International Journal of Pharmaceutics 475 (2014) 408–415

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Thermosensitive liposomes with higher phase transition temperature for targeted drug delivery to tumor

Jun Chen [a,*], Chao-qin He [a], Ai-hua Lin [b], Wei Gu [a], Zhi-peng Chen [a], Wei Li [a], Bao-chang Cai [a,*]

[a] School of Pharmacy, Nanjing University of Chinese Medicine, Nanjing 210023, China
[b] The Second Faculty of Clinical Medicine, Guangzhou University of Chinese Medicine, Guangzhou 510120, China

ARTICLE INFO

Article history:
Received 27 May 2014
Received in revised form 24 August 2014
Accepted 6 September 2014
Available online 10 September 2014

PubChem classifications:
Brucine (PubChem CID:442021)
Calcein (PubChem CID: 65079)
1,2-dipalmitoyl-sn-glycero-3-phosphacholine (PubChem CID:452110)

Keywords:
Hyperthermia
Thermosensitive liposomes
Tumor targeted drug delivery
Calcein
Brucine

ABSTRACT

Thermosensitive liposomes (TSL) in combination with local hyperthermia (HT) represent a promising tool for tumor specific drug delivery. The objective of the study was to investigate the influence of phase transition temperature (T_m) on the properties of TSL. High temperature triggered TSL (HTSL), low temperature triggered TSL (LTSL) and non-TSL (NTSL) were prepared and temperature sensitive release properties were extensively compared in different media. Mouse plasma was determined to have similar effect on the release profiles compared to human plasma, in which complete release were obtained at 38 °C and 40 °C for LTSL and HTSL, respectively. The temperature at which complete release achieved was found to be obviously lower than T_m. Brucine, an antitumor alkaloid, was encapsulated into different TSLs. After HT treatment, the viabilities of SMMC 7721 cells were determined to be 21.3 ± 3.8% and 16.8 ± 3.3% for 127 μM brucine LTSL and HTSL, respectively. Treating the tumor-bearing mice with LTSL, HTSL and NTSL led to significantly increased brucine uptake in the heated tumor site compared to the brucine solution group by 2.30, 3.80 and 2.26-fold, respectively. The results of this study suggested that T_m of TSL should be increased to obtain improved drug delivery efficiency to tumor.

1. Introduction

Liposomes are biocompatible, biodegradable and nonimmunogenic nanocarriers that are particularly suitable for targeted drug delivery. However, liposomes are rapidly captured by the mononuclear phagocyte system (MPS) and removed from the blood circulation after intravenous injection. In early 1990s, grafting poly-(ethylene glycol) (PEG) on the surface of the liposomal carrier was invented to avoid the uptake of MPS, resulting in extended blood-circulation time *in vivo*. By reducing MPS uptake, long-circulating PEGylated liposomes can passively accumulate into solid tumors undergoing angiogenesis. The presence of a discontinuous endothelial lining in the tumor vasculature during angiogenesis facilitates extravasation of liposomes into the interstitial space, where they accumulate due to the lack of efficient lymphatic drainage of the tumor, and function as a sustained drug-release system. The process is known as enhanced permeation and retention (EPR) effect (Immordino et al., 2006). Side effects of cytotoxic drugs like doxorubicin (Dox) are significantly reduced by encapsulation into PEGylated liposomes. However, compared to free drug, despite their preferential accumulation at the tumor site, no improved effectiveness of these clinical approved liposomal formulations has been reported (Judson et al., 2001).

