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Research paper

Pulmonary delivered polymeric micelles – Pharmacokinetic evaluation and biodistribution studies

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ABSTRACT

Polymeric micelles represent interesting delivery systems for pulmonary sustained release. However, little is known about their *in vivo* release and translocation profile after delivery to the lungs. In the present study, curcumin acetate (CA), which is an ester prodrug of curcumin, or the mixture of CA and Nile red was encapsulated into PEG–PLGA micelles by a solvent evaporation method. The micellar formulation increased the stability of CA in water and physiologically relevant fluids and led to a sustained drug release *in vitro*. Following intratracheal (IT) administration to rats, CA loaded micelles achieved not only prolonged pulmonary retention with AUC values almost 400-fold higher than by IV route, but also local sustained release up to 24 h. In addition, IT delivery of micelles appeared to facilitate the uptake into the pulmonary vascular endothelium and efficiently translocate across the air–blood barrier and penetrate into the brain. Co-localization of CA and Nile red confirmed that micelles in lung and brain tissue were still intact. This study is the first to demonstrate that aerosolized PEG–PLGA micelles are a promising carrier for both pulmonary and non-invasive systemic sustained release of labile drugs.

1. Introduction

Curcumin is the major active ingredient of *Curcuma longa* rhizome (popularly known as turmeric). The compound has been associated with anti-oxidant, anti-inflammatory and immunomodulatory pharmacological effects [1]. As an NF-κB inhibitor, curcumin has exhibited protective effects in chronic hypoxic hypercapnic and monocrotaline (MCT) induced pulmonary arterial hypertension (PAH) in rats [2,3] and it is thus considered as a potential therapeutic agent for PAH [4].

PAH is a chronic and intractable disease characterized by an elevation in pulmonary artery pressure that leads to right-sided heart failure and premature death [5]. Various pharmacological treatments, including prostacyclin analogues such as epoprostenol, treprostinil and iloprost, endothelin receptor antagonist such as bosentan, and phosphodiesterase 5 inhibitors such as sildenafil and tadalafil, have been approved for this life-threatening disorder, and these interventions can improve symptoms and quality of life for moderate and severe PAH [5–8]. However, most drugs have a very short half-life, often requiring continuous subcutaneous or intravenous infusion to elicit their therapeutic benefit. Meanwhile, systemic exposure of anti-PAH agents can induce off-target actions and result in minor or sometimes even severe side effects, leading to limited treatment compliance [9]. Consequently, non-invasive delivery of inhaled prostacyclins is considered as the most promising means to minimize the systemic side effects while achieving effective pulmonary vasodilation [9,10]. Yet, the existing inhaled medications require repeated dosing (e.g., 6–9 times for inhaled iloprost) due to rapid pulmonary clearance and commonly lead to cough and throat irritation [11–13]. These limitations of current inhaled therapy necessitate the development of novel inhalable formulations that can achieve pulmonary sustained release, or

Abbreviations: BBB, blood–brain barrier; CA, curcumin acetate; DL, loading content; DPI, dry powder inhaler; DSC, differential scanning calorimetry; EE, encapsulation efficiency; IT, intratracheal; IV, intravenous; LLOD, lower limit of detection; LLOQ, lower limit of quantification; MCT, monocrotaline; MOC, overlap coefficient according to Manders; PAH, pulmonary arterial hypertension; PCC, Pearson's correlation coefficient; PDI, polydispersity index; RSD, relative standard deviations; SD, standard deviation; SDS, sodium dodecyl sulfate; S/N, signal-to-noise ratio; Te, targeting efficiency.

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ideally directly target the pulmonary arterial endothelial cells, and to avoid local irritation.

In this context, microparticles [14–17], liposomes [18–20], nanoparticles [21,22], PEG-lipid micelles [23] and nanocrystals [24] have been investigated, and some different nanocarriers have been used to achieve pulmonary sustained release [24–28].

Due to its poor physicochemical and biopharmaceutical properties including very labile stability, poor oral bioavailability and rapid systemic elimination, the clinical use of curcumin is limited. Systemic delivery of curcumin loaded nanoparticles tends to meet difficulty in achieving therapeutic level of the drug in the lung for anti-PAH since the thickened pulmonary vascular wall under PAH pathological condition restrains nanoparticles from extravasating through the vessels to the lung [29]. Considering the fact that polymeric micelles have the property to extend pulmonary drug release [30] and esterified prodrugs can prolong pulmonary retention [31], mPEG–PLGA micelles might be utilized as carriers for the encapsulation of CA, an acetate prodrug of curcumin.

The main objective of this study was to determine the *in vivo* release and translocation profiles of micelles after delivery to the lungs, which were largely uninvestigated in previous studies on inhaled micellar/liposomal systems. The further objective was to test the hypothesis that CA-loaded mPEG–PLGA micelles via the pulmonary route were effective carriers for providing sustained levels of curcumin in the lung and thus increase the local accumulation of the drug in the pulmonary arteries.

