PHOSPHOLIPID FORMULATION FOR ORAL DRUG DELIVERY

DAE-DUK KIM

College of Pharmacy, Seoul National Univeristy, Seoul 08826, Republic of Korea
E-mail: ddkim@snu.ac.kr

Abstract - Novel proliposomes (PLs) with increased lipid content was prepared and characterized in vitro and in vivo. The solid state PLs were characterized by scanning electron microscopy (SEM), powder X-ray diffractometer (PXRD) and differential scanning calorimetry (DSC). The liposomes were reconstituted, and particle size, zeta potential, entrapment efficiency (EE) and drug loading were evaluated. Particle morphology was determined by transmission electron microscopy (TEM). In vitro dissolution and in vivo pharmacokinetic study of PLs were evaluated comparing with crude drug powder. The phospholipid content of the PLs was increased up to 20% (w/w) without stickiness. The particle size of reconstituted liposomes was 580 ± 12 nm. The zeta potential and EE of drug was -64.2 ± 0.3 mV and 78.4 ± 3.6%, respectively. Crystalline state of drug was transformed to amorphous state by incorporating in PLs. In pharmacokinetic study in rats, PLs showed shorter T_{max}, higher C_{max} and AUC, compared with crude drug powder. The amorphous state of the drug in the PLs resulted in increasing solubility and dissolution rate and extent of the drug incorporated, thereby increasing the oral bioavailability of drug.

Index Terms - Formulation, Drug delivery, Phospholipid, Bioavailability.

I. INTRODUCTION

Liposomes are spherical vesicular structures composed of phospholipid bilayers, whereas proliposomes (PLs) are a dispersed system that can rapidly transform into liposomal structures in an aqueous milieu, of which pharmaceutical characteristics make up for the demerits of liposomes [1]. First of all, their granular or powdery forms are easy to handle and usually have good flowability, whereby even a further modifications were applicable. Secondly, PLs exhibited narrower distributions in their sizes after reconstitution, compared to those of conventional liposomes. Moreover, the preparation methods of PLs are relatively simple and cost-effective than those of liposomes. Along with these advantageous properties, PLs also showed promising results in enhancing the dissolution and oral bioavailability of many drugs. Conventionally, PLs were prepared by coating water-soluble materials with phospholipids using a rotary evaporator, a coating pan, and a fluidized bed. The proliposomal formulations from these techniques generated the micro-sized liposomes without further processes like sonication, which compromised their clinical use and limited their production to lab-scale. Meanwhile, solid dispersion technique was applied to the preparation of PLs, of which reconstitution produced nano-sized liposomes without additional manipulations [2]. The methods consisted of simple mixing, evaporation, and/or lyophilization, which were also suitable for production in a large quantity. Therefore, a novel proliposomal formulation for the oral drug delivery was developed in the present study. Solid dispersion technique was applied to fabricate the drug-loaded PLs. Physicochemical properties of the developed PLs in their solid- and liposomal-state were evaluated and characterized. In vitro Caco-2 permeation studies, in vivo pharmacokinetic studies, and ex vivo histological observations in rats were also performed.

II. MATERIALS AND METHODS

A. Materials

Soy phosphatidylcholine (SPC) was a gift from PHYTOS (Gyeonggi, Korea). Poloxamer 188 was obtained from BASF (Ludwigshafen, Germany). All other reagents were of analytical grade.

B. Preparation of proliposomes

SPC, poloxamer 188 were dissolved in ethanol. The mixture was vortex-mixed to obtain a clear yellow solution. Sorbitol was dissolved in double-deionized water. The two solutions were mixed in a round-bottom flask with gentle stirring for 30 min. The mixture was then evaporated under vacuum for 10 min at 40°C using a rotary evaporator. After the removal of ethanol, the resulting solution was immediately lyophilized for 24 h.

C. Characterization of PLs

The morphology of PLs particles was characterized by field emission-scanning electron microscopy (FE-SEM) using a JSM-6700F (JEOL, Japan). Differential scanning calorimetry (DSC) experiments were conducted to evaluate the physical state in the formulations. Powder X-ray diffractometry (PXRD) was used to assess the degree of crystallinity of excipients in the PLs. The reconstituted PLs dispersion was made by adding water was observed by the transmission electron microscopy (TEM). The mean particle size, polydispersity index (PI), and zeta potential of reconstituted PLs were determined by electrophoretic light-scattering spectrophotometer

(ELS-Z, Otsuka Electronics, Japan).

D. In vivo pharmacokinetic study

The femoral artery of rat was cannulated with Intramedic™ polyethylene tubing (PE-50; Becton Dickinson Diagnostics, MD, USA) under anesthesia with Zoletil® 50 (Virbac, Carros, France) at a dose of 50 mg/kg via intramuscular injection. PLs was encapsulated into #9 hard gelatin capsule (Torpac Inc., Fairfield, NJ, USA) and administered orally. Blood samples (~150 µL) were collected from the femoral artery at predetermined interval. Plasma samples (50 µL) were vortex-mixed with ACN (200 µL) containing IS (valsartan; 100 ng/mL) for 5 min and centrifuged at 16,000 × g for 5 min. Each supernatant was transferred to a sample vial and analyzed using the HPLC-MS/MS. Pharmacokinetic parameters were calculated using a non-compartmental analysis (WinNonlin, version 3.1, NCA 201; Pharsight, CA, USA).).

