BASIC SCIENCE

Nanomedicine: Nanotechnology, Biology, and Medicine
11 (2015) 1621–1631

nanomedicine
Nanotechnology, Biology, and Medicine

ELSEVIER

Original Article

nanomedjournal.com

Biological assessment of self-assembled polymeric micelles for pulmonary administration of insulin

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Received 28 December 2014; accepted 14 May 2015

Abstract

Pulmonary delivery of drugs for both local and systemic action has gained new attention over the last decades. In this work, different amphiphilic polymers (Soluplus®, Pluronic® F68, Pluronic® F108 and Pluronic® F127) were used to produce lyophilized formulations for inhalation of insulin. Development of stimuli-responsive, namely glucose-sensitive, formulations was also attempted with the addition of phenylboronic acid (PBA). Despite influencing the *in vitro* release of insulin from micelles, PBA did not confer glucose-sensitive properties to formulations. Lyophilized powders with aerodynamic diameter (<6 μm) compatible with good deposition in the lungs did not present significant *in vitro* toxicity for respiratory cell lines. Additionally, some formulations, in particular Pluronic® F127-based formulations, enhanced the permeation of insulin through pulmonary epithelial models and underwent minimal internalization by macrophages *in vitro*. Overall, formulations based on polymeric micelles presenting promising characteristics were developed for the delivery of insulin by inhalation.

From the Clinical Editor: The ability to deliver other systemic drugs via inhalation has received renewed interests in the clinical setting. This is especially true for drugs which usually require injections for delivery, like insulin. In this article, the authors investigated their previously developed amphiphilic polymers for inhalation of insulin in an in vitro model. The results should provide basis for future in vivo studies.

Key words: Polymeric micelles; Inhalation; Cytotoxicity; Permeability; Phagocytosis; Protein delivery

The administration of compounds via inhalation has been a common practice since ancient cultures in order to treat diseases. Over the years, inhalation was preferably used for local treatment of diseases affecting the lungs and airways, especially for the administration of bronchodilators to treat asthma[1]; but the paradigm has been changing and inhalation as a route for

There are no conflicts of interest to be reported.

Funding source: Fernanda Andrade gratefully acknowledges Fundação para a Ciência e a Tecnologia (FCT), Portugal for financial support (grant SFRH/BD/73062/2010). This work was also financed by the European Regional Development Fund (ERDF) through the Programa Operacional Factores de Competitividade–COMPETE, by Portuguese funds through FCT in the framework of the project PEst-C/SAU/LA0002/2013, and co-financed by North Portugal Regional Operational Programme (ON.2–O Novo Norte) in the framework of project SAESCTN-PIIC&DT/2011, under the National Strategic Reference Framework (NSRF). BASF is also acknowledged for the supply of Soluplus®, Pluronic® F68, F108 and F127. Doctor Juan Sayos and Doctor Aroa Ejarque from CIBBIM-Nanomedicine, Hospital Universitari Vall d'Hebrón and Vall d'Hebrón Institut de Recerca, Universitat Autónoma de Barcelona, are acknowledge for providing THP-1 and U937 cell lines and assistance in the uptake studies.

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http://dx.doi.org/10.1016/j.nano.2015.05.006
1549-9634/

systemic delivery of a variety of drugs, gene therapy or vaccination gained a new breath.[2] This is especially due to the anatomical and physiological characteristics of the respiratory system that, among others, allow the noninvasive administration of drugs through an epithelium with high absorption area and not subject to hepatic first-pass effect, resulting in a higher bioavailability of compounds compared with other routes of administration.[3]

Despite the advantages presented by pulmonary delivery, the highly complex physiology and defense mechanisms of lungs and airways reduce the efficiency of many available conventional formulations.[4,5] Thus, several research groups have developed new and improved formulations based on advances observed in molecular biology and particle engineering technology. Nanocarriers, including polymeric micelles, have been proposed as advanced inhaled drug delivery systems with optimized pharmacokinetics and pharmacodynamics. Among others, nanocarriers could increase the absorption of compounds through the epithelium, reduce the clearance by mucociliary escalator through the penetration of particles in the mucus, and reduce the recognition of particles by alveolar macrophages.[6,7]

