International Journal of Pharmaceutics 505 (2016) 139–146

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Pharmaceutical Nanotechnology

Pulmonary liposomal formulations encapsulated procaterol hydrochloride by a remote loading method achieve sustained release and extended pharmacological effects

Kohei Tahara[a], Hiromasa Tomida[a], Yousuke Ito[a], Suguru Tachikawa[a], Risako Onodera[a], Hiroyuki Tanaka[b,c], Yuichi Tozuka[d], Hirofumi Takeuchi[a,*]

[a] Laboratory of Pharmaceutical Engineering, Gifu Pharmaceutical University, 1-25-4 Daigaku-Nishi, Gifu 501-1196, Japan
[b] Laboratory of Pharmacology, Department of Bioactive Molecules, Gifu Pharmaceutical University, Japan
[c] Medical Information Sciences Division, United Graduated School of Drug Discovery and Medical Information Sciences, Gifu University, Japan
[d] Laboratory of Formulation Design and Pharmaceutical Technology, Osaka University of Pharmaceutical Sciences, Osaka, Japan

ARTICLE INFO

Article history:
Received 7 December 2015
Received in revised form 23 February 2016
Accepted 19 March 2016
Available online 21 March 2016

Keywords:
Procaterol hydrochloride
Liposome
Lung
Pulmonary administration
Sustained release

ABSTRACT

Drug inhalation provides localized drug therapy for respiratory diseases. However, the therapeutic efficacy of inhaled drugs is limited by rapid clearance from the lungs. Small hydrophilic compounds have short half-lives to systemic absorption. We developed a liposomal formulation as a sustained-release strategy for pulmonary delivery of procaterol hydrochloride (PRO), a short-acting pulmonary β2-agonist for asthma treatment. After PRO-loaded liposomes were prepared using a pH gradient (remote loading) method, 100-nm liposomes improved residence times of PRO in the lungs. PRO encapsulation efficiency and release profiles were examined by screening several liposomal formulations of lipid, cholesterol, and inner phase. Although PRO loading was not achieved using the conventional hydration method, PRO encapsulation efficiency was >60% using the pH gradient method. PRO release from liposomes was sustained for several hours depending on liposomal composition. The liposomal formulation effects on the PRO behavior in rat lungs were evaluated following pulmonary administration *in vivo*. Sustained PRO release was achieved using simplified egg phosphatidylcholine (EPC)/cholesterol (8/1) liposome *in vitro*, and greater PRO remnants were observed in rat lungs following pulmonary administration. Extended pharmacological PRO effects were observed for 120 min in a histamine-induced bronchoconstriction guinea pig model. We indicated the simplified EPC/cholesterol liposome potential as a controlled-release PRO carrier for pulmonary administration.

1. Introduction

Intra-tracheal drug delivery to the lungs by inhalation facilitates effective drug therapy for respiratory diseases such as asthma and chronic obstructive pulmonary disease. Topical administration to the lung by inhalation also allows dose reductions compared with systemic oral or intravenous administration, leading to decreased systemic drug exposures and side effects and improved efficacy. However, the therapeutic efficacy of inhaled drugs is limited by rapid clearance from the lungs (Todoroff and Vanbever, 2011). In particular, hydrophilic small molecule agents quickly diffuse across lung epithelia and are transferred to the bloodstream within minutes (He et al., 2007; Yamamoto et al., 2004), and the short lung residence times of such agents necessitate frequent dosing. Therefore, recommended regimens for corticosteroids and short-acting β2-agonists (SABA) indicate two and four times of daily inhalation, respectively (Loira-Pastoriza et al., 2014).

Drug-loaded carriers for drug delivery systems, such as polymeric nanoparticles and liposomes, provide sustained drug release of inhaled preparations in the lungs and improve patient adherence and therapeutic outcomes (Jaspart et al., 2007; Loira-Pastoriza et al., 2014; Rytting et al., 2010; Schreier et al., 1993). Thus, drug encapsulation into carriers with controlled-release functions allows drug retention in the lungs and local release of therapeutic concentrations. In addition, sustained-release of inhaled formulations may minimize side effects by avoiding systemic distribution.

Liposomes are a particularly suitable drug delivery vehicle for pulmonary administration, owing to biocompatible lipid bilayers

* Corresponding author.
E-mail address: takeuchi@gifu-pu.ac.jp (H. Takeuchi).

http://dx.doi.org/10.1016/j.ijpharm.2016.03.031
0378-5173/

that are safe and well tolerated by the lungs (Beck-Broichsitter et al., 2013). In a previous study, submicron-sized liposomes (approximately 100 nm) improved pulmonary residence times in rat lungs compared with microsized multilamellar vesicles (MLVs; (Murata et al., 2014)). Moreover, previous reports suggest that liposomes can be used as controlled-release devices for drugs encapsulated in the inner phase, and patterns of drug release profiles from liposomes can be manipulated with varying liposomal formulations to include lipids with varying phase transition temperatures and varying cholesterol contents of lipid bilayers (Loira-Pastoriza et al., 2014). Some sustained-release liposomal formulations for pulmonary drug delivery have reached clinical trial stage including Arikace®, which is a liposomal amikacin, and Pulmaquin®, which is a liposomal ciprofloxacin (Loira-Pastoriza et al., 2014). Therefore, in terms of practical use and commercialization, liposomal formulation has advantages as a pulmonary drug delivery system compared with other drug carriers.

