Biomaterials 41 (2015) 1–14

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

# Enhanced retention and anti-tumor efficacy of liposomes by changing their cellular uptake and pharmacokinetics behavior

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ARTICLE INFO

*Article history:*
Received 25 July 2014
Accepted 7 November 2014
Available online

*Keywords:*
Liposomes
Poly(carboxybetaine)
Poly(ethylene glycol)
Accelerated blood clearance phenomenon
Tumor accumulation

ABSTRACT

Although PEGylated liposome-based drug delivery systems hold great promising applications for cancer therapy due to their prolonged blood circulation time, PEGylation significantly reduces their cellular uptake, which markedly impairs the *in vivo* tumor retention and antitumor efficiency of drug-loaded liposomes. Most importantly, it has been proved that repeated injections of PEGylated liposomes with cell cycle specific drug such as topotecan (TPT) in the same animal at certain time intervals will induce "accelerated blood clearance" (ABC) phenomenon, which decreases the tumor accumulation of drug-loaded liposomes and presents a tremendous challenge to the clinical use of liposome-based drug delivery systems. Herein, we developed a zwitterionic poly(carboxybetaine) (PCB) modified liposome-based drug delivery system. The presence of PCB could avoid protein adsorption and enhance the stability of liposomes as that for PEG. Quite different from the PEGylated liposomes, the pH-sensitive PCBylated liposomes were internalized into cells *via* endocytosis with excellent cellular uptake and drug release ability. Furthermore, the PCBylated liposomes would avoid ABC phenomenon, which promoted the tumor accumulation of drug-loaded liposomes *in vivo*. With higher tumor accumulation and cellular uptake, the PCBylated drug-loaded liposomes significantly inhibited tumor growth and provided a promising approach for cancer therapy.



## 1. Introduction

Widely recognized for their ability to produce a prolonged blood circulation time and facilitate tumor accumulation *via* the enhanced permeability and retention (EPR) effect, PEGylated liposome-based drug delivery systems hold great promising applications for cancer therapy [1–4]. Unfortunately, PEGylation significantly reduces the cellular uptake and endosomal/lysosomal escape of the liposomes, and interferes with the tumor retention and antitumor efficacy of liposome-based drug delivery systems [5,6]. Most importantly, it has been proved that repeated injections of PEGylated liposomes with cell cycle specific drugs such as topotecan (TPT) in the same animal at certain time intervals will induce "accelerated blood clearance" (ABC) phenomenon [7,8]. PEGylated drug-loaded liposomes are intended to stimulate the spleen to produce anti-PEG IgM after the first administration, which selectively binds to PEG on the surface of the second administrated liposomes to cause rapid elimination and enhanced hepatic uptake [9,10]. This immune response decreases the tumor accumulation of drug-loaded liposomes and presents a tremendous challenge to the clinical use of liposome-based drug delivery systems.

Advancement in nanotechnology has allowed for the development of delivery systems to avoid the induction of ABC phenomenon through changing the physicochemical properties of the PEGylated liposome-based drug delivery systems [11]. Unfortunately, most of these approaches were accompanied with sacrificing the therapeutic efficacy of PEGylated liposomes. It has been shown that liposomes modified with cleavable PEG-lipid derivatives could avoid the induction of ABC phenomenon.

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http://dx.doi.org/10.1016/j.biomaterials.2014.11.010
0142-9612/

However, the effect on long circulation of the cleavable PEGylated liposomes was worse than that of non-cleavable PEGylated liposomes [12].

