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Proliposomes of exemestane for improved oral delivery: Formulation and *in vitro* evaluation using PAMPA, Caco-2 and rat intestine

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ABSTRACT

The aim of the present study was to develop proliposomal formulations to enhance the oral bioavailability of exemestane by improving solubility, dissolution and/or intestinal permeability. Proliposomal powder formulations were prepared using different ratios of drug (exemestane), distearoyl–phosphatidylcholine (DSPC), cholesterol and dimyristoyl–phosphatidylglycerol (DMPG) by solvent evaporation method. The effect of phospholipid composition and drug:lipid ratio on *in vitro* performance of proliposomes was studied. Proliposomes were characterized for their particle size distribution, thermal characteristics by differential scanning calorimetry (DSC) and dissolution behavior. Further, the formulated proliposomes were subjected to *in vitro* permeation or transport studies using different models such as rat intestine, parallel artificial membrane permeability assay (PAMPA) and Caco-2 cell line. Proliposomes provided enhanced exemestane dissolution due to incorporation into the phospholipid bilayers and change in the physical state from crystalline to amorphous. The *in vitro* transport studies in rat intestine, PAMPA and Caco-2 models revealed that the proliposomes were successful in enhancing the permeation of exemestane. These proliposomal formulations of exemestane could provide improved oral bioavailability due to enhanced solubility, permeability and hence absorption.

1. Introduction

Liposomes are the most promising, broadly applicable, and highly researched of all the novel delivery systems. Liposomal encapsulation can be used to enhance lipophilicity; however the success rate of oral delivery of liposomes was limited due to erratic and unpredictable absorption profiles (Fukunaga et al., 1991; Arien et al., 1993; Chaudhari et al., 1994). Liposomes also suffer from several stability problems such as aggregation, susceptibility to hydrolysis and oxidation (Rajesh et al., 2001). However, these stability problems can be avoided by formulating liposomes as proliposomes (Payne et al., 1986; Betageri, 2005).

Proliposomes are dry, free-flowing granular products, which, upon the addition of water, disperse to form a multi-lamellar liposomal suspension (Payne et al., 1986). The oral delivery of liposomes could be improved by enhancing the ability of liposomes to retain their integrity at the site of absorption which could be achieved by formulating them into proliposomes (Deshmukh et al., 2008). Several studies have been reported which prove the utility of oral proliposomes in providing the enhanced solubility and bioavailability for insoluble/poorly soluble drugs (Brocks and Betageri, 2002). Proliposomal formulations of silymarin were prepared for oral administration. These silymarin proliposomes were reported to be stable and enhance the gastrointestinal absorption of silymarin (Yan-yu et al., 2006). Salmon calcitonin (sCT) proliposomes were prepared and evaluated for oral delivery. The apparent permeability of sCT across Caco-2 cell monolayers was increased as the result of incorporating sCT into the proliposomes, suggesting that the pharmacokinetics of sCT would be modified through the administration of proliposomes (Song et al., 2002). Further, the feasibility of using the sCT and bile salts containing proliposomes to improve the intestinal absorption of sCT was explored using rats and Caco-2 systems (Song et al., 2005).

Oral proliposomes have been extensively investigated in our laboratory to increase the permeation of highly hydrophilic compounds and to increase the solubility of poorly water-soluble drugs (Betageri, 2004, 2005). An insoluble drug glyburide was incorporated in a proliposomal formulation and a 3-fold increase in the dissolution of the drug was observed by our group previously (Rajesh et al., 2001). In another study a proliposomal formulation of halofantrine exhibited an increase of 41–47% in the area under curve, and 90–100% in C_{max} compared with the control groups (Brocks and Betageri, 2002). Phospholipid–surfactant proliposomal beads were formulated for improved oral delivery of cromolyn sodium (Deshmukh et al., 2008). Our previous studies have estab-

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lished that proliposomes can serve as a better carrier for improving the dissolution and permeation of drugs.

Exemestane is a novel oral steroidal aromatase inactivator, which showed promising anti-tumor activity in postmenopausal women with hormonal sensitive (estrogen-dependent) breast cancer (Scott and Wiseman, 1999). Nevertheless, exemestane suffers from poor solubility, first pass metabolism and bioavailability problems (Valle et al., 2005). Oral bioavailability of exemestane is reported to be about 42% and plasma levels found to increase by approximately 40% after a high-fat breakfast. Also, the absorption of exemestane reported to be dependent upon formulation type and food where the suspension (compared to tablets) and fed state (compared to fasted state) provided increased absorption (Valle et al., 2005).

Thus, there is a scope of developing lipid-based formulations of exemestane for enhancing its solubility and bioavailability that result in the improved oral delivery. Recently, self-micro emulsifying drug delivery system (SMEDDS) of exemestane was reported (Singh et al., 2008). In this study, it has been reported that SMEDDS could be explored as potential carrier systems for dissolution enhancement of exemestane. Therefore, the purpose of our present research was to develop proliposomal formulations of exemestane. Another important objective of this study was to perform *in vitro* evaluation of proliposomal exemestane formulations using different models such as rat intestine, parallel artificial membrane permeability assay (PAMPA) and Caco-2 cell line. These *in vitro* studies could provide useful information about dissolution and permeation/absorption aspects of exemestane which further could be correlated to *in vivo* bioavailability studies. We hypothesize that these proliposomal formulations of exemestane might lead to improved oral bioavailability due to enhanced solubility, permeation and, thus absorption.