2. Materials and methods

2.1. Materials

CA and curcumin with a purity >98% (determined by HPLC and differential scanning calorimetry, DSC) were donated by Ding-Guo Biotechnology Co., Ltd., (Beijing, China). Leucine was purchased from Alfa Aesar (Ward Hill, MA, USA). Nile red and coumarin-6 were purchased from Sigma–Aldrich (St. Louis, MO, USA). Aloe-emodin was obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). $MPEG_{2000}$-$PLGA_{5000}$ (LA:GA = 75:25) was purchased from Shandong Dai Gang Biotechnology Co., Ltd (Shandong, China). Acetonitrile (ACN) and tetrahydrofuran (THF) of HPLC grade were obtained from Merck (Darmstadt, Germany) and formic acid of HPLC grade from Dima (Lake Forest, CA, USA). Water was purified by a Milli-Q water purification system (Millipore, Bedford, MA, USA). All other chemicals and reagents were of analytical grade.

2.2. Preparation and characterization of CA-loaded mPEG–PLGA micelles

2.2.1. Preparation

Micelles loaded either with CA or a mixture of CA and Nile red or coumarin-6 (5:1) were prepared according to the previously published solvent evaporation method [32]. Briefly, CA, Nile red/coumarin-6 and $mPEG_{2000}$-$PLGA_{5000}$ (1:40, w/w) were co-dissolved in dichloromethane. The organic solvent was evaporated under vacuum to form a film, followed by the addition of pre-warmed water in the presence of leucine (1% of the polymer) at 50 °C. Finally, the non-incorporated drug was removed by filtering through a 220 nm nylon membrane and the filtrates were subjected to characterization and freeze-drying.

2.2.2. Dynamic light scattering

Before measurements, the micellar dispersions without freeze-drying or the reconstituted dispersions after freeze-drying were subjected to 200-fold dilution. The hydrodynamic diameter of the micelles was measured by dynamic light scattering using a Zetasizer Nano ZS (Malvern Instruments Ltd., Malvern, UK) equipped with a 10 mW HeNe laser at a wavelength of 633 nm an a temperature of 25 °C. Scattered light was detected at 173° angle with laser attenuation and measurement position adjusted automatically by the instrument's software. The particle size was calculated automatically based on the scattered light and the Brownian motion of the particles using the Stokes–Einstein equation

$$Rh = kBT/6\pi\eta D$$

With the radius of the particles being *Rh*, the Boltzmann constant is *kB*, the absolute temperature *T*, the solvent viscosity η, and the diffusion coefficient *D*. Values given are the means ± SD of three different experiments with each experiment comprising three measurements of the same sample with at least 10 runs, as determined by the Zetasizer.

2.2.3. Laser Doppler velocimetry

The zeta-potential was measured with a Zetasizer Nano ZS at 25 °C and a scattering angle of 17° by measuring the electrophoretic mobility with laser Doppler velocimetry. Values given are the means ± SD of three different experiments with each experiment comprising three measurements of the same sample with at least 10 runs, as determined by the Zetasizer.

2.2.4. In vitro release

The *in vitro* release was performed in a dialysis bag (Spectra/Por® molecular weight cut off (MWCO) 8,000–14,000 Da, Spectrum Laboratories, Rancho Dominguez, CA, USA) against water containing 2% sodium dodecyl sulfate (SDS) under continuous 800 rpm magnetic stirring at 37 °C. The presence of SDS could improve the stability of released CA. The amount of CA in the receiving phase was determined by an HPLC assay as described in Section 2.5.1 and the fluorescence activity of Nile red and coumarin-6 was analyzed using a fluorescence microplate reader (Fluoroskan Ascent FL, Thermo Fisher Scientific, Waltham, MA, USA) at excitation and emission wavelengths of 530 nm and 590 nm (Nile red) and 430 nm and 538 nm (coumarin-6), respectively. All experiments were carried out in triplicate.

2.2.5. Encapsulation efficiency

The CA or Nile red/coumarin-6 loading content (DL) and encapsulation efficiency (EE) were determined as reported previously [32].

2.3. Stability of CA in micelles

CA solution in ACN and micellar dispersions were evaluated for stability in water or PBS buffer (pH 7.4) at a final concentration of 100 μg/ml and incubated at 40 °C with continuous magnetic stirring protected from light. The temperature of 40 °C was selected due to the fact that at this temperature, the stability could be differentiated between free drug and encapsulated drug in water or PBS buffer. At predetermined time intervals, samples of 200 μl were withdrawn for HPLC assay (see below).

The stability of CA solution and micellar dispersions in rat plasma at the concentration of 100 μg/ml was performed by incubation in an ice-water bath. At predetermined time intervals, samples of 100 μl were withdrawn and 200 μl ACN with aloe-emodin (internal standard) was added to quench esterase activity. The analytes were vortexed and centrifuged, and the supernatant was injected to HPLC (see below).