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Preparation and characterization of isoniazid-loaded crude soybean lecithin liposomes

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ABSTRACT

Tuberculosis (TB) is a poverty related infectious disease that is rapidly giving rise to public health concerns. Lengthy drug administration and frequent adverse side-effects associated with TB treatment make anti-tubercular drugs (ATDs) good candidates for drug delivery studies. This work aimed to formulate and prepare liposomes as a cost-effective option for ATD delivery. Liposomes were prepared by film hydration using crude soybean lecithin (CL) and not pure phospholipids as in the normal practice. Cholesterol was also used (up to 25% mass ratio), and isoniazid (INH) was encapsulated as model drug using a freeze-thaw loading technique. Purified soybean lecithin (PL) was also used for comparative purposes, under the same conditions. INH-loaded liposomes were characterized for particle size, Zeta Potential (ZP), encapsulation efficiency (EE) and drug release. Physicochemical properties were investigated using thermogravimetric analysis, differential scanning calorimetry, X-ray diffraction and Fourier transform infrared. INH-loaded CL-based liposomes showed high EE ($79 \pm 2.45\%$). The average particle size (813.00 ± 9.21 nm) and ZP (-42.80 ± 4.31 mV) of this formulation are promising for the treatment of TB by pulmonary delivery. These findings suggest the possibility of encapsulating ATDs in liposomes made of crude soybean lecithin that is cheap and readily available.

1. Introduction

Tuberculosis (TB) is one of the top two most devastating infectious diseases worldwide, second only to the human immunodeficiency virus (HIV) infection. In 2015, the world health organization (WHO) reported 10.4 million of new active TB-cases and 1.8 million deaths (World Health Organization, 2016). The current anti-TB regimen offers high cure rates – up to 95% – in the case of drug susceptible TB (Nuermberger et al., 2010). The pharmacological concerns in this field include the poor bioavailability of some anti-TB drugs, rapid first pass metabolism and non-selective biodistribution. These result in extended TB treatment of 6 to 24 months, using multiple drugs. This lengthy therapeutic regimen is associated with several adverse effects and low patient compliance that promotes both the development of drug resistance and therapeutic failure (Sosnik et al., 2010). The firt line anti-TB drugs are isoniazid, rifampicin, pyrazinamide and ethambutol (Kadare et al., 2014). Due to various side effects related to their clinical use, all these antimicrobial agents are good candidates for drug delivery in order to control their pharmacokinetics beneficially.

Liposomes are lipid-based vesicular devices that are widely used as drug delivery systems. Among all the current delivery systems, liposomes represent the most clinically established drug carriers (Guptha, 2015). Liposomes are known to be taken up by the cells of mononuclear phagocytic system, especially macrophages. This

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feature is known as a longstanding inconvenience in terms of circulation half-life, when the site of interest is beyond macrophages (Immordino et al., 2006). However, the causative agent of TB, *Mycobacterium tuberculosis,* resides in macrophages, making treatment difficult (Tăbăran and Catoi, 2014). The biological fate of liposomes therefore enhances their appropriateness as a vehicle for anti-TB drug delivery.

Liposomes are already being extensively studied for the delivery of anti-tubercular drugs. Chimote and Banerjee (2010) encapsulated isoniazid in liposomes using dipalmitoyl phosphatidylcholine, DPPC. These INH-loaded DPPC-liposomes demonstrated a dual therapeutic benefit for the management of TB – pulmonary medication and lung surfactant mimic action. Vyas et al. (2004) developed ligand-anchored and negatively charged liposomal aerosols that enhanced and sustained the release of rifampicin (RIF) in macrophages. This formulation showed higher clearance rate of *Mycobacterium smegmatis* residing in rat alveolar macrophages than non-decorated and neutral RIF-loaded liposomes. Recently, Patil et al. (2015) prepared and characterized freeze-dried RIF-loaded liposomes that demonstrated controlled and sustained release profile by intra-tracheal instillation to Wister rat model. In addition, dry powder formulations based on INH-loaded pro-liposomes have demonstrated attractive flow ability, powder performance and high antimicrobial activity in infected macrophages without any inflammatory or cytotoxic effects (Rojanarat et al., 2011). Other groups have reported similar successful encapsulations of anti-TB drugs in liposomal systems (Singh et al., 2015; Patil-Gadhe et al., 2014; Manca et al., 2012; Pandey et al., 2004).

However, the costly formulation status of liposomes is often due to the use of expensive synthetic or purified natural phospholipids (Li et al., 2015; Yokota et al., 2012), which might preclude their use in the management of poverty related diseases like TB, where many patients cannot afford the long regimen. A recent review by Machado et al. demonstrated the importance of using cost effective naturally occurring phospholipids to make liposomes for food ingredient delivery (Machado et al., 2014). The feasibility of using crude soybean lecithin for food-liposome preparation was established by Yokota et al. This group has successfully encapsulated casein hydrolysate in order to mask its bitter taste and odour (Yokota et al., 2012). The aim of our study was to develop anti-TB drug-loaded liposomes using this FDA-approved naturally occurring phospholipids mixture, crude soybean lecithin (CL), which is cheap and readily available, by studying the properties of CL-based liposomes with anti-TB drugs.

