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Polymeric drug delivery micelle-like nanocarriers for pulmonary administration of beclomethasone dipropionate

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ABSTRACT

In this paper, the potential of novel polymeric micelles as drug delivery systems for Beclomethasone Dipropionate (BDP) administration into the lung is investigated. These nanostructures are obtained starting from α,β-poly(*N*-2-hydroxyethyl)-D,L-aspartamide (PHEA), which was subsequently functionalized with *O*-(2-aminoethyl)-*O*'-methylpolyethylenglycole (PEG_{2000}), ethylenediamine (EDA) and lipoic acid (LA), obtaining PHEA-PEG_{2000}-EDA-LA graft copolymer. Empty and drug-loaded micelles possess adequate chemical-physical characteristics for pulmonary administration such as spherical shape, slightly positive surface charge and mean size of about 200 nm. Besides, BDP-loaded micelles, obtained with a Drug Loading equal to 5 wt%, result to be stable in physiological-mimicking media, protecting the drug from hydrolysis and giving a sustained drug release profile. Moreover, the micelle-like structure and surface characteristics seems to improve drug permeation through the mucus layer. Finally, it is also demonstrated that BDP-loaded PHEA-PEG_{2000}-EDA-LA micelles are able to increase cell uptake of BDP of about 44 wt% compared to Clenil® on 16-HBE cells and possess an higher biocompatibility in comparison with the same commercial formulation.

1. Introduction

Inhalation therapy based on nano-technological approaches represents a promising chance to treat several respiratory diseases, such as bronchial asthma, chronic obstructive pulmonary disease (COPD) or cystic fibrosis (CF), in which a rapid onset of pharmaceutical effects of the administered drugs should be reached by using a lower dose of topically administered formulations [1–4]. In fact, the conventional drug administration by inhalation presents several limitations such as the branched structure of the respiratory tree (that hinders the penetration of the drug in depth), the mucuciliary clearance, the uptake by alveolar macrophages, the poor solubility of the drug in the biological fluids and the presence of a dense and viscous mucus that hinders the absorption of the drug, with consequent need of high doses; all together these drawbacks make very challenging the local drug administration to treat pulmonary inflammation associated to lung diseases [5–8]. Glucocorticoids (GCs) are drugs widely used by inhalation to limit their systemic exposure but, however, their effectiveness after local administration could be useless in consequence of a limited absorption and of a reduced achievement of their site of action [6,9,10]. Among GCs, beclomethasone dipropionate (BDP) is extensively used in asthma and COPD treatment as suspension for inhalation therapy (i.e. Clenil® and Prontinal®) and as spray for nasal therapy (i.e. Becotide®, Rinoclenil®) [9–11]. It is a pro-drug with weak glucocorticoid receptor binding affinity that undergoes to hydrolysis to its more active metabolite beclomethasone 17-monopropionate (BMP) and to other minor inactive metabolites [12,13]. It was demonstrated that its local bioavailability is quite low due to its low water solubility that limits the diffusion through the mucus layer to reach the target site [3]. Indeed, previous studies have suggested that BDP is partially removed from the lower airways, i.e. the primary site of action, by mucociliary clearance prior to its dissolution and absorption [14]. Besides, depending on the pulmonary formulation characteristics, BDP could be removed from the lungs by reaching the gastrointestinal tract where undergo hepatic metabolism and subsequently eliminated in feces, urine and bile [14]. Therefore, an increase of its water solubility and residence time in the lungs could be a valid strategy to maximize effectiveness of BDP and/or decreasing the risks of side effects due to its extra lung absorption. To achieve this goal, BDP should be administered as aerosolized formulations based on drug delivery systems such

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as polymeric micelles, which could solubilize adequate amounts of hydrophobic drugs, promote the passage through the layer of hydrophilic mucus thanks to their hydrophilic shell, protect it from hydrolysis by esterases and also offer a controlled and prolonged duration of effect of the encapsulated drugs as well as a spatial distribution – regional and cell-specific drug targeting – within the lung [3]. In literature, several polymeric micelle-like nanocarriers as drug delivery systems of BDP into the lungs are described, which demonstrated that the encapsulation of drugs can improve protection and efficacy when compared with administration of free drugs, thanks to the ability to keep drug at or near the desired pharmacological site of action and to provide selective and prolonged activity in the lung, thereby reducing systemic toxicity [3,15–22].

In previous papers, we have described the potential of novel drug delivery systems for pulmonary administration of BDP obtained through polymeric derivatives based on α,β-poly-(*N*-2-hydroxyethyl)-D,L-aspartamide (PHEA) [15,23]. PHEA is a synthetic protein-like water soluble polymer, that has been largely used as starting material to realize drug and gene delivery systems [24–33]. Here, we described the preparation, chemical-physical and biological characterization of novel polymeric BDP-loaded micelles by using an amphiphilic derivative of PHEA for a potential local treatment of lung diseases. The latter derivative was obtained by subsequent functionalization reactions with *O*-(2-aminoethyl)-*O*'-methylpolyethylenglycole (PEG_{2000}), ethylenediamine (EDA) and lipoic acid (LA), obtaining PHEA-PEG_{2000}-EDA-LA graft copolymer, whose excellent properties as functional coating for gold nanostars were recently described in literature [34].

2. Experimental section

2.1. Materials

A complete description of materials used are reported in Supplementary data.