E. Statistical analysis

The statistical analyses (Student's t-test and one-way analysis of variance with post-hoc test) were performed using SPSS statistics software (Version 21.0; IBM Corp, NY, USA).

III. RESULTS AND DISCUSSION

PLs was prepared by the solvent evaporation and lyophilization methods. SPC content was increased up to 25.0% (w/w) and poloxamer 188 was added to improve the stability of the liposomes after reconstitution. SEM images of PLs granules were apparently smooth and the characteristic structure of sorbitol (rough-textured surface) was disappeared (Figure 1). DSC analysis showed that characteristic peaks of excipients disappeared, indicating the loss of crystallinity during the preparation process. Characteristic PXRD pattern of crystalline also disappeared in PLs, which is consistent with the DSC study. These results indicate that amorphization took place during the preparation of PLs, which would increase solubility and dissolution rate than that of its crystalline form.

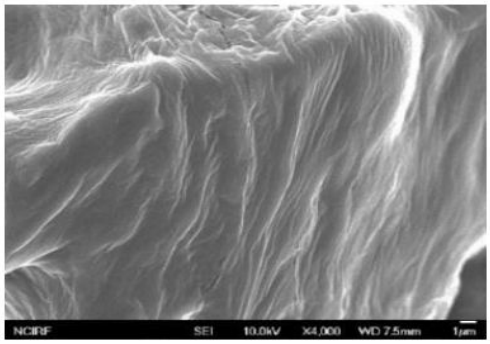


Figure 1. SEM image of PLs. Length of the scale bar is 1 µm.

After the reconstitution of PLs with gentle hand-shaking, the nano-sized dispersion with mean diameters of around 500 nm was obtained. Characteristic vesicular structures, indicating the successful conversion from PLs to liposomes, were confirmed by TEM observation (Figure 2). Negative zeta potential of liposome (around 60 mV) implies it is physically stable after reconstitution.

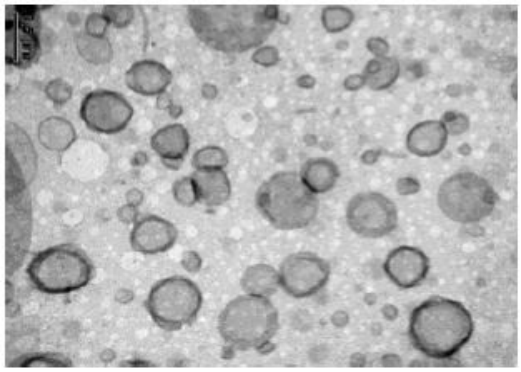


Figure 2. TEM image. Length of the scale bar is 500 nm.

The in vivo pharmacokinetic properties of the celecoxib (CXB) formulations were evaluated in rat model. Each formulation (CXB, Celebrex, or CXBPLs) was administered orally to rats at a CXB dose of 2 mg/kg. The plasma CXB concentration versus time profiles were plotted in Figure 3 and the corresponding pharmacokinetic parameters (AUC, C_{max}, and T_{max}) were listed on Table 1. The mean AUC value of CXBPL-treated group was significantly increased by 1.73-fold ($p < 0.05$), compared to that of CXB powder-treated group.

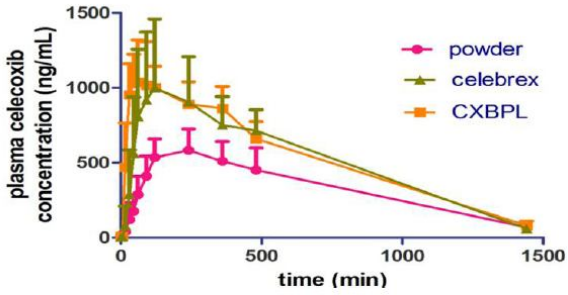


Figure 3. Plasma concentration vs. time profiles of celecoxib (CXB) after a single oral administration of CXB powder, celebrex and CXBPLs at a dose of 2 mg/kg. Data are presented as the mean ± SD (n ≥ 3).

Parameter	CXB	Celebrex	CXBPLs
AUC (µg·min/mL)	464 ± 117	774 ± 165*	803 ± 139*
C_{max} (µg/mL)	0.560 ± 0.165	1.05 ± 0.443	1.12 ± 0.226*
T_{max} (min)	240	165 ± 90.0	63.8 ± 18.9*,+
Bioavailability (%)	100	167	173

*$p < 0.05$, compared to CXB group.
+$p < 0.05$, compared to Celebrex group.
Data are presented as mean ± SD ($n \geq 3$).

Table 3. Pharmacokinetic parameters of CXB after oral administration of CXB, celebrex, and CXBPLs at a dose of 2 mg/kg in rats.

CONCLUSION

A novel proliposomal formulation for the oral drug delivery was successfully prepared using solid dispersion technique which is a biocompatible platform for enhancing the oral bioavailability of drugs with poor-water solubility.

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