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Antimicrobial activity of polymyxin-loaded solid lipid nanoparticles (PLX-SLN): Characterization of physicochemical properties and in vitro efficacy

Patrícia Severino[a,*], Elisânia F. Silveira[a], Kahynna Loureiro[a], Marco V. Chaud[b], Danilo Antonini[c], Marcelo Lancellotti[c], Victor Hugo Sarmento[d], Classius F. da Silva[e], Maria Helena A. Santana[f], Eliana B. Souto[g,h,**]

[a] Laboratory of Nanotechnology and Nanomedicine (LNMed), University of Tiradentes (Unit), and Institute of Technology and Research (ITP), Av. Murilo Dantas, 300, 49010-390 Aracaju, Brazil
[b] Laboratory of Biomaterials and Nanotechnology for the Development and Evaluation of Bioactive Substances, University of Sorocaba, Rodovia, Raposo Tavares km 92.5, 18023-000 Sorocaba, São Paulo, Brazil
[c] Department of Chemistry, Federal University of Sergipe, 49500-000 Itabaiana, SE, Brazil
[d] Institute of Environmental, Chemical and Pharmaceutical Sciences, Federal University of São Paulo, Diadema, Brazil
[e] Biochemical Department, Biology Institute, State University of Campinas-UNICAMP, Campinas, SP, Brazil
[f] Laboratory for the Development of Biotechnological Processes, School of Chemical Engineering, State University of Campinas-UNICAMP, Campinas, SP, Brazil
[g] Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Coimbra (FFUC), Pólo das Ciências da Saúde, Azinhaga de Santa Comba, 3000-548 Coimbra, Portugal
[h] REQUIMTE/LAQV, Group of Pharmaceutical Technology, Faculty of Pharmacy, University of Coimbra, Coimbra, Portugal

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ABSTRACT

Antimicrobial resistance is a current public health concern, limiting the available therapeutic options used for the treatment of common bacterial infections. The development of new drug entities via biotechnological processes is however expensive and time-consuming. Therefore, old antimicrobial agents have been recovered for clinical use. An example of these drugs is polymyxin, which is known for its serious adverse side effects, such as nephrotoxicity, neurotoxicity and promotion of skin pigmentation. To overcome these limitations, the use of biodegradable nanoparticles has been proposed to allow site-specific targeting, increasing the drug's bioavailability and decreasing its side effects. The aim of this work was the development of an optimized pharmaceutical formulation composed of solid lipid nanoparticles (SLN) loading polymyxin B sulphate (PLX) for the treatment of bacterial infections. The PLX-loaded SLN were produced by a double emulsion method (w/o/w), obtaining particles with a mean size of approximately 200 nm, polydispersity of 0.3 and zeta potential of − 30 mV. The encapsulation efficiency reached values above 90% for all developed formulations. SLN remained stable for a period of 6 months of storage at room temperature. The occlusive properties of the SLN was shown to be dependent on the type of lipid, while the antimicrobial properties of PLX-loaded SLN were effective against resistant strains of *Pseudomonas aeruginosa*. Results from the differential scanning calorimetry (DSC), wide angle X-ray diffraction (WAXD) and small angle X-ray scattering (SAXS) analyses confirmed the crystallinity of the inner SLN matrices, suggesting the capacity of these particles to modify the release profile of the loaded drug.

1. Introduction

The increasingly growing number of bacterial infections worldwide is a serious public health problem, given the recognized trend for bacterial drug resistance. Indeed, common infections which have been treatable in the past, are no longer manageable by the classical treatments (Dwivedi et al., 2016; Gupta et al., 2016). Antimicrobial resistance is related to multiple intrinsic and extrinsic mechanisms resistance in bacteria (Dwivedi et al., 2016). The development of new antibiotics is still costly and a long-term process. Among the most common infections, *Pseudomonas* and *Acinetobacter* are major etiologic agents of nosocomial infections (Kim et al., 2016; Labarca et al., 2016;

