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NANOMEDICINE

Formulation, characterization and pulmonary deposition of nebulized celecoxib encapsulated nanostructured lipid carriers

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ABSTRACT

The aim of the current study was to encapsulate celecoxib (Cxb) in the nanostructured lipid carrier (Cxb-NLC) nanoparticles and evaluate the lung disposition of nanoparticles following nebulization in Balb/c mice. Cxb-NLC nanoparticles were prepared with Cxb, Compritol, Miglyol and sodium taurocholate using high-pressure homogenization. Cxb-NLC nanoparticles were characterized for physical and aerosol properties. In-vitro cytotoxicity studies were performed with A549 cells. The lung deposition and pharmacokinetic parameters of Cxb-NLC and Cxb solution (Cxb-Soln) formulations were determined using the Inexpose™ system and Pari LC star jet nebulizer. The particle size and entrapment efficiency of the Cxb-NLC formulation were 217 ± 20 nm and >90%, respectively. The Cxb-NLC released the drug in controlled fashion, and in-vitro aerosolization of Cxb-NLC formulation showed an FPF of 75.6 ± 4.6%, MMAD of 1.6 ± 0.13 μm and a GSD of 1.2 ± 0.21. Cxb-NLC showed dose and time dependent cytotoxicity against A549 cells. Nebulization of Cxb-NLC demonstrated 4 fold higher AUC_t/D in lung tissues compared to the Cxb-Soln. The systemic clearance of Cxb-NLC was slower (0.93 l/h) compared to the Cxb-Soln (20.03 l/h). Cxb encapsulated NLC were found to be stable and aerodynamic properties were within the respirable limits. Aerosolization of Cxb-NLC improved the Cxb pulmonary bioavailability compared to solution formulation which will potentially lead to better patient compliance with minimal dosing intervals.

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1. Introduction

Inhalation drug delivery represents a potential delivery route for the treatment of several pulmonary disorders. Inhalation drug delivery has several advantages over conventional (parenteral and oral) dosage forms such as a) non-invasive b) circumventing first pass metabolism and systemic toxicity c) reduced frequent dosing and d) the inhaled drug reaches directly to the lung epithelium thereby enhancing local drug concentrations. In pulmonary drug delivery systems, surfactants and co-solvents are often used to prepare stable formulations of highly lipophilic active ingredients and inhalation of formulations utilizing excipients cause lung inflammation [1]. It is expected that encapsulation of lipophilic compounds in nanoparticles will improve the stability by protecting the active ingredient from degradation and release the encapsulated drug in a controlled manner for a prolonged period of time [2]. Among several inhalation drug delivery systems, biodegradable nanoparticles have demonstrated several advantages in terms of protecting the active ingredient from degradation and releasing the drug in a controlled manner for prolonged periods of time. Although few attempts have been made to deliver anticancer agents using nanoparticles and liposomes via an inhalation route, the major limitations of these systems are instability during nebulization, biodegradability, drug leakage and associated drug adverse side effects [3,4]. Solid lipid nanoparticles (SLN) have several advantages such as a) good tolerability, b) biodegradability and c) greater stability against the shear forces generated during nebulization [3,5] compared to polymeric nanoparticles [3], liposomes [4] and emulsions [6]. SLN are produced by replacing the oil lipid of an o/w emulsion with a solid lipid or a blend of solid lipids, where the lipid particle matrix being solid at both room and body temperature. Despite greater stability, SLN have some limitations such as low drug loading, risk of gelation and drug leakage during storage caused by lipid polymorphism [7]. Therefore, in order to decrease the degree of organization of the lipid matrix in SLN and increase the drug loading capacity, the nanostructured lipid carriers (NLC) have been developed and reported as the second generation of lipid nanoparticles. NLC nanoparticles are comprised of an inner oil core surrounded by an outer solid shell and hence allow the high payload of a lipophilic drug [8]. NLC nanoparticles have been investigated for topical delivery of lipophilic anti-inflammatory molecules and also in cosmetic products [9,10].

