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Selection of phospholipids to design liposome preparations with high skin penetration-enhancing effects

Pajaree Sakdiset[a,b], Akie Okada[a], Hiroaki Todo[a], Kenji Sugibayashi[a,*]

[a] Graduate School of Pharmaceutical Sciences, Josai University, 1-1 Keyakidai, Sakado, Saitama 350-0295, Japan
[b] School of Pharmacy, Walailak University, 222 Thaiburi, Thasala, Nakhon Si Thammarat 80161, Thailand

A R T I C L E I N F O

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Formulation development*Chemical compounds used in this article:*
DLPC (PubChem CID: 512874)
DLPG (PubChem CID: 46891823)
DMPC (PubChem CID: 5459377)
DMPG (PubChem CID: 46891824)
DPPC (PubChem CID: 452110)
DPPG (PubChem CID: 46891827)
DSPC (PubChem CID: 94190)
DSPG (PubChem CID: 53487811)
DOPC (PubChem CID: 10350317)
DOPG (PubChem CID: 23702460)
Cholesterol (PubChem CID: 5997)
Caffeine (PubChem CID: 2519)

A B S T R A C T

This study attempted to identify a good selection method for phospholipids to design liposome preparations with high skin penetration-enhancing effects. Five kinds of phosphatidylcholines and phosphatidylglycerols each were selected. First, phospholipid aqueous dispersions and liposomes containing caffeine as a model drug were tested for their skin penetration-enhancing effects using excised hairless rat skin. Accordingly, 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC) and 1,2-dipalmitoyl-*sn*-glycero-3-phosphoglycerol, sodium salt (DPPG) dispersions showed high penetration-enhancing ratios (*ERs*), whereas DPPG, 1,2-dilauroyl-*sn*-glycero-3-phosphocholine (DLPC), and 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) liposomes showed high *ERs*, suggesting that liposomes had different skin penetration-enhancing mechanisms from phospholipid dispersions. Next, two experiments were done to clarify the possible mechanism of liposome penetration; excised skin was pretreated for 1 h with caffeine-free phospholipid dispersions and liposomes, and caffeine solution was added to determine its skin permeation. Separately, caffeine permeation experiments were done using physical mixtures of blank liposomes and caffeine solution (caffeine-spiked liposomes) and caffeine-entrapped liposomes (caffeine was entrapped only in liposomes). DPPG was found to be a promising phospholipid candidate for liposome formulations with high skin penetration-enhancing effects, because DPPG phospholipid and liposome vesicles had a combination effect of disrupting the SC lipid barrier to carry both free and entrapped caffeine in the formulation through the skin.

1. Introduction

Liposomes, a type of classical vesicular drug delivery system, have received extensive attention in the field of skin drug delivery due to their ability to entrap drug(s) and enhance the skin penetration of both hydrophilic and lipophilic molecules [1–3]. The main component of liposomes is an amphiphilic molecule, phospholipids, which can spontaneously form closed bilayer vesicles as they confront water [4]. Mechanisms to account for the enhancement of skin penetration of drugs by liposomes have been proposed including (1) free drug operation, (2) intact vesicular penetration, (3) vesicle adsorption to and/or fusion with the stratum corneum (SC) and (4) their penetration-enhancing effect [5]. However, conflicting results for these mechanisms have been found in spite of extensive efforts made by many research groups.

One of the well-accepted mechanisms for the skin penetration-enhancement by liposomes is the penetration of the amphiphilic components into the skin barrier and their perturbatory actions on the packing of SC lipids [6,7]. From this reason, liposome composition must be an important parameter for the enhancing effect of liposomes on the skin penetration of drugs. In the formulation design of liposomes, many researchers have already focused on the optimization of liposomal characteristics; *i.e.*, morphology, vesicular size, surface charge, entrapment efficiency, transition temperature, or elasticity of liposomes by changing the liposome compositions or their preparation procedure [8–10]. Moreover, novel classes of vesicular carriers have been developed to obtain the high skin penetration-enhancing effect of liposomes, by addition of edge activators or chemical penetration enhancers into classical liposomes [11–13]. However, the skin penetration-enhancing effects of the main phospholipid compositions in liposomes have not been fully clarified.