* Correspondence to: P. Severino, Program in Industrial Biotechnology, Laboratory of Nanotechnology and Nanomedicine (LNMED), Institute of Technology and Research (ITP), Tiradentes University (UNIT) - Aracaju, Av. Murilo Dantas, 300, Farolândia, Aracaju, Sergipe, Brazil.
** Correspondence to: E. B. Souto, Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Coimbra (FFUC), Pólo das Ciências da Saúde, Azinhaga de Santa Comba, 3000-548 Coimbra, Portugal.
E-mail addresses: patricia_severino@itp.org.br (P. Severino), ebsouto@ff.uc.pt (E.B. Souto).

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MacVane, 2016; Xia et al., 2016). In the case of gram negative bacteria, it has been necessary to recover old antimicrobial agents for clinical use, including polymyxins (Harm et al., 2016; Heming et al., 2016). Polymyxins are effective antibiotics for gram negative and their clinical use is increasing, despite their serious adverse side effects, such as nephrotoxicity and neurotoxicity (Gallardo-Godoy et al., 2016; Myint et al., 2016; Rigatto et al., 2016), as well as the risk of promoting skin pigmentation (Gothwal et al., 2016; Mattos et al., 2016). They are widely used in urinary tract infections, lung, eye and ear infections, in meningitis, mucositis, otitis, wound infections, periodontitis and ocular infections (Arnold et al., 2007; Henao and Ghaffari, 2016; Myint et al., 2016; Rigatto et al., 2016). Oral mucositis is a comorbidity that affects patients undergoing head and neck cancer treatment, for which conventional treatments available (i.e. local anesthesia, cryotherapy, and/or involve the local use of anti-inflammatories and antibiotics) are only palliative. The therapeutic efficiency is reduced by the low residence time at the action site (Richards, 2006). It is therefore desirable to increase the therapeutic efficiency of polymyxins by developing controlled release formulations for longer residence time of the drug at the site of action.

The use of nanoparticles to load antimicrobial drugs is a suitable approach to decrease toxicity and improve the drug's bioavailability. Examples include the loading of polymyxin B in silver nanoparticles (Lambadi et al., 2015), in liposomes (Alipour and Suntres, 2014) and in nanomicelles (Brandenburg et al., 2012).

Biodegradable SLN have been developed by our group as non-viral delivery systems (Severino et al., 2015c, 2015d), to load hydrophilic biomolecules (Severino et al., 2014a, 2014b). We have successfully developed SLN loaded with PLX complexed with sodium alginate to achieve a controlled release profile of the drug (Severino et al., 2015a). The present work focuses on the evaluation of the effect of the SLN lipid composition on the PLX solubility, immediate and long-term physicochemical stability of the drug-loading particles, their matrix crystallinity, PLX encapsulation efficiency, occlusion effect and antimicrobial properties in oral mucosa.

2. Material and methods

2.1. Material

Crodapearl® past, behenic acid, crodacol® C90, crodacol® CS90, crodamol® SS, crodacol® S95, arlacel® 170, crodamol® MM, crodamol® CP were kindly provided by Croda (Campinas/Brazil). Pluronic® F68 was purchased by Synth (Campinas/Brazil). Polymyxin B sulphate (PLX) was donated by Cristália (Itapira/Brazil) and Lipoid® S75 was received by Lipoid (São Paulo/Brazil). Double distilled water was used after filtration in a Millipore system (home supplied).

2.2. Methods

2.2.1. Determination the presence of crystals

The determination the presence of crystals solubility of PLX has been studied in different lipids (crodapearl® past, behenic acid, crodacol® C90, crodacol® CS90, crodamol® SS, crodacol® S95, arlacel® 170, crodamol® MM, crodamol® CP) and evaluated by mixing the drug with increasing concentrations (0.010, 0.5 and 1 w/w) added to the melted lipid at 90 °C. The presence of crystals was determined visually by checking the presence or absence of crystals of the drug every 15 min, for a period of 1 h at 90 °C.

