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Research paper

Progesterone lipid nanoparticles: Scaling up and in vivo human study

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ABSTRACT

This investigation describes a scaling up study aimed at producing progesterone containing nanoparticles in a pilot scale. Particularly hot homogenization techniques based on ultrasound homogenization or high pressure homogenization have been employed to produce lipid nanoparticles constituted of tristearin or tristearin in association with caprylic-capric triglyceride. It was found that the high pressure homogenization method enabled to obtain nanoparticles without agglomerates and smaller mean diameters with respect to ultrasound homogenization method. X-ray characterization suggested a lamellar structural organization of both type of nanoparticles. Progesterone encapsulation efficiency was almost 100% in the case of high pressure homogenization method. Shelf life study indicated a double fold stability of progesterone when encapsulated in nanoparticles produced by the high pressure homogenization method. Dialysis and Franz cell methods were performed to mimic subcutaneous and skin administration. Nanoparticles constituted of tristearin in mixture with caprylic/capric triglyceride display a slower release of progesterone with respect to nanoparticles constituted of pure tristearin. Franz cell evidenced a higher progesterone skin uptake in the case of pure tristearin nanoparticles. A human in vivo study, based on tape stripping, was conducted to investigate the performance of nanoparticles as progesterone skin delivery systems. Tape stripping results indicated a decrease of progesterone concentration in *stratum corneum* within six hours, suggesting an interaction between nanoparticle material and skin lipids.

1. Introduction

Recent research efforts have demonstrated that nanotechnology achieves dramatic results in different scientific area, including drug formulation and delivery. Particularly, the advantages of lipid based nanoparticles, over conventional formulations, include: (a) the control of drug release and/or drug targeting; (b) the reduction of toxicity problems thanks to the use of biocompatible excipients (i.e. lipids) and avoidance of organic solvents, (c) the reduction of number of administrations and drug dosage, (d) the ability to solubilize poorly water-soluble drugs and finally (e) the vast range of administration routes [1–4].

For instance, the variation of lipid composition offers the possibility to produce nanoparticles with different properties; the use of lipids that are solid at room temperature, such as glyceryl tristearate results in the formation of the so called "solid lipid nanoparticles" (SLN), while employing mixtures of solid and liquid lipids results in the obtainment of nanostructured lipid carriers (NLC). NLC can be considered as second generation of SLN, in which the presence of liquid regions (within the nanoparticle matrix) increases the ability to solubilize lipophilic drugs and prevents drug leakage during storage [5,6].

Hot homogenization technique is by far the most widely used nanoparticle production approach; the method is based on dispersion of a melted lipid phase in a hot aqueous surfactant solution under stirring by high-shear mixing device. The obtained emulsion is then submitted to hot homogenization and cooling, finally obtaining nanoparticles by re-crystallization of lipids [7,8].

The homogenization step is mandatory to reduce size of droplets constituting the inner oil phase of the initial emulsion. The homogenization can be achieved by ultrasound, or by high pressure. Ultrasound homogenization (UH) is based on the

Abbreviations: SLN, solid lipid nanoparticles; NLC, nanostructured lipid carriers; PRG, progesterone; x-gum, xanthan gum; UH, ultrasound homogenization; HPH, high pressure homogenization; cryo-TEM, cryogenic transmission electron microscopy; PCS, photon correlation spectroscopy; EE, encapsulation efficiency; LC, loading capacity.

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transmission of high power ultrasound waves in a liquid media by a probe. Ultrasound waves generate small vacuum bubbles which implodes, resulting into high shear forces [9]. On the one hand, an important advantage of this method is low cost of instrumentation, on the other a drawback is formation of lipid agglomerates during cooling.

High pressure homogenization (HPH) is an ongoing process widely used in pharmaceutical and food industry, based on reduction of droplet/particle size and uniformity under conditions of extreme pressure by a piston-gap homogenizer. To produce lipid nanoparticles, the initial coarse emulsion is forced through a special homogenization valve at extremely high pressures (up to 150 MPa), at temperatures above the lipid melting point, leading to a secondary thin emulsion. Several homogenization cycles can be performed at 50–150 MPa, enabling to obtain lipid nanoparticles free from agglomerates. Notwithstanding its high cost, high-pressure homogenizer lab-scale and pilot-scale models can operate with limited sample volumes and produce the same results as large models, ensuring scalability of results. Thus, HPH enables to scale-up nanoparticle volume from lab to pilot and industrial scale [10,11].

Progesterone (PRG) is a steroid hormone present in different medicines with various indications such as the control of habitual abortion, suppression and synchronization of oestrus, regulation of ovulation and menstruation (e.g. to treat amenhorrea), treatment of infertility by luteal phase support and pevention of endometrial hyperplasia [12,13]. For these applications, PRG is typically administered orally, rectally, intramuscularly, subcutaneously or intravaginally [12,13].

Subcutaneous or intravaginal administered formulations for PRG are also commercially available; such medicines were designed to avoid first-pass metabolism, observed in the case of oral administration, to achieve higher concentrations.

