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Can lipid nanoparticles improve intestinal absorption?

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ABSTRACT

Lipid nanoparticles and their multiple designs have been considered appealing nanocarrier systems. Bringing the benefits of these nanosystems together with conventional coating technology clearly results in product differentiation.

This work aimed at developing an innovative solid dosage form for oral administration based on tableting nanostructured lipid carriers (NLC), coated with conventional polymer agents. NLC dispersions co-encapsulating olanzapine and simvastatin (Combo-NLC) were produced by high pressure homogenization, and evaluated in terms of scalability, drying procedure, tableting and performance from *in vitro* release, cytotoxicity and intestinal permeability stand points.

Factorial design indicated that the scaling-up of the NLC production is clearly feasible. Spray-drying was the method selected to obtain dry particles, not only because it consists of a single step procedure, but also because it facilitates the coating process of NLC with different polymers. Modified NLC formulations with the polymers allowed obtaining distinct release mechanisms, comprising immediate, delayed and prolonged release. Sureteric:Combo-NLC provided a low cytotoxicity profile, along with a ca. 12-fold OL/3-fold SV higher intestinal permeability, compared to those obtained with commercial tablets. Such findings can be ascribed to drug protection and control over release promoted by NLC, supporting them as a versatile platform able to be modified according to the intended needs.

1. Introduction

Oral administration is regarded as the most commonly accepted route of drug administration, offering several advantages, including convenience, ease of compliance, and cost-effectiveness. However, this route is associated to numerous limitations, mostly due to the different environmental conditions found along the gastrointestinal tract (GIT). Such drawbacks are linked to the extensive drug metabolism in the liver first pass effect, to an unpredictable absorption as a result of the gastrointestinal conditions (e.g., the acidic medium in the stomach, the presence of enzymes that can degrade the drugs, the presence of food that can affect gastric emptying), and to irritation of the gastric mucosa, promoting nausea and vomiting, and delayed onset of action. Not surprisingly, a sufficiently high oral bioavailability is one of the most important considerations for the bioactive compounds to ensure the respective pharmacological effect. A poor oral bioavailability will affect drug performance, resulting in high intra- and inter-patient variability (Tong, 2008). One of the possibilities to enhance bioavailability and prevent the adverse conditions throughout GIT relies on the use of nanocarrier systems, such as lipid nanoparticles (LN) (Yuan et al., 2007). LN are a promising nanosystem for drug delivery, which has raised particular interest in comparison to conventional colloidal systems. Their biocompatible and biodegradable nature, physico-chemical stability, control over drug release, cost-effectiveness and ease of scale-up are some of their advantages (Manjunath and Venkateswarlu, 2005; Wissing et al., 2004). In addition, LN have a solid matrix at both room and body temperature, allowing controlled release and chemical protection of the drug. The benefits of LN for oral administration have already been reported (Muchow et al., 2008). Their use increases bioavailability, drug protection from hydrolysis, controlled release of the drug and, therefore, dose reduction (Wissing and Muller, 2002). Such

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improvements can be achieved, essentially, by two different mechanisms: the adhesiveness promoted by a close interaction of LN with the gut wall, allowing the delivery of the drug released exactly in the place where it should be absorbed, and an increased lymphatic absorption that avoids the liver first pass, as a result of their lipid nature (Bargoni et al., 1998; Muchow et al., 2008). This last mechanism is called by "Trojan Horse"(Muller et al., 2011).

Within LN, nanostructured lipid carriers (NLC) have been described as a second and smarter generation (Müller et al., 2002). Their difference from the first generation LN, the so-called solid lipid nanoparticles (SLN), relies on the introduction of a liquid lipid in the matrix (Muchow et al., 2008). This structural modification enables them to overcome stability issues related to polymorphic transitions, thus avoiding the expulsion of the drug during the storage period (Muller et al., 2011). Numerous methods of NLC production have been described, although the hot HPH technique is the most generally used (Ahmed et al., 2012; Akkar et al., 2004; Al-Haj and Rasedee, 2009; Jelvehgari and Montazam, 2012; Mehnert and Mader, 2012; Mei et al., 2003; Trotta et al., 2003). In contrast with other techniques, this method is not associated to scale-up problems and allows to obtain formulations with particle concentration up to 40% (w/w), and a homogeneous size distribution (Bergstrom et al., 2004; Muller et al., 2011). Besides, it does not require the use of organic solvents, which minimizes the toxicological concerns of the end product. After the preparation of NLC, they may be submitted to secondary processes, such as freeze-drying (Doktorovova et al., 2014; Schwarz and Mehnert, 1997) and spray-drying (Harsha et al., 2015; Heng et al., 2011; Jinno et al., 2006; Lee et al., 2011) for the conversion of the liquid dispersion into a dry product. These techniques contribute to an increase in physicochemical stability, although based on different principles. Freeze-drying removes water by sublimation under vacuum. Sometimes, the use of a cryoprotector is deemed necessary to prevent particle aggregation and improve the redispersion properties of the powder obtained. In turn, the spray-drying technique employs high temperatures, but the drying procedure occurs in a single step. Protectors may also be used in this process to avoid lipid matrix melting. Such diverse procedures open a broad scope of applications for oral delivery, either as aqueous dispersions or as solid dosage forms (e.g. tablets, pellets or capsules).