Cyclooxygenase-2 (COX-2) enzyme is over-expressed among various human malignancies and is thought to have a potential role in the pathogenesis of non-small cell lung cancer (NSCLC) [11]. Celecoxib (Cxb), a lipophilic COX-2 inhibitor has shown a synergistic

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anticancer activity in combination with other anticancer agents such as docetaxel [11]. The preclinical data suggest that the COX-2/prostaglandin E2 signaling pathway plays an essential role in conferring the malignant phenotype in non-small cell lung cancer by stimulating angiogenesis, inhibiting apoptosis and suppressing the immune response. It has been also shown that Cxb inhibits NF-kB activation through inhibition of IKK and Akt activation which leads to down-regulation of COX-2 synthesis and other genes needed for inflammation, proliferation, and carcinogenesis [12]. In lung cancer patients, Cxb is also known to inhibit the overproduction of prostaglandin E2, as well as modulate the IL-10 production in the lung microenvironment [13]. Cxb is a poorly water soluble drug (7 μg/ml) with a partition coefficient of 3.68 [14] and necessitates use of surfactants and a co-solvent such as ethanol to formulate an aerosol formulation. Our earlier studies showed that Cxb formulations prepared using tocopheryl polyethylene glycol succinate and ethanol resulted in improved solubility and enhanced the anticancer activity of nebulized Cxb [15,16]. However, these formulations were un-stable and precipitation was observed on long term storage. Therefore, it is expected that encapsulation of Cxb in NLC nanoparticles will improve the stability and alter its pharmacokinetics by enhanced retention [17] and releasing the Cxb in a controlled fashion.

In the present investigation, we explored the feasibility of NLC nanoparticles as a novel carrier system for inhalation drug delivery of Cxb. Therefore in this study, we examined the effect of Cxb-NLC on the release of Cxb, aerodynamic properties and in-vitro cytotoxicity against A549 NSCLC cells. The present investigation was also aimed to evaluate the in-vivo pulmonary deposition and systemic availability of aerosolized Cxb loaded NLC (Cxb-NLC) nanoparticles in Balb/c mice utilizing an Inexpose® exposure chamber.

2. Material and methods

2.1. Materials

The Cxb was a generous gift from Pfizer (Skokie, IL). The triglyceride Miglyol 812 was obtained from Sasol Germany GmbH (Witten, Germany) and Compritol® 888 ATO was a kind gift sample from Gattefosse (Saint Priest, France). The taurocholic acid sodium salt was procured from Sigma-Aldrich Chemicals (St. Louis, MO). Dialysis tubing (molecular weight cut-off: 6000–8000 Da and flat width of 23 mm) was obtained from Fisher Scientific (Pittsburg, PA). The polyoxyethylene-20 oleyl ether or Volpo-20 (Oleth-20) was a kind gift from Croda Inc (New Jersey, USA). Vivaspin centrifuge filters (molecular weight cut-off: 10,000 Da) were procured from Sartorius Ltd, (Stonehouse, UK). Fetal bovine serum (FBS), antibiotics and DID-oil (a lipophilic fluorescent dye with excitation 644 nm, emission 665 nm) were procured from Invitrogen Corp (Eugene, OR). The A549 human NSCLC cell line was obtained from American Type Culture Collection (Rockville, MD, USA). A549 cells were grown in F12K medium (Sigma, St. Louis, MO, USA) supplemented with 10% FBS. All tissue culture media contained an antibiotic antimycotic solution of penicillin (5000 U/ml), streptomycin (0.1 mg/ml), and neomycin (0.2 mg/ml). The cells were maintained at 37 °C in the presence of 5% CO_2 in air. All other chemicals used in this research were of analytical grade.

2.2. Animals

Male Balb/c mice (20–25 g; Charles River Laboratories) were utilized for the studies. The protocol for in-vivo experiments was approved by the Animal Care and Use Committee, Florida A&M University. The animals were acclimated to laboratory conditions for one week prior to experiments and were on standard animal chow and water *ad libitum*. The temperature of the room was maintained at 22 ± 1 °C and the relative humidity of the experimentation room was found in the range of 35–50%. For nebulization studies, 4–5 days prior to the start of the experiment animals were trained by nebulizing water for 30 min. This is to acclimate the nebulization environment and prevent any discomfort during the formulation nebulization.

2.3. Nanoparticle preparation

A Cxb-NLC formulation was prepared by the hot melt homogenization technique [9]. In brief, 0.02% w/w of Cxb was dissolved in dichloromethane and mixed with lipid phase comprised of Compritol (7.0% w/w) and Miglyol (3.0% w/w). Later, organic phase was removed on a rota evaporator for 2–3 h at 80 °C and to the heated lipid phase the aqueous solution (40 ml) containing sodium taurocholate (1.5% w/w) surfactant was added at the same temperature under high speed mixing using a Cyclone IQ2 with a Sentry™ Microprocessor (USA) at 20,000 rpm for 1 min. The resultant oil-in-water dispersion was passed through a Emulsiflex-C5 (Avestin, Ottawa, Canada) high-pressure homogenizer at 5000 psi for 5 cycles. Throughout the process temperature was maintained at 80 °C. The Cxb-NLC and placebo NLC (without Cxb) formulations were prepared for comparison.

2.4. Characterization of nanoparticles

The particle size of the NLC nanoparticle formulation was measured using a BI-90 particle sizer (Brookhaven Instruments, Boston, USA), which is based on the principle of Dynamic Light Scattering and Zeta potential measurement was carried with Zeta plus (Brookhaven Instruments). In order to verify the total amount of drug present in the system, 0.1 ml of the Cxb-NLC formulation was dissolved in 0.9 ml of tetrahydrofuran and subsequent dilutions were made with acetonitrile. Prior to HPLC analysis for Cxb content, samples were centrifuged at 13,000 rpm for 15 min and 100 μl of supernatant was injected into HPLC. Entrapment efficiency was determined using vivaspin centrifuge filters as per reported method [18]. In brief, the Cxb-NLC (0.5 ml) formulation was placed on top of the vivaspin centrifuge filter membrane (molecular weight cut-off 10,000 Da) and centrifuged at 3500 rpm for 15 min. The aqueous phase collected at the bottom of vivaspin filter membrane was subjected to high-performance liquid chromatography (HPLC) analysis to determine the Cxb content. To determine the drug loading, 1.0 ml of the Cxb-NLC formulation was centrifuged at 16,000 *g* for 1.5 h and the pellet was dissolved in tetrahydrofuran. The amount of Cxb present in the pellet was estimated by HPLC. Drug loading was calculated based on the amount of Cxb identified in a measured amount of NLC using the following equation [19]

$$\textit{Drug Content}\ (\%\ \mathrm{w}/\mathrm{w}) = (\textit{mass of Cxb in NLC} \times 100) \div (\textit{mass of NLC recovered}) \quad (1)$$

2.5. Differential Scanning Calorimetry

The interaction of Cxb with lipids and the association of Cxb in the NLC nanoparticle formulation were determined using a DSCQ100 (TA instrument, DE). Approximately 10 mg of the formulation was weighed into an aluminum pan and sealed hermetically, and the thermal behavior was determined in the range of 10 to 225 °C at a heating rate of 5 °C min^{-1}. Baselines were determined using an empty pan, and all the thermograms were baseline-corrected. Transition temperatures were determined from the endothermic peak minima while transition enthalpies were obtained by integration of the endothermic transitions using linear baselines.

2.6. In-vitro drug release studies

In-vitro drug release studies were conducted with a cellulose membrane (6000–8000 molecular weight cut-off) using a USP 1 (basket) dissolution apparatus (Vankel, NC) for 72 h with the help of