Formulation Development And Characterization Of Liposomes For Sorafenib Delivery

Rangasamy Pasupathy, Pitchaimuthu Pandian, Subramanian Selvamuthukumar

Abstract: Sorafenib is an anticancer drug for the treatment of hepatocellular carcinoma. The present work involves the formulation development and characterization of sorafenib loaded liposomes. Sorafenib loaded liposomes were prepared by the thin-film hydration method. Morphology was examined by scanning electron microscopy. Particle size and zeta potential were determined by laser-scattering method. Drug and excipients compatibility study was performed by ATR-FTIR, and the study revealed that there was no interaction between drug and excipients. The encapsulation efficiency (EE) of sorafenib liposomes was determined using the HPLC method. The in vitro release profile of sorafenib loaded liposomes was determined using a dialysis method. The sorafenib liposomes expected a spherical shape with a uniform particle size (97 nm), negative zeta potential (-18.3 mV), and polydispersity index (0.474). % EE was found to be 97 %. A slow and sustained drug release was observed in vitro. This result suggests that sorafenib loaded liposomes can be considered a novel chemotherapeutic approach for treating liver cancer.

Index Terms: Sorafenib, Hepatocellular carcinoma, Liposomes, Lipophilic drug, Thin film hydration, Tumor drug delivery, Tyrosine kinases.

1 INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common and leading human malignancy, causing cancer-related deaths worldwide [1,2]. Sorafenib (SRF) is the first drug approved by the USFDA to treat advanced Hepatocellular carcinoma. Sorafenib as a potent competitive multi-kinas inhibitor of the RAF/MEK/ERK signalling pathway, suppress tumor cell proliferation, survival, and angiogenesis by competitively binding to VEGFR-2, VEGFR-3, and PDGFR-b tyrosine kinases [3,4]. A required daily dose of 400-800 mg of sorafenib is required for optimum efficacy when it is given orally. However, sorafenib has low aqueous solubility and undesirable side effects, which limit its clinical application [5-8]. Thus the problems associated with oral administration made us design the novel system to deliver sorafenib with an alternate route of administration. Liposomes are novel drug delivery carriers and too versatile in delivering diverse drug molecules [9]. Liposomes are vesicles with spherical in shape and comprised of phospholipid bilayers, conventionally made by hydration of thin lipid film. By the characteristic structure, liposomes structure allows the incorporation of different types of drugs as lipophilic drugs are mainly entrapped within the lipid bilayer, while lipophobic drugs are encapsulated in the inner aqueous compartments [10, 11]. Extensive studies reported that the liposomes are a target-based drug delivery system that enhances the solubility and gastrointestinal (GI) permeability of the drugs, ultimately results in an improved oral bioavailability [10, 12, 13]. Liposomes can be prepared as Lipoid S 75 (phosphatidylcholine (PC) >75%) from soybean lecithin. Amphiphilic lipid S 75 is comprised of a lipophobic head domain and lipophilic tail domain. This can come from the liposome in the aqueous media having a structure of a hydrophilic surface and a hydrophobic inner layer. It would be an ideal core material to prepare carriers for a nutrient that has the biological activity itself [14].

Cholesterol improves the stability of the lipid bilayers by forming ordered and highly rigid phase with fluid-like characteristics depending on the type of phospholipid involved in the liposome [15]. In the present study, Sorafenib loaded liposomes were fabricated by the method called, thin-film hydration using Lipoid S75 and cholesterol followed by extrusion. Sorafenib liposomes were characterized by morphology, particle size, and zeta potential. The drug release behaviour of sorafenib was performed with a dialysis bag diffusion method.

2 MATERIAL AND METHODS

A gift sample of Sorafenib drug was received from Hetero labs limited. (Jadcherla, India), Cholesterol was purchased from Nice chemicals, and Fat-free soybean phospholipids with 70% phosphatidylcholine (SPC) (Lipoid S75) were gifted from Lipoid (GmbH, Germany). The other chemicals and reagents used during the work were of analytical grade.

2.1 Preparation of Sorafenib Liposomes

Liposomes were prepared with a slight modification to the described method earlier [16]. Briefly, 240 mg of soybean phosphatidylcholine, 16 mg of cholesterol, and the drug were firstly dissolved in 2 ml of chloroform: ethanol (2:1) and placed in a round-bottom flask. Chloroform and ethanol were eliminated by vaporization via rotary evaporator at 40° C, and the traces solvent were eliminated by continuing the lipid film evaporation under a vacuum overnight. About 16 ml deionized water was added to hydrate the dried lipid film, the contents were gently shaken and sonicated using probe-type sonicator (Make: Sonics-Vibra cell) for 10 min. Liposomes suspension was then stored at 4° C in a refrigerator.

