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Dextran–protamine coated nanostructured lipid carriers as mucus-penetrating nanoparticles for lipophilic drugs

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ABSTRACT

The main objectives of the present study were (i) to evaluate the effect of the mucus layer on saquinavir-loaded nanostructured lipid carriers (SQV-NLCs) uptake and (ii) to evaluate the mucopenetrating properties of dextran–protamine (Dex–Prot) coating on NLCs as per SQV permeability enhancement. Three different NLC formulations differing on particle size and surfactant content were obtained and coated with Dex–Prot complexes. SQV permeability was then evaluated across Caco-2 cell monolayers (enterocyte-like model) and Caco-2/HT29-MTX cell monolayers (mucus model). In the Caco-2 monolayers, Dex–Prot–NLCs increased up to 9-fold SQV permeability in comparison to uncoated nanoparticles. In the Caco-2/HT29-MTX monolayers, Dex–Prot–NLCs presenting a surface charge close to neutrality significantly increased SQV permeability. Hence, Dex–Prot complex coating is a promising strategy to ensure successful nanoparticle mucus-penetration, and thus, an efficient nanoparticle oral delivery. To our knowledge, this is the first time that Dex–Prot coating has been described as a nanoparticle muco-penetration enhancer across the intestinal mucus barrier.

1. Introduction

Nanoparticle-based drug delivery systems have been reported as a promising strategy to deliver poorly-water soluble drugs by the oral route (Couvreur, 2013; Elgart et al., 2012; Harde et al., 2011; Huynh et al., 2009; Merisko-Liversidge and Liversidge, 2011; Mora-Huertas et al., 2010; Zhang et al., 2008). Nanoparticles possess the ability to overcome multiple biological barriers due to their nanometer size and present a sustained and controlled delivery of drugs while preserving them from their degradation. However, for these nanoparticles to be effectively delivered to mucosal surfaces, it is important they avoid the rapid mucus clearance mechanism and, penetrate quickly through the mucus layer to reach the underlying epithelium (Lai et al., 2009). Mucus represents a barrier against nanoparticle penetration to epithelial surfaces by means of two major mechanisms: either particles are retained by interacting with the mucus components or the size of the mucus mesh might hinder nanoparticle penetration (Cone, 2009; Sigurdsson et al., 2013).

The development of mucus-penetrating nanoparticles for the oral delivery of poorly water-soluble drugs and peptides is of widespread interest and still remains a challenge (Behrens et al., 2002; des Rieux et al., 2013; Ensign et al., 2012; Lai et al., 2007; Plapied et al., 2011). The surface characteristics claimed to provide mucopenetrating properties are somehow controversial. Behrens et al. (2002) examined the effect of mucus on the uptake of nanoparticles with different physicochemical surface properties in mucus-secreting MTX-E12 cells by comparing bioadhesive *versus* non-bioadhesive nanoparticles. They concluded that the internalization of bioadhesive chitosan nanoparticles was larger compared to polystyrene nanoparticles and demonstrated that chitosan could penetrate the mucus. However, Wang et al. (2008) hypothesized that both hydrophilicity and neutral charge are desirable towards mucus-penetrating nanoparticles. More recently, Lai et al. (2007) have demonstrated that, unlike the mucus mesh-pore size limitation (range 10–200 nm), larger nanoparticles

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(200–500 nm), if properly coated, can rapidly penetrate the mucus barrier, questioning nanoparticle size limitation in order to penetrate the mucus barrier.

Nanostructured lipid carriers (NLCs) are a second generation of solid lipid nanoparticles (SLNs) presenting a solid matrix mixed to a liquid lipid (oil) forming an unstructured matrix, which provides them with a higher stability and drug loading capacity. In a previous study (Beloqui et al., 2013a), we showed that the permeability of saquinavir (SQV) (a low solubility drug, BSC class IV) across intestinal cells increased when formulated in NLCs. As mentioned above, the diffusion of the particles through the mucus can affect their transport (Ensign et al., 2012). However, the mucus penetrating properties of lipid-based nanoparticles, including NLCs, have not been extensively studied yet.

Dextran (Dex) is a polyanion biocompatible polysaccharide that hampers interactions with other components such as serum proteins and can reduce the complement activation induced by the particles (Hirsjärvi et al., 2013). Delgado et al. (2012a) described that, when incorporated on the surface of SLNs, dextran decreased hemagglutination in a test *in vitro* and, after intravenous administration to mice, the long circulation stay of the SLNs in the bloodstream was partially related to the presence of dextran. Protamine (Prot) is a peptide that enhances cell penetration (Martínez Gómez et al., 2008; Delgado et al., 2011). The combination of Dex–Prot might provide mucophilic properties to NLCs by helping these avoiding NLCs-mucin interactions and enhancing cell penetration, and thus, providing an efficient delivery of NLCs to the intestinal site.

The aim of this work was, first, to investigate the effect of the mucus layer on SQV-NLCs uptake by comparing SQV permeability across Caco-2 (enterocyte-like model) and Caco-2/HT29-MTX (mucus model) monolayers. In a second step, to obtain a successful delivery of NLCs at the intestinal site, we hypothesized that a Dex–Prot complex coating could provide NLCs with mucopenetrating properties, and thus, we evaluate its role as a SQV permeability enhancer across the mucus barrier.