2.2.2. Solid lipid nanoparticles production

SLN were prepared using the w/o/w emulsion technique. Briefly, 2.5% (w/v) Crodacol® CS90 (F1 and F2) or Crodacol® C90 (F3 and F4) and 1.25% (w/v) Lipoid® S75 was solubilized in ethanol (oil phase) and then added with PLX (1 mg/mL) as the internal aqueous phase. The primary w/o emulsion was prepared by a high shear homogenization for 5 min and 20.000 rpm (Ultra-Turrax® - IKA, T25 impeller 10G). The primary emulsion was re-emulsified with the external aqueous phase containing 1% Pluronic® F68 using a high shear homogenization for 5 min and 10.000 rpm, after the formulation was processed in a microfluidizer (Microfluidics®, Santa Clara, CA, USA) using 500 bar and 5 cycles. Finally, the sample spent 24 h under magnetic stirring to evaporate the alcohol. Formulations F1 and F3 represent PLX-free SLN, whereas F2 and F4 represent the PLX-loaded SLN. The hydrodynamic diameter and polydispersity index of the SLN were determined by quasi-elastic scattering of light by photon correlation spectroscopy (PCS) using high power laser. The surface charge density of the produced SLN was assessed by measuring the zeta potential (ζ). The measurements were performed at 25 °C, using the Malvern Autosizer NS.

Table 1
Assessment of presence of crystals of PLX in different lipids at different time-points.

Lipids	15 min	30 min	60 min	90 min
Crodapearl®	−	+	+	+
Ácido behénico	+	+	+	+
Crodacol® C90	+	+	+	+
Crodacol® CS90	+	+	+	+
Crodamol® SS	−	−	−	−
Crodacol® S95	−	−	−	−
Arlacel® 170	−	−	−	−
Arodamol® MM	−	−	−	−
Crodamol® CP	−	−	−	−

Note: (+) absence of PLX crystals; (−) presence of PLX crystals.

2.2.3. Stability after lyophilization

Approximately 5.0 g of each formulation was added with 1 mL to glycerin (cryoprotectant). The samples were frozen at − 80 °C, and after freezing they were lyophilized for 48 h using a vacuum pump followed by a steam condenser. After lyophilization, the formulations were reconstituted with Milli-Q water (5 mL) and sonicated for 60 min (Cole-Parmer, Illinois, USA) to allow a complete rehydration of the particles. The hydrodynamic diameter, polydispersity index and zeta potential were determined as described above.

2.2.4. Encapsulation efficiency

SLN were centrifuged in cooled centrifuge tubes using high-speed centrifugation ultrafilter, having a molecular weight cutoff of 100 KD, 5.000 rpm for 45 min a 4 °C (Kushwaha et al., 2013). The amount of PLX in the supernatant was determined at 210 nm using UV–Vis. The methodology was standardized by Severino et al. (Severino et al., 2015b). The encapsulation efficiency (EE, %) was calculated using the following equation:

$$\text{Encapsulation efficiency (EE,\%)} = \frac{(M_{initial} - M_{final})}{M_{initial}} \times 100 \quad (1)$$

where $M_{initial}$ stands for the mass of drug added to the formulation and M_{final} for the non-loaded drug (i.e. mass of drug determined in the supernatant).

2.2.5. Occlusion test

The occlusion test was performed in vitro, following the description by Souto et al. (Souto et al., 2004). Firstly, beakers of 100 mL were filled with 50 mL of water and covered with filter paper to seal them. Then, 200 mg of the sample were spread onto the filter paper (area). The samples were stored at 32 °C and 50–55% relative humidity for 48 h. The samples were weighed at time zero and at 6, 24 and 48 h, thus giving the amount of water evaporated at each interval. Beakers covered with a filter paper, but without the application of sample were used as control. The occlusion factor was calculated according to the following equation: