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Research Paper

Optimization of elastic transfersomes formulations for transdermal delivery of pentoxifylline

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ABSTRACT

Pentoxifylline (PTX) is a xanthine derivative indicated in treatment of intermittent claudication and chronic occlusive arterial diseases. It has low oral bioavailability and short half-life; thus, it was considered as a good candidate drug for the transdermal transfersomes formulation. In the present study, an attempt has been made for development, *in-vitro* and *in-vivo* evaluation of transdermal transfersomes using sodium cholate (SC) and non-ionic surfactants as edge activators. The optimal formulation, $F4_{(Gcholate)}$, exhibited drug entrapment efficiency of 74.9 ± 1.6%, vesicles elasticity of 145 ± 0.6 ($mg\ s^{-1}\ cm^{-2}$), zeta potential of −34.9 ± 2.2, average vesicle diameter of 0.69 ± 0.049 μm with PDI of 0.11 ± 0.037 and permeation flux of 56.28 ± 0.19 $\mu g\ cm^{-2}\ h^{-1}$. It attained a prolonged drug release where 79.1 ± 2.1% of PTX released after 10 h of the run. The drug release kinetic obeys Higuchi model ($R^2 = 0.997$) with Fickian diffusion mechanism. Moreover, the formula enhanced drug permeation through the excised rat's skin predominantly via the carrier-mediated mechanism by 9.1 folds in comparison with the control. Results of the *in vivo* pharmacokinetics study in male volunteers showed that $F4_{(Gcholate)}$ transfersomes formulation increased PTX absorption and prolonged its half-life comparing to the commercial oral SR tablets. Hence, the elastic transfersomes formulation of PTX possesses admirable potential to avoid drug metabolism, improve PTX bioavailability and sustain its release.

1. Introduction

Transfersomes are a special type of liposomes, consisting of bilayer former as phospholipid and edge activator (EA). These vesicles are more elastic than the conventional liposomes [1]. Elasticity in these vesicles is accredited to the presence of an edge activator, which is a single chain surfactant with a high radius of curvature, able of weakening the lipid bilayers of the vesicles and increasing their deformability and flexibility [2]. Sodium cholate, sodium deoxycholate, Spans, Tweens and dipotassium glycyrrhizinate were employed as edge activators [3]. Because of these deformable properties, transfersomes overcome the skin penetration difficulty by squeezing themselves through channels of the stratum corneum that are less than one-tenth the diameter of the transferosomes [4].

Transfersomes are biocompatible and biodegradable, as they are made from natural phospholipids similar to liposomes. They have been efficiently used to protect the drugs from metabolic degradation [5], release drugs in controlled and gradual way [6], and improve the transdermal flux of various drugs such as analgesics, corticosteroids, sex hormone, and chemotherapeutics [7–9].

Pentoxifylline (PTX) is a dimethyl xanthine derivative (Fig. 1), indicated specifically in the treatment of intermittent claudication and other forms of chronic occlusive arterial diseases of the limbs. PTX decreases blood viscosity and increases the blood flow to the affected microcirculation [10]. PTX is a water-soluble and has a partition coefficient (Log*P*) equal to 0.3. Although PTX is completely absorbed after oral administration it undergoes first-pass metabolism results in a low bioavailability (20%) and short half-life (0.4–0.8 h). It is metabolized in the liver to give two active

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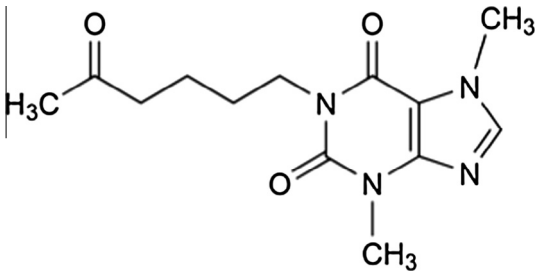

Fig. 1. Chemical structure of PTX.

compounds, which extend its pharmacological half-life by 1–1.6 h [11]. Thus, the present study focused on using the elastic transfersomes formulations to avoid excessive PTX hepatic metabolism as well to enhance its bioavailability through the skin.

2. Materials and methods

2.1. Materials

Pentoxifylline (PTX) was purchased from (Slovakopharma, Slovenia), Sodium cholate was obtained from Sigma–Aldrich, UK. Phospholipon 90G (L-α-Phosphatidylcholine (94% purity)) from soybean is a kind gift from Lipoid-Switzerland. Egg yolk L-α-Phosphatidylcholine (91% purity) and soybean L-α-Phosphatidylcholine (97% purity) were bought from (Shenyang Tianfing, China). All other chemicals were of analytical grade.

2.2. Preparation of PTX transfersomes

PTX Transfersomes were prepared by a modified vortexing-sonication method [12], as shown in Fig. 2. A blend of lipid, edge activator (EAs) and PTX was dispersed in phosphate buffer (pH 7.4) and vortexed for 5 min to attain a milky suspension. The suspension was sonicated by LUC 410 power sonicator (LabTech, Korea) for 30 min followed by freezing at −20 °C for 18 h and thawing at room temperature for 6 h, for three consecutive cycles. The suspension was extruded through 0.2 μm Sartorius® membrane filter (GMBH, Germany) for five times at 50 °C.