In the present study, different types of phospholipids were first tested for their skin penetration-enhancing effects of a model hydrophilic drug, caffeine, through excised hairless rat skin using a currently established assay system comprised of multiple-diffusion cells as a screening approach, because phospholipids in the liposome composition can play an important role in the skin penetration-enhancing effect

* Corresponding author. Faculty of Pharmacy and Pharmaceutical Sciences, Josai University, 1-1 Keyakidai, Sakado, Saitama 350-0295, Japan.
E-mail address: sugib@josai.ac.jp (K. Sugibayashi).

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of liposomes. Then, several kinds of phospholipids were selected to prepare liposomes and tested for their skin penetration-enhancing effects, and the obtained results were compared with those for their phospholipid dispersions, in order to design suitable liposomes with high skin penetration-enhancing effects. Next, two further permeation experiments were carried out in order to clarify the possible modes of action of each phospholipid in their skin penetration-enhancing effects. 1) The effect of 1 h of pretreatment of excised skin was evaluated with caffeine-free phospholipid dispersions and liposomes. Caffeine solution was added after pretreatment to assess general skin permeation. 2) Caffeine permeation experiments were performed using a physical mixture of blank liposomes and caffeine solution (caffeine-spiked liposomes) and caffeine-entrapping liposomes (caffeine was entrapped only inside of the liposomes).

These results were used to survey phospholipid(s) to design liposomes having a high skin penetration-enhancing effect of a model hydrophilic drug, caffeine.

2. Materials and methods

2.1. Materials

Phospholipids including: 1,2-dilauroyl-*sn*-glycero-3-phosphocholine (DLPC; as below); 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC); 1,2-dimyristoyl-*sn*-glycero-3-phospho-glycerol, sodium salt (DMPG); 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC); 1,2-dipalmitoyl-*sn*-glycero-3-phosphoglycerol, sodium salt (DPPG); 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC); 1,2-distearoyl-*sn*-glycero-3-phosphoglycerol, sodium salt (DSPG); 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC); and 1,2-dioleoyl-*sn*-glycero-3-phosphoglycerol, sodium salt (DOPG) were purchased from NOF Corporation (Tokyo, Japan). 1,2-Dilauroyl-*sn*-glycero-3-phosphoglycerol, sodium salt (DLPG) was obtained from Olbracht Serdary Research Laboratories (Toronto, ON, Canada). Table 1 summarizes the abbreviations, the number of carbon atoms and double bonds in the alkyl chains of these phospholipids .

Cholesterol was purchased from Sigma-Aldrich (St. Louis, MO, USA). Caffeine, chloroform, methanol, and ethanol were purchased from Wako Pure Chemicals Industries, Ltd. (Osaka, Japan). These reagents were used without further purification.

2.2. Experimental animals

Male WBN/ILA-Ht hairless rats, weighing between 200 and 260 g, were obtained from the Life Science Research Center, Josai University (Sakado, Saitama, Japan) and Ishikawa Experimental Animal Laboratories (Saitama, Japan). Rats were bred in a room maintained at 25 ± 2 °C, in which the on and off times for the lighting were 07:00 and 19:00, respectively. Animals had free access to water and food (MF, Oriental Yeast Co., Ltd., Tokyo, Japan).

All breeding procedures and experiments on the animals were performed in accordance with the guidelines of the Animal Experiment Committee of Josai University.

Abdominal skin from hairless rats was excised under anesthesia by *i.p.* injection of anesthesia containing medetomidine (0.375 mg/kg), butorpharnol (2.5 mg/kg), and midazolam (2 mg/kg). The hairless rats were then sacrificed immediately by injection of pentobarbital sodium (40 mg/kg).

Table 1
Abbreviations, numbers of carbon atoms and double bonds in the alkyl chain of the phospholipids used in the present study.

Full name	Abbreviation	Carbon length: double bond
1,2-dilauroyl-*sn*-glycero-3-phosphocholine	DLPC	12:0
1,2-Dilauroyl-*sn*-glycero-3-phosphoglycerol, sodium salt	DLPG	12:0
1,2-dimyristoyl-*sn*-glycero-3-phosphocholine	DMPC	14:0
1,2-dimyristoyl-*sn*-glycero-3-phosphoglycerol, sodium salt	DMPG	14:0
1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine	DPPC	16:0
1,2-dipalmitoyl-*sn*-glycero-3-phosphoglycerol, sodium salt	DPPG	16:0
1,2-distearoyl-*sn*-glycero-3-phosphocholine	DSPC	18:0
1,2-distearoyl-*sn*-glycero-3-phosphoglycerol, sodium salt	DSPG	18:0
1,2-dioleoyl-*sn*-glycero-3-phosphocholine	DOPC	18:1
1,2-dioleoyl-*sn*-glycero-3-phosphoglycerol, sodium salt	DOPG	18:1

2.3. Preparation of liposomes

Liposomes were prepared using phospholipids and cholesterol in a ratio of 4:1 w/w. The compositions were dissolved in chloroform:methanol (2:1 v/v) in a round-bottomed flask, and the solvent was evaporated to form a thin film using a rotary evaporator under reduced pressure. The obtained film was purged with nitrogen gas and kept overnight to remove traces of organic solvent. The flask was then immersed in a water bath at 90 °C for annealing of the thin film for 30 min, and then 100 mM caffeine in phosphate-buffered saline (PBS) pH 7.4 solution was added to adjust the phospholipid concentration to 3% (w/v). The thin film was hydrated for 30 min and the resulting liposomes containing caffeine were then sonicated using a probe sonicator (VCX-750, Sonics & Materials Inc., Newtown, CT, USA) for 30 s. Next, four freeze-thaw cycles were performed by immersing the flask in liquid nitrogen and in a 90 °C water bath for 3 min each. The obtained liposomes were further extruded using a mini-extruder (Avanti Polar Lipids, Inc., Alabaster, AL, USA) assembled with a membrane filter (with pore sizes of 400, 200, and 100 nm, Whatman® track-etched membranes, GE Healthcare Japan, Tokyo, Japan). All final liposome formulations containing caffeine were kept at 25 °C and freshly used for skin permeation experiment within a day after preparation. In the final formulations, caffeine must be contained both inside and outside of the liposomes. These liposome formulations were used to evaluate caffeine permeation through the skin.

Caffeine-free liposomes (blank liposomes) was also prepared using the same procedure but without addition of caffeine.

2.4. Characterizations of liposomes

2.4.1. Particle size and zeta potential

The particle size and zeta potential of liposomes were measured after 100-fold dilution with PBS using a Zetasizer Nano ZS (Malvern Instruments Ltd., Malvern, UK). The size measurements were performed at 25 °C and a scattering angle of 90°. Individual zeta potential measurement was repeated for at least 10 readings from each liposome sample.

2.4.2. Caffeine distribution in liposome formulations

The entrapment efficiency (*EE*) of caffeine in each liposome sample was determined using an ultracentrifuge technique to evaluate the caffeine distribution either inside or outside of the liposome formulations. The final liposome suspension (400 µL) was placed in a centrifuge tube and centrifuged using a micro-ultracentrifuge (Himac CS120GXII, Hitachi Koki Co., Ltd., Tokyo, Japan) at 289,000 × *g*, 4 °C for 20 min to separate the liposome pellet (entrapped drug, E_{drug}) from the supernatant (unentrapped drug, U_{drug}). The supernatant was collected, and the free caffeine content was determined after 10-fold dilution with ethanol followed by 10-fold with PBS. In addition, the entrapped drug content inside the liposomes was determined by dispersing the packed liposome pellet with 400 µL PBS and further disrupting with 10-fold

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