European Journal of Pharmaceutical Sciences 85 (2016) 123–131

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

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

Optimization of the process variables of tilianin-loaded composite phospholipid liposomes based on response surface-central composite design and pharmacokinetic study

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ARTICLE INFO

Article history:
Received 12 October 2015
Received in revised form 11 February 2016
Accepted 11 February 2016
Available online 13 February 2016

Chemical compounds studied in this article:
Tilianin (PubChem CID: 5,321,954)
Phosphatidylcholine
(PubChem CID: 45,266,626)
Lecithin Hydrogenated
(PubChem CID: 10,147,142)
Cholesterol (PubChem CID: 5997)

Keywords:
Tilianin
Composite phospholipid liposomes
Response surface-central composite design
In vitro drug release
Pharmacokinetic

ABSTRACT

Tilianin is attracting considerable attention because of its antihypertensive, anti-atherogenic and anticonvulsive efficacy. However, tilianin has poor oral bioavailability. Thus, to improve the oral bioavailability of tilianin, composite phospholipid liposomes were adopted in this work as a novel nanoformulation. The aim was to develop and formulate tilianin composite phospholipid liposomes (TCPLs) through ethanol injection and to apply the response surface-central composite design to optimize the tilianin composite phospholipid liposome formulation. The independent variables were the amount of phospholipids (X_1), amount of cholesterol (X_2) and weight ratio of phospholipid to drug (X_3); the depended variables were particle size (Y_1) and encapsulation efficiency (EE) (Y_2) of TCPLs. Results indicated that the optimum preparation conditions were as follows: phospholipid amount, 500 mg, cholesterol amount, 50 mg and phospholipid/drug ratio, 25. These variables were also the major contributing variables for particle size (101.4 ± 6.1 nm), higher EE (90.28% ± 1.36%), zeta potential (−18.3 ± 2.6 mV) and PDI (0.122 ± 0.027). Subsequently, differential scanning calorimetry techniques were used to investigate the molecular interaction in TCPLs, and the in vitro drug release of tilianin and TCPLs was investigated by the second method of dissolution in the Chinese Pharmacopoeia (Edition 2015). Furthermore, pharmacokinetics in Sprague Dawley rats was evaluated using a rat jugular vein intubation tube. Results demonstrated that the C_{max} of TCPLs became 5.7 times higher than that of tilianin solution and that the area under the curve of TCPLs became about 4.6-fold higher than that of tilianin solution. Overall, our results suggested that the prepared tilianin composite phospholipid liposome formulations could be used to improve the bioavailability of tilianin after oral administration.

1. Introduction

Tilianin (acacetin 7-glucoside, Fig. 1) is initially extracted and isolated from the herb of the traditional Uyghur and Tibetan folk medicine (Traditional Chinese medicine) *Dracocephalum moldavica* L. (Maimaitiyiming et al., 2014), which is widely used in the treatments of heart disease, blood pressure, angina, neuralgia and atherosclerosis (Jiang et al., 2014). Currently, tilianin has attracted much attention for its pharmacological and biological properties, such as angina pectoris, ischemia myocardial, anti-atherogenic, antihypertensive and other related diseases (Hernández-Abreu et al., 2013). In addition, tilianin mainly reduces the atherosclerotic lesion formation through the inhibition of cytokine-induced IκB kinase activation, which shows a significant decrease in both systolic and diastolic blood pressure on spontaneously hypertensive rats in acute model (Li et al., 2015a,b). In summary, the difficulty observed in tilianin is the glycosyl group on the ring, which has low hydrophilic, poorly absorbed and low permeability after oral administration (Nam et al., 2006; Dai et al., 2015). These characteristics strongly influence the oral bioavailability and limit its therapeutic efficacy tilianin.

Composite phospholipid liposomes (CPLs) have been used as a novel nanoformulation to improve the oral bioavailability of water-insoluble drugs (Kan et al., 2006). CPLs have also been applied in pharmaceuticals to improve the release characteristics of drug (water-insoluble) and enhance the drug loading of poorly soluble drugs (Zeng et al., 2015). CPLs are vesicular structures made of different lipids (soybean lecithin, SPC and hydrogenated lecithin, HSPC) that are formed in phosphate buffered saline (PBS, pH = 7.4) solutions. Structurally, they resemble the

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http://dx.doi.org/10.1016/j.ejps.2016.02.007
0928-0987/

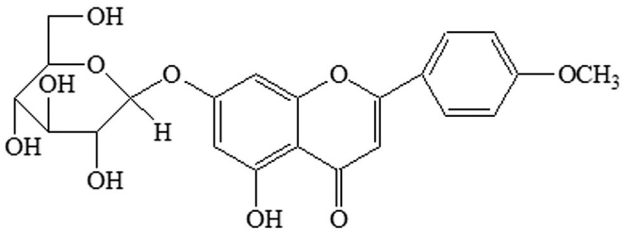

Fig. 1. Chemical structure of tilianin.

lipid membrane of living cells. The characteristic (amphiphilic nature) of different lipids (SPC and HSPC) allows them to form organized structures, such as carriers or membranes of lipid bilayer, when injected into an aqueous environment (stirring) (Zhu et al., 2015). Composite phospholipid liposome features include higher encapsulation efficiency (EE) and oral bioavailability, non-toxicity, and better biocompatibility and stability compared with traditional liposomes (Koshiyama et al., 2015; Zheng, 2014). To overcome the poor solubility and oral absorption of tilianin, we provide a liposome-based nanodelivery system that can encapsulates large amounts of hydrophobic compounds and improve the stability and bioavailability of drugs.