In the present study, we demonstrate prolonged drug retention in the lung and improved topical availability of inhaled compounds, reflecting synergy of retention of 100-nm liposomes in the lung and subsequent sustained-release of drug. Procaterol hydrochloride hydrate (PRO) is an intermediate-acting β2-adrenergic receptor agonist that is commercially available (Meptin® nebulizer, pMDI, and DPI/Clickhaler) as an inhaled formulation for the treatment of asthma (Itazawa et al., 2013; Mirza et al., 1998). Commercially available inhalation PRO (Meptin®), which has a short acting pharmacological effect because of its rapid clearance from the lungs, and patients need several daily inhalations of the drug owing to the small size of the molecule and the hydrophilic property of PRO. Therefore, the development of long-acting PRO formulation could possibly lead to be more convenient for patients, thereby improving patient adherence. In the present study, PRO, which is a weakly basic drug, was used as a model active pharmaceutical ingredient (API) to attain sustained-drug release and test the effect of the liposome.

The hydrophilic API, such as PRO, is generally loaded with low efficiency in liposome prepared by the conventional thin-layer hydration method (Eloy et al., 2014), and the encapsulation method of PRO into liposome has not yet been established. To encapsulate PRO into liposomes, we have developed a procedure using an ammonium salt gradient method, which is available for active encapsulation, with a high efficiency for weakly basic drugs (Fritze et al., 2006). Subsequently, drug release profiles from engineered liposomal formulations were evaluated *in vitro*, drug distribution and retention times were determined in rat lungs, and pharmacological effects were investigated in a histamine-induced guinea pig model of asthma.

2. Materials and method

2.1. Materials

Egg phosphatidylcholine (EPC, COATSOME NC-10S) and hydrogenated soybean phosphatidylcholine (HSPC, COATSOME NC-21) were purchased from Nippon Oil and Fats Co., Ltd. (Tokyo, Japan). PRO (MW 335.83, $pK_{a1} = 7.35$, $pK_{a2} = 9.37$; Fig. 1) was provided by Otsuka Pharmaceutical Co., Ltd. (Tokushima, Japan). Cholesterol (Chol) and 5(6)-carboxyfluorescein (CF, MW 376.32) were purchased from Sigma-Aldrich (St. Louis, MO, USA), and 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI) was purchased from Lambda Probes (Graz, Austria). All other chemicals were commercial products of reagent grade.

2.2. Preparation of liposomes using the ammonium salt pH gradient method

Lipid (EPC or HSPC; 10.2 mM) and cholesterol were dissolved in a small amount of chloroform in a round-bottom flask and were dried in a rotary evaporator under reduced pressure at 40 °C to form a thin lipid film. Fluorescence-labeled liposomes were generated by dissolving DiI into lipid solution with cholesterol to a final concentration of 75 μg/ml. Films were dried in a vacuum oven overnight to ensure complete removal of solvent. Subsequently, lipid films were hydrated at 70 °C by vortexing with 120 mM ammonium salt solutions. The resulting multilamellar vesicles (MLVs) were frozen and thawed four times using a freezer and a water bath maintained at 40 °C. Submicron-sized liposomes were prepared using an extruder (LipoFast™-Pneumatic; Avestin, Inc., Ottawa, Canada) with a size-controlled polycarbonate membrane (0.1-μm membrane filter pore size).Extrusion was performed 41 times under nitrogen pressure (200 psi). To generate ammonium salt concentration gradients across liposome membranes, ammonium salts of the external liposome medium were replaced with phosphate buffer saline (PBS, pH 7.4) in two dialysis steps. PRO solutions in PBS were then mixed with liposomes to final concentrations of 300 μg/ml. Remote loading was performed by incubation of liposomes for 10, 60, or 180 min at 37 °C or 60 °C (Fujisawa et al., 2012; Tsukamoto et al., 2013).

2.3. Characterization of PRO-loaded liposomes

Particle sizes of liposomes were measured in aliquots of liposome suspensions diluted in large volumes of distilled water using the dynamic light scattering method (Zetasizer Nano ZS, Malvern, Worcestershire, UK). Liposome zeta potentials were measured using the laser Doppler method (Zetasizer Nano ZS). To determine the efficiency of liposomal PRO, PRO-loaded liposomes were separated from free PRO using ultracentrifugation (231,000*g*, 45 min) at 4 °C. PRO concentrations in supernatants were then determined at 254 nm following high-performance liquid chromatography (HPLC) with a COSMOSIL $5C_{18}$-MS-II column (Nacalai Tesque, Tokyo, Japan) and a mobile phase containing methanol, 5 mM sodium 1-pentanesulfate and acetic acid at 23:76:1.

2.4. In vitro *PRO release test*

PRO release was determined in phosphate-buffered saline (PBS) at pH 7.4. The whole PRO liposomal formulation including unentrapped drug (0.5 ml) were transferred into dialysis bags (MWCO = 12,000 – 14,000) and were soaked in PBS at 37 °C (50 ml). At predetermined times, 0.2 ml aliquots of receptor phase were

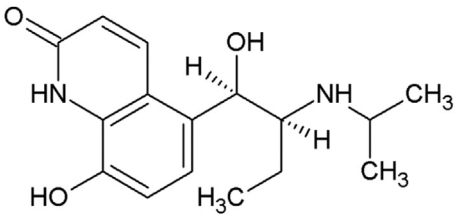

Fig. 1. Chemical structure of PRO.