2. Materials and methods

2.1. Materials

Saquinavir mesylate (SQV) was kindly provided by Roche (Mannheim, DE). Coumarin-6, protamine sulphate salt from salmon (Grade X), dextran (M_n = 3260), and Triton® X-100 were purchased from Sigma–Aldrich (Madrid, SP). Precirol ATO®5 was kindly provided by Gattefossé (Madrid, SP). Polysorbate 80 (Tween® 80) was purchased from Vencaser (Bilbao, SP). Poloxamer 188 (Lutrol® F68) was a gift from BASF (Madrid, SP). Miglyol 812 N/F was purchased from Sasol (Hamburg, DE). Potassium dihydrogen phosphate (KH_2PO_4) disodium hydrogen phosphate (Na_2HPO_4) and cetyl trimethyl ammonium bromide (CTAB) were obtained from Panreac (Barcelona, SP). Acetonitrile (gradient HPLC grade) was purchased from Scharlau (Barcelona, SP). Dulbecco's Modified Eagle Medium (DMEM) and Hank's Balanced Salt Solution (HBSS) were purchased from Gibco™ (Invitrogen, Barcelona, SP). Ultrapure water was used throughout and obtained from a Milli-Q® Plus apparatus (Millipore).

2.2. Preparation of the formulations

2.2.1. SQV-NLCs preparation

SQV-NLCs were prepared by the high-pressure homogenization technique as previously described (Beloqui et al., 2013a, 2014). Briefly, Precirol ATO®5 (10%) (w/v) (solid lipid) and Miglyol 812 (1%)(w/v) (liquid lipid) were melted at 75 °C until a uniform and clear oil phase was obtained, and the lipophilic drug SQV (50 mg) was homogenously dispersed in the above solution. The surfactant solution was composed of polysorbate 80 (1%) (w/v) and poloxamer 188 (1 or 0.5%) (w/v), which provide negative charge. The hot aqueous phase was then added to the oil phase, and the mixture was sonicated for 15 s to form a hot pre-emulsion, which was subsequently homogenized at 80 °C and 500 bar using a Stansted nG12500 homogenizer (SFP, Essex, UK) for ten homogenization cycles. To obtain NLCs with an increased particle size, one of the batches was not homogenized, and the pre-emulsion was used. Table 1 summarizes the different formulations assayed.

In order to track the entry of nanoparticles into the cells, SQV-NLCs were labeled with the fluorescent dye coumarin-6. Briefly, 5 mg of coumarin-6 was incorporated in the lipid phase of the formulation, and the preparation continued as aforementioned.

2.2.2. Dextran–protamine coatings

In order to prepare Dex–Prot–NLCs, Dex–Prot complexes were prepared as previously described by Delgado et al. (2012b). An aqueous solution of dextran (1 mg/mL in H_2O Milli-Q) was mixed with and aqueous solution of protamine (2 mg/mL in H_2O Milli-Q) to form the Dex–Prot complexes at w/w ratio of 1:20 and mantained under agitation for 15 min. Then, the NLCs were mixed with the Dex–Prot solution and mantained under agitation for 30 min to prepare Dex–Prot–NLCs at w/w/w ratio 1:20:5 by self-assemblying (Thu et al., 2012). The same ratio was maintained for all formulations regardless of their surface charge or size in order to compare the differences in permeability using the same Dex–Prot coating ratios.

2.3. NLC characterization

2.3.1. Size and zeta potential measurements

The size of the NLCs was determined using photon correlation spectroscopy (PCS), and the zeta potential was measured using Laser Doppler Velocimetry (LDV) with a Malvern Zetasizer Nano ZS (Malvern Instruments Ltd., Worcestershire, UK) before and after Dex–Prot coating. Samples were diluted in Milli-Q™ water before measurement (Table 1).

2.3.2. Drug encapsulation efficiency

The encapsulation efficiency (EE) of SQV-NLCs was calculated as previously described by Beloqui et al. (2013b) by determining the amount of free drug using a filtration technique. Briefly, the SQV-NLCs suspension was placed in the upper chamber of Amicon® centrifugal filters (molecular weight cutoff, MWCO, 100,000 Da, Millipore, Spain) and centrifuged for 20 min at 1500 × *g*. The unencapsulated SQV in the filtrate was determined using HPLC. The total drug content in the SQV-NLCs was determined by

Table 1
Summary of formulation composition and particle size, zeta potential and polydispersity index (PI) per formulation (n = 3; data are expressed as mean ± SD), before and after Dex–Prot coating.

Uncoated-NLCs	A	B	C
Polysorbate 80 (%)	2	1	1
Poloxamer 188 (%)	1	0.5	0.5
Homogenization	Yes	Yes	No
Size (nm)	152 ± 1	272 ± 5	936 ± 1
Zeta (mV)	−29 ± 7	−36 ± 6	−22 ± 4
PI	0.25 ± 0.03	0.34 ± 0.10	0.58 ± 0.21
Coated-NLCs	**A**	**B**	**C**
Size (nm)	244 ± 1	1113 ± 1	1326 ± 1
Zeta (mV)	−0.1 ± 5	±0.5 ± 4	+12 ± 4
PI	0.411 ± 0.16	0.5 ± 0.15	0.4 ± 0.38