Response surface methodology (RSM) is a combination of mathematical and statistical techniques, which is useful for modelling and analysis; in this method, a response of interest is influenced by several independent variables, and the goal is to optimize this response (Xu et al., 2014). In the optimization process, RSM plays the role of an important tool; practical research on the optimization of preparation mainly focuses on central composite design (CCD) under RSM. However, CCD is frequently used in the optimization method for the preparation of technology because of its advantages in optimizing multifactor problems with optimum number of experimental runs (no limitation on the increased number of experiments) (Rose et al., 2015). This method is practically suitable for comparing theoretical models with experimental methodology and includes the interactive effects of the variables, as well as the overall effects of the parameters on the process (Varshosaz et al., 2010).

To date, fewer studies about TCPL are available; neither the optimization process of RSM nor the in vivo evaluation (rat jugular vein intubation tube) has been reported by any study on the effectiveness of CPLs as a novel nanoformalution delivery system to enhance the oral bioavailability of tilianin. Consequently, the present research mainly aims to optimize the preparation process of TCPLs and assess the oral bioavailability of TCPLs in vivo.

2. Materials and methods

2.1. Materials

Tilianin (>94%, HPLC grade) was provided by Xinjiang Institute of Materia Medica (China). SPC (injection grade, phosphatidylcholine accounts for 96%), HSPC (>98%), Cholesterol and Sephadex G-50 were purchased from Lipoid Company (Germany), Shanghai Advanced Vehicle Technology Pharmaceutical Ltd. (Shanghai, China), Hui Xing Biochemical Reagent Co., Ltd. (Shanghai, China) and Beijing Pharmacia company (China), respectively. Other chemicals and reagents used were chromatographic or analytical grade.

2.2. Preparation of TCPLs

TCPLs were prepared via ethanol injection. Briefly, tilianin (0.4 mg/mL), SPC, HSPC and cholesterol were dissolved in 5 mL of ethanol. The mixture was completely homogenized using ultrasound, injected into about 20 mL of PBS (pH = 7.4) and stirred for 45 min by magnetic stirrer. Finally, the resulting mixture was sonicated for 5 min through probe sonication for 1 min cycle (1 s working and 2 s rest) at 400 W (Ningbo Xinzhi Bio-tech Co. Ltd., China). The resulting tilianin composite phospholipid liposome suspension was extruded through sterile Millipore Express (PES, Millipore, US) with 0.22 μm pore size.

2.3. Characterization of TCPLs

When the samples were placed in plastic disposable cuvettes and equilibrated at 25 °C, the particle size, PDI and zeta potentials of TCPLs were determined by laser diffractometry (NanoZS90, Malvern Instruments Ltd., England). EE was determined using gel filtration method with SephadexG-50 column; in addition, tilianin encapsulation was determined following the solubilization of carriers in ethanol and analysed by a validated high performance liquid chromatography (HPLC) method (r^2: 0.9998). The quantitative determination of tilianin was performed using HPLC (UltiMate3000, Thermo Fisher Scientific, US). The system consisted of a UV detector and Shim-pack ODS (4.6 mm × 250 mm, 5 μm). The mobile phase consisted of 0.5% methanol and acetonitrile (75:25, *v*/*v*), and the flow rate was 1 mL/min. The column temperature was maintained at 35 °C, and the detection wavelength was set at 324 nm. About 10 μL of tilianin solution, TCPLs and CPLs of blank were injected, and no interferences were observed.

$$EE\ (\%) = M_{in}/M_{total} \times 100$$

where M_{in} is the amount of drug entrapped, and M_{total} is the total drug amount used in the preparation.

2.4. RSM design and optimization of the preparation conditions

RSM was developed to acquire the optimal preparation conditions by describing the relationships between the variables and the response. According to the preliminary experiments (single factor test results), the ranges of independent variables, including the amount of phospholipids (X_1, mg), amount of cholesterol (X_2, mg) and weight ratio of phospholipid to drug (X_3), were identified as key factors responsible for size and EE. The range and central point values of the three independent variables used in these studies are summarized in Table 1. The central point was replicated six times to determine the system error. The central composite design experiments were carried out in a randomized order. The data were statistically analysed by ANOVA.

Experimental data were analysed using Design-Expert software (Version 8.05). The fitted polynomial equations were expressed in 3D response surfaces.

2.5. Transmission electron microscopy (TEM)

The morphologies of the TCPLs and CPLs of blank were observed using a TEM (H-600, Hitachi, Japan). After dilution with distilled water, the samples were negatively stained with 2% (*w*/*v*) phosphotungstic acid for observation.

2.6. Differential scanning calorimetry (DSC)

The samples (tilianin, physical mixture, CPLs of blank and TCPLs) were loaded individually into a standard aluminium sample pan and

Table 1
Factors and responses in face-centred central composite design.

Independent variables			Levels		
	−1.682	−1	0	+1	1.682
X_1	100	262.2	500	737.8	900
X_2	20	32.2	50	67.8	80
X_3	10	16.1	25	33.9	40
Dependent variables			Constraints		
Y_1 = particile size (nm)			Minimize		
Y_2 = EE (%)			Maximize		