Once liposomes have accumulated in targeted site, effective release of contents from these vesicles is a prerequisite for improved drug bioavailability in tumor. In 1978, triggered drug release from thermosensitive liposomes (TSL) was first introduced by Yatvin et al. (1978). Since then, different TSL have been described and by careful selection of the lipid composition their temperature sensitivity can be successfully tuned between 41 and 43 °C that is clinically preferable. Typical TSL have been prepared from 1,2-dipalimitoyl-*sn*-glycero-3-phosphocholine (DPPC) as the primary lipid, because its phase transition temperature (T_m) occurs at 41.5 °C. The gel-to-liquid transition at the T_m is a result of a conformational change in the alkyl chains of the lipids, which leads to an increase in the volume occupied by the hydrocarbon chains in the membrane and thus an increase in the permeability of the lipid bilayer. Moreover, with the incorporation of lysophosphatidylcholines (lyso-PC, *e.g.* 1-stearoyl-2-hydroxy-*sn*-glycero-3-phosphocholine,

* Corresponding authors. Tel.: +86 25 86798281; fax: +86 25 86798281.
E-mail addresses: chenjun75@163.com (J. Chen), bccai@126.com (B.-c. Cai).

http://dx.doi.org/10.1016/j.ijpharm.2014.09.009
0378-5173/

MSPC) into the liposomal membrane, it is possible to further accelerate encapsulated drug at T_m (Mills and Needham, 2005).

Hyperthermia (HT) treatment involves the heating of tumors to temperature of up to 43 °C and is usually combined with radiation or chemotherapy to enhance the antitumor efficacy. Presently, HT technology has been perfected and can be locally applied by focusing electromagnetic or ultrasound energy on the tumor area (Koning et al., 2010). For example, radiofrequency ablation (RFA) is one of the common strategies for HT treatment. In RFA, imaging techniques such as ultrasound, computed tomography or magnetic resonance are used to help guide a needle electrode into a tumor. By exerting radio-energy with frequency of approximately 460–480 kHz, ionic agitation and frictional heat in the surrounding tissue occur (Ng et al., 2003).

The most clinically advanced TSL formulation containing Dox known as ThermoDox®, is composed of DPPC: MSPC: DSPE-PEG2000 (86:10:4, molar ratio) described as low temperature triggered TSL (LTSL). ThermoDox® in combination with mild HT (42 °C) has been tested in clinical trials against liver cancer (Poon and Borys, 2009). Unfortunately, the effectiveness of LTSL in clinical trials was less than expected. In 2013, Celsion Corp., announced that its late-stage drug ThermoDox® in combination with RFA failed the study, unable to demonstrate a significant improvement in progression-free survival (http://investor.celsion.com).

Fine-tuning drug release kinetics of TSL for the HT strategy is crucially important (Park, 2014). Clinical applications of LTSL, such as ThermoDox®, may not achieve desirable outcomes simply because of the significant difference between *in vitro* and *in vivo* release profiles. In our previous study, it was found that T_m of liposomes was about 2 °C higher than the actual release temperature (Chen et al., 2013). Hossann et al. (2012) also found that 90%–100% Dox released from LTSL (T_m = 42.6 °C by DSC) in plasma or serum at 39–40 °C. Therefore, for LTSL, it is possible that all contents might release from the vesicles below 41–42 °C and significant leakage under body temperature could not be avoided in blood circulation *in vivo* before the accumulation of vesicles in the targeted tumor site.

In the present paper, high temperature triggered TSL (HTSL) composed of DPPC, hydrogenated soy phosphatidylcholine (HSPC), MSPC and DSPE-PEG2000 was firstly introduced. The T_m of HTSL was determined to be about 44 °C. The limitation of LTSL is the major benefits of HTSL. With higher T_m, less leakage before accumulation in tumors is expected under body temperature. Furthermore, since phase separation was visualized for liposomes composed of mixed PCs (Korlach et al., 1999), the bilayer of HTSL might be more permeable to contents on T_m. Therefore, compared to LTSL, the MSPC amounts in HTSL could be decreased significantly to obtain to obtain rapid and complete release profile on T_m. The temperature sensitive release and targeted drug delivery properties of LTSL, HTSL and non-TSL (NTSL) were intensively investigated and compared.

It should be noted that HTSL was different from traditional TSL (TTSL). TTSL was also composed of DPPC and HSPC (2:1). But in TTSL lipid composition, 15% cholesterol (Chol) was included and no lyso-PC was added (de Smet et al., 2010). Consequently, the release of HTSL at T_m could be more complete since lyso-PC could stabilize pores in the grain boundary regions of the partially melted solid phase and lead to permeability enhancement of liposomal membrane (Mills and Needham, 2005).