2. Materials

Distearoyl–phosphatidylcholine (DSPC) and dimyristoyl–phosphatidylglycerol (DMPG) were obtained from Genzyme Pharmaceuticals (Cambridge, MA, USA). Cholesterol was purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). Caco-2 cells were obtained from the ATCC (American type culture collection) cell repository (Manassas, VA, USA) and transwell 6-well cell culture plates were obtained from Corning Corp. (Corning, NY, USA). Fetal bovine serum was purchased from Invitrogen (Carlsbad, CA, USA). L-*R*-phosphatidylcholine (for PAMPA) and *n*-dodecane were purchased from Sigma Chemical Co. (St. Louis, MO, USA). MultiScreen-IP PAMPA assay plates and PTFE Receptor plates were purchased from Millipore Corp. (Billerica, MA, USA). Dulbecco's MEM, nonessential amino acids, penicillin, and L-glutamine, EDTA, and trypsin/EDTA were all purchased from Sigma Chemical Co. (St. Louis, MO, USA). Sprague–Dawley rats were obtained from Harlan Inc. (Indiana polis, IN, USA). All the organic solvents were of HPLC grade and were obtained from Fisher Scientific (Pittsburgh, PA, USA).

3. Methods

3.1. Preparation of proliposomal powder

Proliposomal powder formulations were prepared with different ratios of drug (exemestane), distearoyl–phosphatidylcholine (DSPC), cholesterol and dimyristoyl–phosphatidylglycerol (DMPG). The lipid composition of the formulations with their corresponding formulation code is depicted in Table 1. The drug, lipid(s) and cholesterol were dissolved in ethanol and ethanol was evaporated to get a dry powder. The dried material was passed through the #60 mesh sieve to obtain a free-flowing proliposomal powder. The exemestane content of the proliposomes is determined using the analytical method described in the following section.

Table 1
Formulation composition of proliposomes.

Formulation code	Lipid composition[a]			Formulation composition[a]
	DSPC	Cholesterol	DMPG	
I	1	0	0	1:1, drug:lipid
II	1	1	0	1:1, drug:lipid
III	7	2	1	1:1, drug:lipid
IV	1	0	0	3:1, drug:lipid
V	1	1	0	3:1, drug:lipid
VI	1	1	0	1:3, drug:lipid

[a] Molar ratio.

3.2. Analysis of exemestane

Drug analysis was carried out by reversed phase HPLC method using the method reported by Breda et al. (1993) with minor modifications with respect to mobile phase. Water's HPLC system with the UV–visible detector was used for the analysis. The mobile phase consisted of 70:30 acetonitrile:0.02 M phosphate buffer (pH 4.0) set at a flow rate of 1.0 ml/min (isocratic method). The elution was done on a Prodigy C-18 column (5 μm, 250 × 4.66 mm) and the chromatographic analysis was carried out at 247 nm.

3.3. Particle size analysis of proliposomes after hydration

Proliposomes (powder) were hydrated in pH 7.4 phosphate buffered saline (PBS) by sonication and vortex to obtain liposomes, these liposomes were then analyzed for the size distribution. Particle size analysis was performed by dynamic light scattering (DLS) using a NICOMP 370 particle sizer.

3.4. Differential scanning calorimetric (DSC) analysis

DSC studies were performed to comprehend the physical state of the drug in the proliposomal formulations. The analysis was carried out using PerkinElmer Pyris 1 differential scanning calorimeter (with Pyris Manager software) (PerkinElmer Corporation, CT, USA). The peak transition temperature (T_m) and heat of fusion (enthalpy) (ΔHf) were determined and used in the analysis. Indium (T_m = 159.2 °C; ΔHf = 28.8 J/g) was used as a standard for routine calibration. An empty aluminum pan was used as reference, and nitrogen (purity > 99.99%) was used as the purge gas. The samples were scanned at 10 °C/min from 20 °C to 400 °C and DSC chromatograms were recorded for comparison.

3.5. Dissolution studies

Dissolution studies were conducted using USP type-II apparatus. The dissolution medium used was 900 ml of pH 7.4 PBS. The studies were carried out at 100 rpm and at 37 ± 0.5 °C. Each experiment was performed in triplicate and the samples were withdrawn at fixed time intervals up to 3 h. After appropriate dilutions, the samples were analyzed. Cumulative percent of drug released was calculated, and mean of three samples was used in the data analysis.

3.6. Rat intestinal permeation studies

Intestinal permeability of the formulations was assessed by using mucosal sheets from the rat intestine at 37 °C in pH 7.4 PBS. On the day of the experiment, Sprague–Dawley rat was anesthetized and a midline abdominal incision was made and the entire length