Another approach has focused on the use of alternative polymers to extend the circulation time of liposome-based drug delivery systems [13]. In our previous study, it has been proved that zwitterionic polymer poly(carboxybetaine) (PCB) has superior ability in extending the blood retention without interfering with the cellular uptake and endosomal/lysosomal escape of the liposomes, which is quite different from that of PEGylation [14]. However, whether liposome-based drug delivery systems modified with PCB would avoid ABC phenomenon *in vivo* has not been verified. Herein, to address the challenge, the performances of PCBylated liposome-based drug delivery systems in cellular uptake, pharmacokinetics and tumor therapy were investigated. Our findings demonstrated that PCBylation could change the cellular uptake behavior and avoid ABC phenomenon of drug-loaded liposomes, which facilitated the tumor accumulation and therefore enhanced the antitumor activity of liposome-based drug delivery systems (Scheme 1).

## 2. Materials and methods

### 2.1. Materials

1,2-Distearoyl-*sn*-glycero-3-phosphoethanolamine-*n*-[methoxy (polyethylene glycol)-2000] (DSPE-PEG 2000), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC), cholesterol were purchased from Advanced Vehicle Technology Ltd., Co. (Shanghai, China). Doxorubicin (DOX) and topotecan hydrochloride (TPT) were obtained from Melonepharma (Dalian, China). (5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT), FITC-phalloidin and 4′,6-diamidino-2-phenylindole dihydrochloride (DAPI) were obtained from Sigma–Aldrich. LysoTracker Green and Annexin V-FITC apoptosis detection kit were obtained from Invitrogen. Dulbecco's Modified Eagle Medium (DMEM), penicillin (10,000 U/mL), streptomycin (10 mg/mL), trypsin-EDTA and fetal bovine serum (FBS) were purchased from Thermo. All other reagents used were obtained commercially at analytical grade.

Male SD rats weighting 200–250 g and male BALB/c nu/nu mice weighting 20–25 g were purchased from the Academy of Military Medical Sciences of China. The animal had free access to water and animal chow. All procedures involving experimental animals were carried out in accordance with the protocols approved by the Institutional Animals Care and Use Committee of Peking University.

### 2.2. Preparation and characterization of empty and drug-loaded liposomes

The empty liposomes modified with distearoyl phosphoethanolamine-poly(-carboxybetaine)$_{20}$ (DSPE-PCB$_{20}$) were prepared by thin lipid film method. Briefly, the mixture of POPC, cholesterol and DSPE-PCB$_{20}$ (50: 40: 10, molar ratio) were dissolved in 20 mL of chloroform and dried to a thin lipid film under a stream of $N_2$ gas, followed by incubation overnight under vacuum to remove residual solvent. The dried lipid films were subsequently hydrated in 10 mL of 200 mM ammonium sulfate. After sonication at 37 °C for 30 min, the solution was extruded 5 times using EmulsiFlex-C5 high-pressure homogenizer (Avestin, Canada).

The liposomes with drugs of DOX or TPT were prepared using the method of ammonium sulfate gradient. The external buffer of the liposomes was exchanged by dialyzing the empty liposomes against PBS (pH = 7.4) for 3 h. Subsequently, the liposomes and DOX or TPT solution (10:2, weight ratio) were incubated for 30 min at 60 °C. Non-entrapped free DOX or TPT was removed by dialyzing the liposomes against PBS (pH = 7.4) for 24 h at room temperature. The DSPE-PEG 2000 liposomes with the same drugs were prepared by the same way.