2. Materials and methods

2.1. Materials

Soybean lecithin granules used in this study were sourced from Health Connection Wholefoods (USA). According to the manufacturer, these granules (100 g) contained mainly phosphatidylcholine (23 g), phosphatidylinositol (14 g), polyunsaturated fat (35 g), saturated fat (13 g), glycaemic carbohydrates (8 g), sodium (0.11 g) and total energy up to 2940 kJ. Cholesterol used was from Carlo Erba/Divisione Chimica (Italy). Isoniazid, mono- and dibasic sodium phosphate were purchased from Sigma Aldrich (Germany). Ethyl acetate and acetone used were from Protea Chemicals (South Africa) while chloroform was from BM Scientific/Parow Industria (South Africa). Methanol from Merck (Germany) and acetonitrile from Ranbaxy Fine Chemicals Ltd (India) were of analytical high performance liquid chromatography (HPLC) grade. Unless indicated, all materials were used without further purification.

2.2. Methods

2.2.1. Purification of soybean lecithin

Crude soybean lecithin was purified according to the procedure described by Mertins et al. (2008) with a slight modification. Briefly, the crude soybean lecithin (10 g) was dissolved in ethyl acetate (100 ml), after which HPLC grade water (4 ml) was added while stirring slowly in order to prevent the formation of two phases. After allowing the liquid mixture to stand, the supernatant was decanted and the bottom phase, which looked like a gel, was dispersed in acetone (60 ml). Any agglomerates that formed were disrupted using a glass rod. Thereafter, the acetone was decanted and a further portion of acetone (60 ml) added, repeating the crushing process. Finally, the precipitate was filtered under vacuum and dried in a desiccator connected to a vacuum pump for 48 h. The purified lecithin (7.99 g) was collected and kept at room temperature. Further purification using liquid chromatography did not improve the quality of the lipid mixture significantly and was not considered in further studies.

2.2.2. Validation studies

An HPLC method for the quantification of isoniazid (INH) was validated for linearity, accuracy, repeatability (intraday precision) and intermediate precision (inter-day precision). The chromatographic system consisted of an Agilent HP1100 LC–MSD and equipped with a quaternary pump, in-line degasser, DAD detector, 1100 MSD and ChemStation for collection and analysis of data. A ZORBAX Elipse Plus C18 4.6 i.d.x 150 mm × 5 μm column was used for reversed-phase HPLC analysis. The mixture of aqueous solution of monobasic sodium phosphate 0.01 M and acetonitrile (90:10) was used as mobile phase on isocratic elution mode. From a 1000 μg/ml stock solution of INH in HPLC grade water, five different concentrations (5–500 μg/ml) were prepared and filtered using 0.45 μm Millipore filters before their injection.

Over six days of experiments, three daily injections of 20 μl for each INH fresh solution were performed the HPLC instrument. The flow rate of the mobile phase was 1 ml/min and the UV detection was set at 254 nm. The duration of each elution was 5 min and the retention time for INH was found at 1.2 min. The average peak areas obtained were plotted against respective concentrations to construct the calibration curve in order to determine the linearity range, regression equation and correlation coefficient. The percentage recovery and relative standard deviations (RSD) were calculated in order to evaluate accuracy and precision respectively. The limits of detection (LOD) and quantification (LOQ) were established as the ratio of the standard deviation for the lower concentration in the calibration curve to the slope of the regression equation times 3 for LOD and 10 for LOQ (ICH Experts, 2005; CLSI, 2004).

2.2.3. Preparation of liposomes

The crude soybean lecithin (CL) or the purified version (PL) was used in combination with cholesterol at the mass ratio of 3:1. Basically, 0.10 g of lipid components was dissolved in 1 ml of chloroform in an ultra clean 25 ml round bottom flask. The obtained solution was dried at 50 °C under vacuum using a rotary evaporator (Büchi Rotavapor R-205, Switzerland) at 200 rpm for 5 min. The round bottom flask was removed and stored in a vacuum desiccator at room temperature overnight. Phosphate buffer (3 ml, pH 7.0) in HPLC grade water was added to the thin lipid film. The mixture was heated for an hour at 60 °C under stirring at 400 rpm to hydrate the lipids. The medium was then homogenized in a bath sonicator (Digital Ultrasonic Cleaner, Spellbound 909) at 60 °C for 20 min to produce a suspension of CL-based liposomes or PL-based liposomes. On the other hand, 3 ml of