We propose the use of amphiphilic polymers for the development of powder formulations intended for the delivery of proteins by inhalation, using insulin as a model protein. In a previous work,[8] we successfully developed and characterized insulin-loaded micelles of Soluplus® (SOL), Pluronic® F68 (F68), Pluronic® F108 (F108) and Pluronic® F127 (F127). Lyophilized formulations were easily dispersed in liquid forming micelles of low size (<300 nm), able to retain high percentages of the native-like structure of insulin, and shown to be physically stable upon storage up to 6 months both at 4 and 20 °C, with negligible loss of the secondary structure of the protein.[8] Phenylboronic acid (PBA) was incorporated in the formulations to provide them with glucose-sensitive properties, since boronic acid derivatives have been proposed as excipients to control the release of insulin from formulations, especially hydrogels, depending on glucose concentration.[9] At body pH, it is expected a change in the release of insulin in response to changes in the hydrophilicity of formulations when the neutral moieties of PBA convert to anionic boronate esters upon reaction with the diol group of glucose.[9]

In this work, powders composed by polymeric micelles were produced and their suitability as delivery systems for inhalation of insulin was assessed *in vitro* using pulmonary epithelial and macrophage cell lines. The development of stimuli-sensitive formulations was also attempted with the addition of PBA to the systems.

Materials and methods

Materials

SOL (mw 90,000-140,000 g/mol), F68 (mw 7680-9510 g/mol), F108 (mw 12,700-17,400 g/mol) and F127 (mw 9840-14,600 g/mol) were kindly provided by BASF (Ludwigshafen, Germany). Lyophilized human insulin (potency ≥ 27.5 U/mg), PBA, phosphate buffer saline pH 7.4 (PBS), 5-([4,6-dichlorotriazin-2-yl] amino)fluorescein hydrochloride (5-DTAF) and D-glucose were purchase from Sigma-Aldrich (St. Louis, MO, USA). The other reagents used were methanol and ethanol absolute from analytical grade, and type 1 ultrapure water (18.2 MΩ.cm at 25 °C, Milli-Q®, Billerica, MA, USA).

Production of micelles

Micelles were prepared using the thin-film hydration technique as described previously.[8] The production of micelles is detailed in Supplementary material, section S1.

For uptake studies, empty fluorescent-labeled micelles were prepared with 5-DTAF-conjugated polymers in the same way as for non-fluorescent micelles. Full description and schematic representation of the conjugation are detailed in Supplementary material, section S1.

Determination of size, zeta potential, association efficiency and osmolality of liquid and lyophilized formulations

Micelles mean hydrodynamic diameter and polydispersity (PdI) were measured without dilution of the samples by dynamic light scattering (DLS) at both 25 and 37 °C using a detection angle of 173°, and zetapotential was assessed by laser Doppler micro-electrophoresis using a NanoZS (Malvern Instruments, UK). Each type of formulation was produced and analyzed at least in triplicate.

The osmolality of formulations was determined at room temperature using a Micro-Osmometer M3320 (Advanced Instruments, Inc., MA, USA). Triplicates of each formulation were analyzed. Association efficiency (AE%), i.e. the amount of insulin associated with the micelles, and the loading capacity (LC%), i.e. the mass percentage of insulin of the total mass of the particles were calculated according to Eqs. (1) and (2), respectively. The free insulin in filtrates was recovered after filtration of the formulations by centrifugation for 10 minutes at 10,000 rpm and 37 °C, using 30 k pore filters (Nanosep® Centrifugal Devices, Pall Corporation, Spain). Insulin was quantified by HPLC-UV. Full description of the HPLC-UV method is detailed in the Supplementary material, section S1.

$$\mathrm{AE\%} = \frac{\text{total amount of insulin} - \text{free insulin in filtrate}}{\text{total amount of insulin}} \times 100 \quad (1)$$

$$\mathrm{LC\%} = \frac{\text{total amount of insulin} - \text{free insulin in filtrate}}{\text{total weight of micelles}} \times 100 \quad (2)$$

Lyophilization

After production, micelles were lyophilized in an AdVantage 2.0 BenchTop Freeze Dryer (SP Scientific, Warminster, PA, USA) in order to obtain solid formulations with increased stability. The cycle used was as follows: samples were frozen at −30 °C and the temperature maintained for 60 minutes, the primary drying was set at 20 °C for 480 minutes at 150 mTorr and the secondary drying for 480 minutes at 30 °C and 100 mTorr.[8]

Powders particle size distribution and aerodynamic diameter

The geometrical particle size distribution of 20 mg samples of lyophilized formulations was determined by laser diffraction