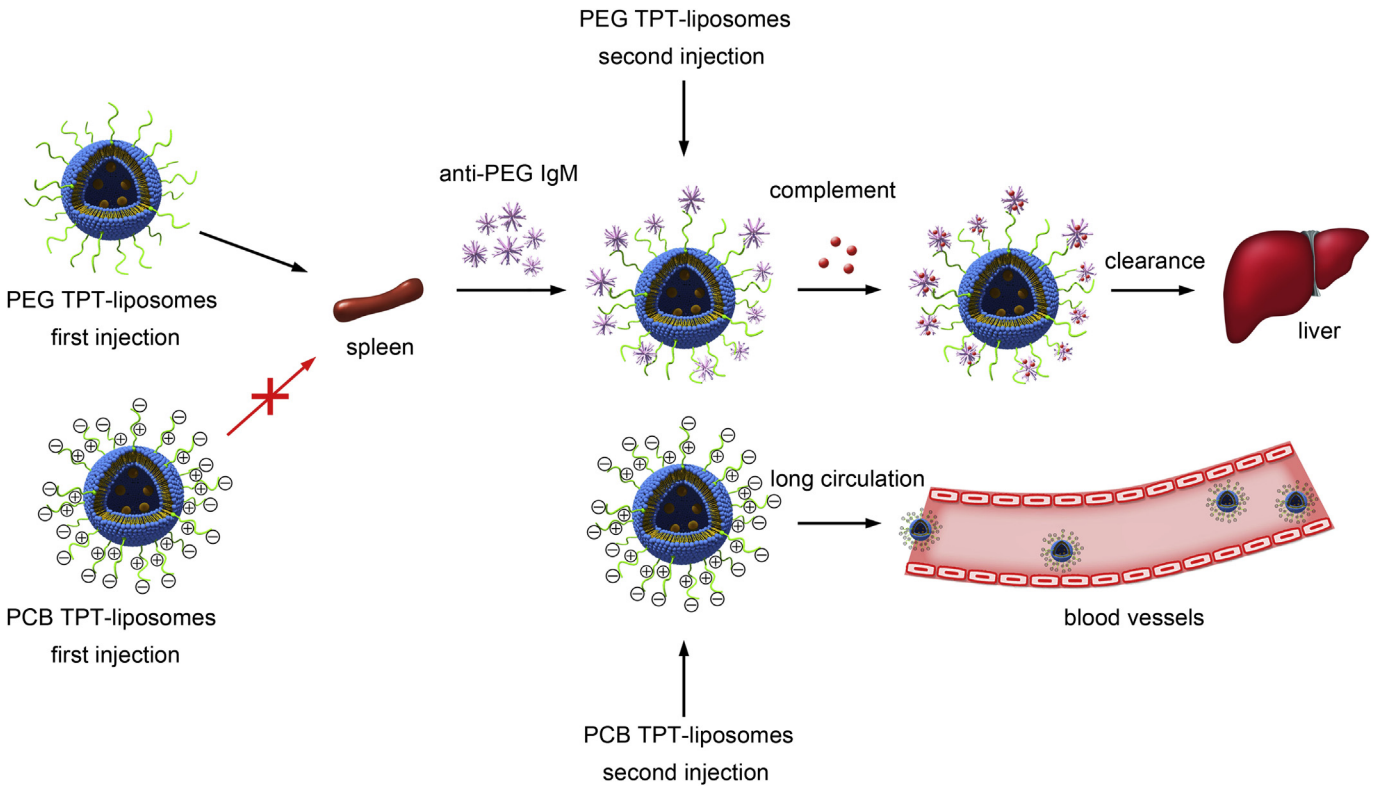
The mean particle diameters of the empty and drug-loaded liposomes were detected by dynamic light scattering (DLS), and the surface charge was analyzed by the zeta potential using a Zetasizer Nano ZS instrument (Malvern Instruments). Further morphological analysis was carried out by cryogenic transmission electron microscopy (Cryo-TEM, FEI Tecnai 20, Netherlands).

### 2.3. Encapsulation efficiency (EE) and drug loading content of liposomes

The encapsulation efficiency and drug loading content of DOX or TPT in liposomal samples were assessed by its UV absorption at the wavelength of 485 nm (DOX) or 360 nm (TPT) on a UV spectrophotometer (TU-1810, Beijing, China). The calibration curves were generated using known concentration of DOX or TPT. The drug loading content and encapsulation efficiency were calculated using the formula:

$$\text{Encapsulation efficiency (\%)} = W_2/W_1 \times 100\%$$

$$\text{Drug loading content (\%)} = W_2/W_0 \times 100\%$$




**Scheme 1.** Schematic illustration of the ABC process of the PCBylated and PEGylated drug-loaded liposomes.