2.2 Characterization of Sorafenib liposomes:

Particle size and Zeta potential measurement

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Scanning electron microscope

Scanning electron microscopy (SEM) was used to visualize the structure of the liposomes, and the images were recorded. The sample was gold sputter-coated for 5 min, and the images were obtained using a scanning electron microscope (JEOL...
Quantification of Sorafenib in liposomes by HPLC
The quantitative determination of Sorafenib in liposomes by High-performance liquid chromatography (HPLC) was performed as described previously. The HPLC system comprised of a quaternary gradient pump, a PDA detector, a standard autosampler, a column oven, and a chromatography workstation (Shimadzu Technologies) following this HPLC method: A reverse phase Shim-Pack GIST C18 column was used (150 mm × 4.6 mm, 5 μm). The mobile phase composition was 70% acetonitrile and 30% ammonium acetate (20 mM). The samples (50 μL) were injected into the HPLC column with a mobile phase as a diluent, with 1.0 ml/min of flow rate and temperature of the column at 40°C. The drug peaks were detected at 260 nm, and the sorafenib concentration was calculated using an external standard calibration curve. The linear calibration curves were plotted using the concentration range of 2, 4, 6, 8, 10, and 12 μg/ml with the regression coefficient of 0.999.

Encapsulation efficiency of Sorafenib liposomes
The drug encapsulation efficacy was determined by quantifying sorafenib concentration in 1 ml of liposomes. Liposomes were further added to 4 ml of methanol to rupture the vesicles. The solution was further diluted with the mobile phase. The diluted samples were filtered by a 0.45-μm nylon membrane and injected into HPLC for content analysis. Sorafenib concentration was quantified by using a calibration curve, and encapsulation efficiency (%EE) was determined as follows:

\[ \% EE = \frac{\text{amount of drug in liposomes}}{\text{amount of initially added drug}} \times 100 \]

Fourier transform infrared spectroscopy (FTIR)
Sorafenib, drug loaded liposomes, and their formulation were subjected to ATR-FTIR studies between 4000 cm\(^{-1}\) to 650 cm\(^{-1}\) using Agilent cary 630 FTIR spectrometer operated with Agilent Resolutions Pro software.

In-vitro drug release study
The release of sorafenib from the liposomes was determined using a dialysis method [18, 19]. The equal quantity of sorafenib and sorafenib liposomes were placed in a dialysis membrane bag. The in-vitro release study was initiated by keeping the sealed dialysis bag in 20 ml of phosphate buffer saline (pH 7.4) containing 1 % tween 80, at 37°C and 100 rpm. The sample at regular intervals was withdrawn and replaced with the equal quantity of fresh phosphate buffer saline to maintain sink condition. The concentration of sorafenib in the withdrawn samples was determined by HPLC method described previously. The in vitro release study was performed in triplicate and the results were expressed as mean±SD (n=6).

3 RESULTS AND DISCUSSION

3.1 Preparation and Characterization of Liposomes Formulation
Sorafenib loaded liposomes were synthesized by thin film hydration method. Table 1 summarizes the average diameter, zeta potential, PDI values, and encapsulation efficiency of sorafenib loaded liposomes and blank liposomes obtained by dynamic light scattering. The mean diameters of both liposomes were presented less than 100 nm without significant difference the low particle size distribution was possible to avoid filtration by the kidney, minimize specific sequestration by sinusoids in the spleen and fenestra in the liver [20]. The zeta potential values indicated that the liposomes obtained were -16.57 and -18.3 mV for blank and sorafenib loaded liposomes samples, respectively, thus no statistical differences found in the zeta potential between both. The low PDI values for the liposomes ensure uniform formed particles in the formulation. Sorafenib loaded liposomal formulation achieved a high encapsulation efficiency of over 97.91%.

<table>
<thead>
<tr>
<th>Liposome Formulations</th>
<th>Avg. Diameter ± SD (nm)</th>
<th>Zeta potential ± SD (mV)</th>
<th>PDI ± SD</th>
<th>Encapsulation efficiency (% EE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank Liposomes (Placebo)</td>
<td>95.8 ± 0.68</td>
<td>-16.57 ± 0.65</td>
<td>0.432 ± 0.085</td>
<td>–</td>
</tr>
<tr>
<td>Sorafenib loaded Liposomes</td>
<td>97.9 ± 0.75</td>
<td>-18.3 ± 0.93</td>
<td>0.474 ± 0.093</td>
<td>97.91 ± 0.13</td>
</tr>
</tbody>
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3.2 Surface morphology properties of sorafenib liposomes
The surface morphology of sorafenib loaded NBs was measured using scanning electron microscopy (SEM). The SEM image of liposomes showed the spherical shape with a smooth surface (Fig. 1).

Fig. 1. SEM image of Sorafanib Liposomes

3.3 Fourier transform infrared spectroscopy (FTIR)
The FTIR analysis with the Attenuated total reflectance (ATR) method was performed to investigate the intermolecular interactions among the components consisting of sorafenib loaded liposomes. The FTIR spectra of sorafenib, SPC, cholesterol, physical mixture, and sorafenib loaded liposomes

JSM-5610LV, 30 kV).

The equal quantity of fresh phosphate buffer saline (pH 7.4) containing 1 % tween 80, at 37°C and 100 rpm. The sample at regular intervals was withdrawn and replaced with the equal quantity of fresh phosphate buffer saline to maintain sink condition. The concentration of sorafenib in the withdrawn samples was determined by HPLC method described previously. The in vitro release study was performed in triplicate and the results were expressed as mean±SD (n=6).

Table 1. Physico-chemical characteristics of Liposomes formulations. Results are shown as mean ± SD (n=3).

3.3 Fourier transform infrared spectroscopy (FTIR)
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are shown in Fig.2. The SPC spectrum showed peaks at 2922 cm⁻¹ (aliphatic C-H stretching), 2853 cm⁻¹ (olefinic C-H stretching), 1056 cm⁻¹ (C-O stretching), and 968 cm⁻¹ (C-C stretching) [21]. The pure sorafenib sample showed the main characteristic bonds at 3308 cm⁻¹ (N-H stretching), 3114 cm⁻¹ (C-H stretching), 1723 cm⁻¹ (C=O bond, uried group), 1688 cm⁻¹ (C=O bond, amide group), 1400-1600 cm⁻¹ (C=C, N-H, C-N stretching) [22]. The characteristics peaks of cholesterol represented at 3421 cm⁻¹ (O-H bond vibration of free hydroxyl group), 2926 and 2850 cm⁻¹ (asymmetric vibration of C-H bonds of methyl groups), 1461 cm⁻¹ (C=C vibration), 1371 cm⁻¹ (bending and stretching of O-H and C-O-H ), 1051 cm⁻¹ (C=O vibrations) and 955 cm⁻¹ (C=C backbone vibrations) [23]. All these characteristic peaks and associated wavenumbers were recorded without any detectable interactions at a 1:1 ratio in case of physical mixtures and formation of sorafenib loaded liposomes. The presence of broad and strong absorption peak at 3332 cm⁻¹ was due to Hydrogen bond formation of the amide with phosphate groups of SPC, and thus the disappearance of the characteristic peak of sorafenib as the sorafenib is liposomes encapsulated.

The slow-reaction characteristics of sorafenib from its liposomes were expected due to the lipophilic nature of sorafenib with lower aqueous solubility that made it more stick to the lipid bilayer and interact with the nonpolar chains of phospholipids. Sorafenib is a hydrophobic drug, has been known to exhibit slow, steady, and sustained release from nano-liposomes. The results implied that the constant therapeutic amount of sorafenib might be maintained for a long period. The enhanced stability of the nanomedicine was favorable for transportation in the bloodstream.

4 CONCLUSIONS
In conclusion, sorafenib liposomes were developed for tumor drug delivery and HCC treatment. The sorafenib loaded liposomes were formulated by thin-film hydration technique with extrusion, and they appeared as spherical globules in shape. The specific characters comprised of sorafenib liposomes are uniform particle size distribution, negative zeta potential, high encapsulation efficiency with drug loading, and the slow but sustained release sorafenib through liposomes vesicles. The developed liposomes system could be a biocompatible platform for enhancing the oral bioavailability of drugs with poor water solubility like sorafenib.

5 ACKNOWLEDGMENT
The authors are most grateful and appreciate towards Lipoid (GmbH, Germany) for gift sample of Lipoid S 75 sample. Authors also acknowledge Department of Pharmacy, Annamalai University, Chidambaram, Tamilnadu for providing necessary facility to success this project.

6 REFERENCES

Fig. 2. FTIR spectrum of SPC, Cholesterol, Sorafenib, Physical mixture 1:1 ratio (SPC:Chol:SRF), and Sorafenib loaded liposomes.

3.4 In-vitro drug release study
The drug release profile of sorafenib (pure drug) and sorafenib loaded liposomes was determined in vitro. At 48 hrs, the cumulative release rates of sorafenib (pure drug) and sorafenib liposomes were 71.31% and 33.21%, respectively (Fig 3). Sorafenib release was high in the first hours of study from liposomes. This could be due to the drug release from the surface or near to the surface in the bilayer. However, the drug was released very slowly later, probably due to slower drug diffusion through the core of the formulation.

Fig. 3. In vitro release profile of pure sorafenib drug and sorafenib liposomes in pH 7.4 PBS containing 1% w/v Tween-80 at 37±1°C (n=6).