α,β-poly(*N*-2-hydroxyethyl)-DL-aspartamide (PHEA) was prepared and purified according to a procedure elsewhere reported [36]. The weighted average molecular weight ($\bar{M}_w$) of PHEA used in this study was 41.2 kDa ($\bar{M}_w/\bar{M}_n = 1.76$), as determined by size exclusion chromatography (SEC) analysis [28,31].

PHEA-PEG_{2000}–EDA-LA graft copolymer was synthesized and purified according to a procedure described elsewhere [34,35]. The degree of derivatization of PHEA-PEG_{2000}–EDA-LA graft copolymer was calculated by ^{1}H NMR and resulted to be 2.5 ± 0.5 mol% of repeating units for PEG molecules (DD_{PEG}), 40 ± 2 mol% of repeating units for EDA chains (DD_{EDA}) and 6.3 ± 0.5 mol% for LA molecules (DD_{LA}). DD_{LA} was further confirmed by Thannhauser's assay, a colorimetric assay able to determine the amount of disulfide bridges present in the copolymer [36].

$\bar{M}_w$, determined by SEC analysis, resulted to be 33.2 kDa ($\bar{M}_w/\bar{M}_n = 1.86$).

The synthesis and the characterization of PHEA-PEG_{2000}, PHEA-PEG_{2000}-EDA and PHEA-PEG_{2000}-EDA-LA and their characterization can be found in Supplementary data.

2.2. Critical aggregation concentration determination by fluorescence spectroscopy

The Critical Aggregation Concentration (CAC) for PHEA-PEG_{2000}-EDA-LA graft copolymer have been determined by pyrene assay [15]. Briefly, a stock solution of pyrene (6.0×10^{-2} M) was prepared in acetone and stored at 5 °C. To obtain the fluorescence spectrum, the pyrene solution was diluted with bi-distilled water to a pyrene concentration of 12×10^{-7} M. The solution was then distilled under vacuum at 60 °C for 1 h to remove acetone. The acetone-free pyrene solution was then mixed at 1:1 v/v with PHEA-PEG_{2000}-EDA-LA aqueous dispersions, at concentrations in the range from 1×10^{-5} to 7.5 mg/mL. The final concentration of pyrene in each sample was 6.0×10^{-7} M. The solutions were placed in quartz cuvettes and outgases by bubbling with oxygen-free nitrogen for 5 min before recording the spectra. The fluorescence spectra were recorded with a RF-5301PC spectrofluorometer (Shimadzu, Italy) by monitoring the change of the pyrene excitation intensities ratio at 333 nm and at 336 nm ($\lambda_{emission} = 390$ nm) and also the change of the pyrene emission intensities ratio at 373 nm and at 384 nm ($\lambda_{excitation} = 333$ nm).

2.3. Surface tension measurements

The surface tension measurements have been carried out with a KSV Sigma 70 tensiometer by using the Whilelmy plate method. The tensiometer was equipped with an automatic solution dispenser and interfaced to a computer. The critical micelle concentration of PHEA-PEG_{2000}-EDA-LA was estimated as intersection of the two linear plots, above and below the c.m.c., of surface tension as a function of log [P].

2.4. Micelle characterization: mean size and ζ potential

The mean diameter, width of distribution (polydispersity index, PDI) and ζ potential measurements of the obtained micelles were determined by photon correlation spectroscopy (PCS) by using a Zetasizer Nano ZS (Malvern Instruments).

The measurements were carried out at a fixed angle of 173° at a temperature of 25 °C by using twice-distilled water, NaCl 0.9% w/v and phosphate buffer saline (PBS) aqueous solutions at pH 7.4 as suspending media. Each dispersion, prepared in filtered media at a micelle concentrations equal to 3, 1 and 0.5 mg mL^{-1}, was kept in a cuvette and analyzed in triplicate.

2.5. Preparation of BDP-loaded PHEA-PEG_{2000}-EDA-LA micelles

BDP containing micelles have been prepared by closely mixing in mortar through a pestle, of PHEA-PEG_{2000}-EDA-LA graft copolymer (50 mg) and drug (50 mg) [15]. Aliquots of 1 mL of twice-distilled water were added to the mixture under mixing until 10 mL of volume. The obtained suspension was sonicated for 10 min and then centrifuged at 6000 rpm for 5 min at 25 °C. The supernatant was filtered on 0.45 μm cellulose membrane and freeze dried for 5 days.

2.6. Determination of drug loading (DL%)

High-performance liquid chromatography (HPLC) analyses have been carried out using Waters Breexe System Liquid Chromatograph equipped with a Waters 717 Plus Autosampler (40 μL injection volume) and a Shimadzu UV–vis HPLC detector on line with a computerized workstation, monitored at 238 nm. As a column was used a C_{18} column (Chromolitic, 5 μm, 250mm × 46 mm i.d., obtained from Waters), a mobile phase constituted by a mixture methanol:water 70:30 (v/v) with a flow rate of 1 mL/min. 20 mg of drug loaded micelles were dissolved in 10 mL of MeOH at room temperature for 30 min, filtered on 0.2 μm nylon filter and analyzed by HPLC.

The obtained peak area ($t_r = 7.2$ min) corresponding to BDP amount blended in PHEA-PEG_{2000}-EDA-LA micelles was compared with a calibration curve obtained by plotting areas versus standard solution concentrations of BDP in methanol in the range of 0.1–0.0125 mg mL^{-1} ($y = 69786x$, $R^2 = 0.998$). The amount of BDP loaded into micelles was expressed as Drug Loading capacity (DL%),