2. Materials and methods

2.1. Materials

The PCs, hydrogenated soy phosphatidylcholine (HSPC), 1,2-dipalmitoyl-*sn*-glycero-3-phosphacholine (DPPC) and 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-(carbonyl-methoxypolyethyleneglycol-2000) (DSPE-PEG2000), were purchased from Lipoid Corp., (Ludwigshafen, Germany). 1-stearoyl-2-hydroxy-*sn*-glycero-3-phosphocholine (MSPC) was purchased from Avanti Polar Lipids (Alabaster, AL, USA). Cholesterol (Chol) was purchased from Huixing Biochemistry Reagent Company (Shanghai, China). Calcein and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenylterazolium bromide (MTT) were obtained from Sigma–Aldrich Inc., (St. Louis, MO, USA). Sephadex G-50 was purchased from Pharmacia Biotech (Uppsala, Sweden). Fetal bovine serum (FBS) and bovine calf serum (BCS) were purchased from Tianhang-Bio Inc., (Huzhou, China). Pooled human plasma was a gift of the blood bank of the Affiliated Drug Tower Hospital of Nanjing University Medical School (Nanjing, China). Plasma of different species was from Laboratory Animal Center of Nanjing University of Chinese Medicine (Nanjing, China). The internal standard huperzine A was supplied by National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). HPLC-grade acetonitrile was obtained from Tedia Company Inc., (Fairfield, OH, USA). All other reagents were used commercially available and of analytical grade.

2.2. Preparation of calcein-loaded liposomes

Different phospholipid compositions were used to prepare liposomes by the thin film hydration method. The calculated amounts of phospholipid were dissolved in chloroform in a round bottom flask. The solvent was evaporated using a rotary evaporator to form a thin layer of lipid in the round bottom flask. The dried lipid films of LTSL [DPPC:MSPC:DSPE-PEG2000 (86:10:4)]; HTSL [DPPC:HSPC:MSPC:DSPE-PEG2000 (73.6:18.4:4:4)]; and NTSL [HSPC:Chol:DSPE-PEG2000 (75:50:4)] were hydrated by 5 mL of 90 mM calcein dissolved in pH 7.4 phosphate buffer saline (PBS) at 60 °C for 40 min to form multilamellar vesicles. Unilamellar vesicles were obtained by ten times extrusion through polycarbonate nanopore filters of 100 nm pore size using a LIPEX™ (Northern Lipids Inc., Burnaby, Canada) extruder at 60 °C. Unencapsulated calcein was removed by gel filtration using Sephadex G50 column (1 × 27 cm) with pH 7.4 PBS as eluent at a flow rate of 1 mL/min.

2.3. Temperature dependent release of calcein form liposomes

Temperature dependent release properties were evaluated according to previously described methodology (Chen et al., 2013; Hossann et al., 2012) with minor modifications. The release of calcein was determined by measuring the increase of fluorescence signal as a result of de-quenching. Calcein-loaded liposomes (0.1 mL) were added to 2.9 mL medium and incubated at 37 °C under magnetic stirring (500 rpm) for 1 h. Then 20 μL of the medium containing liposomes was transferred to a fresh tube and immediately incubated in another pre-heated magnetic stirrer at the desired temperature for 20 min to measure the temperature dependent calcein release. After rapidly cooling down in an ice-bath, the fluorescence of the sample was measured with a fluorimeter (F96S, Lengguang, Shanghai, China) using 490 nm excitation wavelength and 515 nm emission wavelength. Complete release of calcein was achieved by the addition of Triton X-100 (final concentration, 1%). The percentage of calcein release was calculated using the following equation:

$$\%\text{release} = 100 \times (F_t - F_0)/(F_{100} - F_0)$$

where F_t is the fluorescence at the time point t, F_0 is the initial fluorescence of calcein liposomes at the start of the incubation, and F_{100} is the fluorescence of Triton X-100 treated samples.