Further formulations for PRG include those applied on the skin to treat deficiency associated with menopause and perimenopause, mastodynia or diffuse fibrocystic mastopathy.

In addition, PRG is present in cutaneous formulations (i.e. gels and creams) to treat dermatological disorders such as acne and ageing [14,15].

Regarding the pharmaceutical application of lipid nanoparticle, no formulations are on the market up to now, even though research studies have demonstrated that both SLN and NLC are suitable for many administration ways, including subcutaneous, vaginal and skin application [1,7]. Specifically, SLN and NLC are proposed for mucosal and skin applications, since they are non-irritant and non-toxic [6,10]. In this respect, the current study describes the encapsulation of PRG in SLN and NLC produced by UH and HPH methods. The obtained nanoparticle suspensions have been further formulated in viscous gels obtained by xanthan gum (x-gum) addition. Tape stripping experiments have been conducted to compare the performances of SLN and NLC gels applied on skin. Particularly PRG amount in *stratum corneum* has been evaluated after cutaneous application of the different nanoparticulate gels.

2. Materials and methods

2.1. Materials

The copolymer poly (ethylene oxide) (a) –poly (propylene oxide) (b) (a = 80, b = 27) (poloxamer 188) was a gift of BASF ChemTrade GmbH (Burgbernheim, Germany). Miglyol 812 N, caprylic/capric triglycerides (miglyol) was a gift of Cremer Oleo Division (Witten, Germany). Tristearin, stearic triglyceride (tristearin), progesterone (PRG) and HPLC solvents were purchased from Sigma-Aldrich, Merck (Darmstadt, Germany).

2.2. Preparation of lipid nanoparticles

Lipid nanoparticles were alternatively prepared by hot homogenization techniques based on ultrasound or high pressure. In both cases the lipid phase (5% with respect to the whole weight of the dispersion) was constituted of pure tristearin (in the case of SLN preparation) or a mixture of tristearin and miglyol in a 2:1, w/w ratio (in the case of NLC preparation), while the aqueous phase was a poloxamer 188 solution (2.5, % w/w).

2.2.1. Ultrasound homogenization (UH) method

As first step an emulsion was obtained by addition of the aqueous phase (4.75 ml) heated at 80 °C to the molten lipid phase (250 mg) followed by mixing at 15,000 rpm, 80 °C for 1, 2 or 3 min (IKA T25 digital ultra-turrax) (data in brief, Fig. S 1A). As second step, the emulsion was subjected to ultrasound homogenization at 6.75 kHz for 5, 10 or 15 min (Microson ultrasonic Cell Disruptor-XL Minisonix) (data in brief, Fig. S 1B) and let cooling at 25 °C. Lipid nanoparticle dispersions were stored at room temperature.

2.2.2. High pressure homogenization (HPH) method

As first step, the aqueous phase (950 ml) heated at 80 °C was added to the molten lipid phase (50 g) and subjected to stirring at 15,000 rpm, 80 °C for 1, 2 or 3 min (data in brief, Fig. S 1C). The emulsion was homogenized using high pressure homogenizer for 1–3 cycles at 100 MPa (Panda Plus2000/GEA Niro Soavi, Parma, Italy) to form o/w nanoemulsion (data in brief, Fig. S 1D). The nanoemulsion was then cooled by a coil employed as heat exchanger directly connected to the instrument for solidification and formation of lipid nanoparticles. Lipid nanoparticle dispersions were stored at room temperature.

Both for UH and HPH methods, in the case of PRG loaded nanoparticles (named SLN-PRG and NLC-PRG), the drug (0.1%, w/w with respect to the whole dispersion; 0.02%, w/w with respect to the lipid phase) was added to the fused lipid phase before emulsification step.

2.3. Photon correlation spectroscopy (PCS) analysis

Submicron particle size analysis was performed using a Zetasizer 3000 PCS (Malvern Instr., Malvern, England) equipped with a 5-mW helium neon laser with a wavelength output of 633 nm. Glassware was cleaned of dust by washing with detergent and rinsing twice with water for injections. Measurements were made in triplicate at 25 °C at an angle of 90°, periodically from 0 to 6 months after nanoparticle production. Data were interpreted using the "CONTIN" method [16].

2.4. Cryogenic transmission electron microscopy (cryo-TEM) analysis

Samples were vitrified as previously described [17]. The vitrified specimen was transferred to a Zeiss EM922Omega transmission electron microscope for imaging using a cryoholder (CT3500, Gatan). The temperature of the sample was kept below −175 °C throughout the examination. Specimens were examined with doses of about 1000–2000 e/nm^2 at 200 kV. Images were recorded digitally by a CCD camera (Ultrascan 1000, Gatan) using an image processing system (GMS 1.9 software, Gatan). In addition, size distribution of nanoparticles was performed by measuring 1000 nanoparticles for each cryo-TEM image by the digital analyzer ImageJ 1.48v.