouse CD62P mAbs were obtained from Biolegend (San Diego, CA, USA). Paclitaxel was purchased from J&K Scientific Ltd. (Beijing, China). The mouse breast cancer cell line 4T1 was purchased from American Type Culture Collection (ATCC). 4T1 cells were cultured in DMEM supplemented with 10% FBS at 37 °C incubator with 5% CO_2.

2.2. Animals

Female BALB/c mice with 4–6 weeks of age were obtained from Charles River (Beijing, China) and raised in a specific pathogen-free environment with free access to food and water. All animals received care in compliance with the guidelines outlined in the Guide for the Care and Use of Laboratory Animals. All procedures were approved by the Jilin University Animal Care and Use Committee.

2.3. Preparation and characterization of cSLNs with different surface charges or PEG densities

The cSLNs with different surface charges or PEG densities were prepared by a W/O emulsion-solvent evaporation technique as described previously [29]. Briefly, 3 ml aqueous solution of mPEG-DSPE was added into 0.5 ml of chloroform containing PLGA and DOTAP. The amounts of PLGA, DOTAP and mPEG-DSPE for each sample were shown in Table 1. The mixture was emulsified by probe sonication (VCX130, Sonics & Materials, Inc. Newtown, CT, USA) for 1 min at 60 W over an ice bath. Afterwards, the chloroform was removed using a rotary evaporator (R-210, Buchi, Switzerland). The nanoparticles were collected by centrifugation (30,000 × g; 30min) and washed twice with sterile water. The final stock suspension of cSLNs was prepared by dispersing them in sterile water at concentration of 65 mg/ml. For preparing fluorescent dye (DiD or DiR)-labeled cSLNs, fluorescent dye was added into chloroform at 0.1% weight ratio to PLGA during cSLNs synthesis. PTX loaded cSLN3-3 was prepared by adding PTX (1.25% weight ratio to PLGA) into chloroform during cSLN3-3 synthesis. Size and zeta potential of the prepared cSLNs were monitored by a dynamic light scattering (Zetasizer NanoZS90, Malvern Instruments, Southborough, UK) at 25 °C. The morphologies of cSLNs were observed by a TEM operated at 120 kV (HT7700, Hitachi, Tokyo, Japan).

Table 1
The composition of cationic solid lipid nanoparticles (cSLNs) with different surface charges and PEG densities.

Name	DOTAP/PEG-DSPE/PLGA	
	Wt/Wt/Wt	Molar ratio
cSLN1-1	1/5/8	2.15/1.5/1
cSLN1-2	1/3/8	2.15/0.9/1
cSLN1-3	1/2/8	2.15/0.6/1
cSLN2-1	2/5/8	4.3/1.5/1
cSLN2-2	2/3/8	4.3/0.9/1
cSLN2-3	2/2/8	4.3/0.6/1
cSLN3-1	3/5/8	6.45/1.5/1
cSLN3-2	3/3/8	6.45/0.9/1
cSLN3-3	3/2/8	6.45/0.6/1

2.4. In vitro hemolysis analysis

The hemolysis analysis was performed as described previously [30]. Briefly, blood from BALB/c mice was collected into heparin-coated tubes. RBCs were obtained by centrifugation at 900 × g for 5 min and washed with PBS for three times. The purified RBCs were resuspended at a concentration of 2×10^8 cells/ml. cSLNs in PBS were mixed with RBCs solution at a final concentration of 5 mg/ml. Distilled water and PBS were used as positive and negative control, respectively. The mixture was incubated at 37 °C for 30 min, and then centrifuged at 900 × g for 5 min. The supernatant was collected for spectroscopic analysis; the cell pellet was resuspended in PBS and examined for morphology under an optical microscope.

2.5. In vitro platelet activation assay

The effect of cSLNs on platelet activation was determined by flow cytometry. Mouse platelets were prepared according to a previously reported method [31]. Briefly, blood was collected from the BALB/c mice into heparin-coated tubes. Platelet-rich-plasma (PRP) was prepared by collecting the supernatant buffy coat after the centrifugation at 100 × g for 20 min. Platelets were separated from PRP by centrifugation at 900 × g for 5 min and resuspended in modified Tyrode's buffer at a concentration of 2.5×10^8 platelets/ml. Then, cSLNs were added into freshly isolated platelets suspension at a final concentration of 1 mg/ml and incubated at 37 °C for 1 h. After that, the platelets were recovered by centrifugation at 900 × g for 5 min and stained with anti-mouse CD41-APC and anti-mouse CD62P-FITC (CD41 and CD62P detect total and activated platelets, respectively [32]). Samples were collected using a BD LSR Fortessa (BD Biosciences) and data were analyzed with FlowJo software.

2.6. Serum protein binding assay

To assess the serum protein binding, hydrodynamic size and surface charge of each cSLNs were measured before and after