The aim of this work is the development and modulation of oral formulations based on NLC. In particular, the potential of these nanocarriers for the co-encapsulation of drugs that are complementary from a therapeutic point of view, olanzapine and simvastatin, is explored. OL is an antipsychotic that belongs to secondary generation class of derivate antipsychotic agents (Callaghan et al., 1999; Kapur et al., 1998). It is indicated for the treatment of schizophrenia and bipolar disorders (Narasimhan et al., 2007). In addition, due to the chronic nature of the treatment, the drug is used for a long period of time. This results in weight gain and dyslipidemia. Given these conditions, the association with a statin will mitigate the alterations in the lipid profile (Kolovou et al., 2008).

The feasibility of the scale-up process of a NLC formulation prepared by hot HPH was firstly inspected, using a full factorial planning. The optimal formulation in terms of particle size, polydispersity index and zeta potential was subsequently submitted to freeze and spray-drying procedures and thoroughly evaluated. Finally, tablets based on a combo-NLC formulation (OL-SV-NLC) with different coatings were prepared and characterized. The tablets performance was assessed through *in vitro* release, permeability, cytotoxicity and cellular uptake studies. Thus, this work intended to highlight NLC as a versatile nanoplatform whose quality product target profile is prone to be tailored according to the unmet medical needs.

2. Materials and methods

2.1. Materials

Simvastatin was kindly provided by Labesfal (Santiago de Besteiros, Portugal). Olanzapine was purchased from Zhejiang Myjoy (Hangzhou, China). Glyceryl tripalmitate (tripalmitin, T8127, melting point 66 °C), polysorbate 80 (Tween® 80), and polyvynilpyrrolidone K 30 (PVP K 30) were provided by Sigma. Oleic acid, trehalose, starch, lactose, microcrystalline cellulose were acquired from Fluka. Sureteric® and Eudragit® RL 30D were kindly donated from Colorcon® and Evonik, respectively. Water was purified (Millipore®) and filtered through a 0.22 μm nylon filter before use. The compounds used for the preparation of the Krebs Bicarbonate Ringer's (KBR) solution were acquired from Merck KGaA (Darmstadt, Germany), Riedel-de Haen AG (USA) and Fluka. All other reagents and solvents were from analytical or high performance liquid chromatography (HPLC) grade.

2.2. Methods

2.2.1. Preparation of aqueous NLC dispersions

The NLC were prepared by the hot high pressure homogenization (HPH) technique, as previously described (Vitorino et al., 2013a). This was carried out at 80 °C, a temperature above the melting point of the solid lipid. Firstly, the melted lipid phase (7.5% w/V), containing the oleic acid and tripalmitin (at a ratio of 50:50), was emulsified in an aqueous solution of Tween® 80 (3% w/V) at the same temperature, using an Ultra-Turrax for 1 or 3 min (Ystral GmbH D-7801, Dottingen, Germany). A pre-emulsion was obtained. Secondly, the pre-emulsion was processed in a pre-heated high pressure homogenizer (HPH) (Emulsiflex®-C3, Avestin, Inc., Ottawa, Canada), at a pressure of 500 or 1000 bar for 2.5 min or 12.5 min, according to the defined factorial planning variables (see Table 1). In the optimal drug loaded formulation, Combo-NLC, the addition of both drugs was carried out in the initial lipid melted phase. NLC containing 0.20% (w/w), in relation to lipid content, of fluorescence dyes, Nile Red (NR) and Fluorescein-5-Isothiocyanate (FITC), were prepared using the same conditions.

2.2.2. Experimental design

A two-level full factorial design, 2^k, with three-variables was used to accomplish the optimization of the scale-up process. This mathematical tool allows to obtain a high amount of information using a reduced number of experiments, when the study of several factors effect is deemed necessary. The mathematical model that follows was applied to describe the main effects and interaction among the variables under consideration

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3$$

where y is the response, β_0 is the arithmetic mean of the response, β_1-β_3 are the coefficients of the respective independent variables and β_{12}, β_{13} and β_{23} are the interaction terms.

For each variable, a high (+1) and a low (−1) level were established. The choice of these limits was based on previous work

Table 1
Experimental design independent variables and respective codification.

Production conditions	Independent variables	Level −1	Level +1
Pre-emulsion time (min)	x_1	1	3
Batch size (mL)	x_2	30	150
HPH pressure (bar)	x_3	500	1000