Then formulations were centrifuged in a refrigerated centrifuge (Hettich Zentrifugen, Germany) for three hours at 20,000 rpm and 4 °C. PTX transfersomes at the bottom of the tubes were washed with normal saline to remove the free drug and diluted with phosphate buffer up to 10 mL. Samples were stored at 4–8 °C in amber tightly closed containers for further investigations. Blank transfersomes were obtained without adding PTX during the preparation process. Table 1 demonstrates the composition of the prepared PTX-loaded transfersomes, where Phospholipon 90G, Egg yolk L-α-Phosphatidyle choline (91% purity) and soybean L-α-Phosphatidyle choline (97% purity) were used as lipid components, besides sodium cholate (SC), Tween 21, Tween 20, Span 80 and Span 20 as edge activators.

2.3. Characterization of PTX transfersomes

2.3.1. Morphological and size characterization of PTX transfersomes

An optical microscope (Memmert, Germany) fitted with a digital camera (Micam, China) was used to observe and photograph the prepared vesicles before extrusion under magnification 40×. The surface morphology of the selected formula was identified by Inspect S50 scanning electron microscope (Philips Electronics, Netherland).

The mean diameter and the polydispersity index (PDI) of the transfersomes were determined by ABT9000 Nano Laser Particle Size Analyzer (Angstrom, USA). Measurements were performed at a scattering angle of 90°, a temperature of 25 °C and a laser wavelength of 635 nm. PDI was determined as a measure of vesicles size distribution. PDI value of less than 0.1 was considered as a homogeneous distribution of vesicles, whereas a value of greater than 0.3 was of higher heterogeneity.

2.3.2. Zeta potential measurement of PTX transfersomes

The zeta potential of the prepared PTX transfersomes was measured using a zetasizer Nano ZS (Malvern, UK). The zetasizer capillary cell was cleaned with 90% ethanol and distilled water before analyses. The measurement was performed at 25 °C after an appropriate dilution with distilled water. All of the measurements were repeated at least three times and zeta potential was determined as a mean ± SD.

2.3.3. Drug entrapment efficiency

Samples of PTX transfersomes were centrifuged at 20,000 rpm and 4 °C for 3 h. 1 mL of the supernatant was collected, diluted up to 100 mL with PBS pH 7.4 and analyzed for the drug content spectrophotometrically at 272 nm. Percent of the entrapped PTX (EE%) was calculated according to Eq. (1) [13]:

$$EE\% = (\text{Entrapped drug}/\text{Total drug}) \times 100\% \quad (1)$$

2.3.4. Elasticity measurement

This study was carried out by extrusion method at 7.5 psi pressure through 0.2 μm polycarbonate filter membrane (Sigma Chemicals, UK) fixed to stainless steel pressure holder of 50 mL capacity barrel. Half milliliter of the suspension was diluted up to 10 mL with PBS pH 7.4 and then extruded for 10 min. through the filter medium. The elasticity of transfersomes was calculated as per Eq. (2) [14]:

$$E = J_{\text{Flux}}(r_v/r_p)^2 \quad (2)$$

where E is elasticity value of transfersomes (mg s^{-1} cm^{-2}); J_{Flux} is the rate of penetration through a permeable barrier (mg s^{-1} cm^{-2}); r_v is vesicles size (after extrusion); and r_p is pore size of the barrier. The amount of the suspension was measured through 10 min.

2.3.5. FTIR study

FTIR study by disk method was performed in order to investigate possible interaction of PTX with the excipients of transfersomes formulation at the molecular level. The pure drug, SC, lipids, and PTX-lipids physical mixtures were mixed separately with infrared grade KBr in the ratio of 1:100 and the corresponding disks were prepared by applying 15,000 lb of pressure in a hydraulic press. Samples were scanned over a wave number range of 4000–500 cm^{-1} using 8400S FTIR (Shimadzu, Japan).

In case of PTX-loaded transfersomes, samples of 5 mL were placed in 100 mL glass containers, frozen at −40 °C for 12 h, and then lyophilized by triad system lyophilizer (Labconco, USA). Sublimation lasted for 36 h under a vacuum pressure of 54×10^{-3} bar at −54 °C. The lyophilized powder samples were prepared as KBr disks and scanned in the FTIR instrument.

2.4. In-vitro drug release

PTX *in-vitro* release from the loaded transfersomes was studied using dialysis bag (Slide-A-Lyzer dialysis cassette, USA), as a donor compartment fitted with a dialysis membrane, and molecular weight is 12,000. One milliliter of the formulation was accurately placed inside the bag, which was sealed with a closure clip to prevent leakage. The dialysis bag was placed in a receptor com-