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Research paper

Improved tacrolimus skin permeation by co-encapsulation with clobetasol in lipid nanoparticles: Study of drug effects in lipid matrix by electron paramagnetic resonance

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ABSTRACT

Combined therapy with corticosteroids and immunosuppressant-loaded nanostructured lipid carriers (NLC) could be useful in the treatment of skin diseases. To circumvent NLC loading capacity problems, loaded drugs should have different physicochemical characteristics, such as tacrolimus (TAC) and clobetasol (CLO). Therefore, in the present study, TAC and CLO were encapsulated in NLC (TAC-NLC, CLO-NLC and TAC+CLO-NLC), coated or otherwise with chitosan. Electron paramagnetic resonance (EPR) spectroscopy of different spin labels was used to investigate the impact of drug and oil incorporation on the lipid dynamic behavior of the lipid matrices. In addition, the impact of co-encapsulation on drug release and skin permeation was evaluated. Entrapment efficiency was greater than 90% for both drugs, even when the maximum drug loading achieved for TAC-NLC and CLO-NLC was kept at TAC+CLO-NLC, because TAC is more soluble in the solid lipid and CLO in the liquid lipid. EPR data indicated that both drugs reduced the lipid fluidity near the polar surface of the lipid matrix, which suggests their presence in this region. In addition, EPR data showed that liquid lipid is also present in more superficial regions of the nanoparticle matrix. CLO was released faster than TAC from TAC+CLO-NLC, probably because it is more soluble in the liquid lipid. TAC skin penetration was affected by CLO. A 5-fold increase in TAC penetration was observed from TAC+CLO-NLC when compared to TAC-NLC formulations. Coating also increased TAC and CLO permeation to deeper skin layers (1.8-fold and 1.6-fold, respectively). TAC +CLO-NLC seems to be an effective strategy for topical delivery of TAC and CLO, and thus constitutes promising formulations for the treatment of skin diseases.

1. Introduction

Inflammatory skin diseases have been commonly treated with clobetasol propionate (CLO) and tacrolimus (TAC) (Fig. S1 - supplementary material). The combined use of CLO and TAC in semi-solid formulations has also shown significant improvement in lupus discoid treatment when compared to using each drug separately [1]. These studies obtained almost complete resolution of the plaque lesions in severe recalcitrant discoid lupus. Complete remission probably did not occur because of the hyperkeratotic condition which impedes the drugs from reaching the deeper skin layers and thus yield more efficient treatment [2], especially for molecules with high molecular weight (>500 Da), such as TAC [3] (Fig. S1B - supplementary material). We believe that recalcitrant lupus could be completely treated with an appropriate formulation and the permanent scars, which have a negative psychological impact on a patients' life, could be avoided [4].

Abbreviations: TAC, tacrolimus; CLO, clobetasol; NLC, nanostructured lipid carriers; C, chitosan oligosaccharide; EPR, electron paramagnetic resonance spectroscopy; SC, stratum corneum; *RS*, remaining skin.

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Nanostructured lipid carriers (NLC) are comprised of oil and solid lipid and seem to be interesting formulations for topical application, since they can increase drug penetration through the skin by interacting with stratum corneum (SC) lipids [5]. These nanoparticles also increase skin hydration, and hence, drug permeation, by forming an occlusive film on the skin surface [6]. They can accumulate in skin follicles and release the drug in a controlled manner [7].

The coating of lipid nanoparticles with chitosan or its derivatives has also demonstrated enhanced drug skin permeation due to the increase in nanosystem interaction with the skin surface, thereby modifying drug distribution in the skin layers [8]. Thus, co-encapsulation of TAC and CLO in NLC, whether coated or otherwise, could favor drug skin penetration in hyperkeratotic conditions.

The idea of the combined therapy is very promising and surprising results have been reported for different therapies. Some studies have suggested the co-encapsulation of drugs for cancer treatments [9–14]. Antioxidants have also been co-encapsulated for use in topical applications [15,16]. However, the development of nanosystems with this proposal is quite difficult, especially when lipid nanoparticles are considered, as they have limited loading capacity due to their particle size and lipid polymorphic structure [17].

It is well known that loading capacity is directly related to molecule accommodation in the lipid matrix [18]. Thus, if a drug is added up to its maximum load to a lipid matrix, it is difficult to maintain this maximum load, when another drug is added to the system, i.e., the addition of two drugs to the same lipid matrix can reduce the drug loading of one or both drugs loaded. Indeed, the co-encapsulation of doxorubicin-complex (with hydrolyzed polymer of epoxidized soybean oil) and mitomycin C in lipid nanoparticles resulted in the reduction of mitomycin encapsulation efficiency from 66.6 to 36.2%. Both mitomycin and doxorubicin-complex are lipophilic at pH 7.4 [10], which can lead to competition for accommodation in the nanoparticle matrix, and result in decreased mitomicyn loading.

To circumvent these problems, we hypothesized that, for successful co-encapsulation, the drugs should have different physicochemical characteristics, such as Log P. This may facilitate the drugs accommodating in different regions of the nanoparticles and result in maximum drug loading for both drugs. Recent studies with EPR of fatty acid spin labels, undertaken by our research group, demonstrated that the encapsulated drug could be localized more deeply in the lipid matrix, in the peripheral region or at the surfactant interface [19,20]. Drug localization not only interferes in nanoparticle loading, but could also modify drug release and skin permeation from the lipid nanoparticles [19]. Dexamethasone spin-labeled EPR spectroscopy has recently been used to study the interaction of the drug loaded in NLP composed of gelucire and witepsol as solid lipids, and capryol as liquid lipid; the EPR results suggested that the spin-labeled dexamethasone is located on the surface of the NLP at the lipid-water interface [21].

In this context, it seems that CLO and TAC are strong candidates for co-encapsulation in lipid matrix. Besides their importance for hyperkeratotic lupus treatment, these drugs present different Log P values (6.09 and 3.0) and molecular weight (822 and 466 g/mol) for TAC and CLO, respectively [22,23], which seem to be relevant for this purpose.

Thus, the aim of this study was to encapsulate TAC and CLO and verify if the maximum drug loading of both drugs could be maintained with the co-encapsulation in lipid nanoparticles. EPR spectroscopy of two spin labels, analogs of stearic acid, incorporating doxyl groups at C-5 and C-16 positions along the fatty acid chain, and a spin-labeled phosphatidylcholine (PC-TEMPO), in which the choline of the head group bears the nitroxide moiety, was used to investigate whether the drug induced lipid dynamic changes at these different positions in lipid matrices. To this end, lipid nanoparticles were obtained with the drugs separately and with co-encapsulation, whether coated or otherwise with chitosan oligosaccharide. We also studied the influence of lipid dynamic behavior (nanoparticle flexibility) on drug release and skin permeation.

2. Materials and methods

2.1. Reagents

TAC (min. 99%) was purchased from AK Scientific Laboratories (Union City, USA) and CLO (min. 99%) from Sigma-Aldrich (St. Louis, USA). HPLC grade acetonitrile were purchased from J. T. Baker (Phillipsburg, USA). Stearic acid (mp 58.77 °C, Vetec, Brazil), oleic acid (Sigma-Aldrich, St. Louis, USA), soy lecithin (Lipoid S100 - Ludwigshafen, Germany), sodium taurodeoxycholate (Sigma-Aldrich, St. Louis, USA) and chitosan oligosaccharide (5000 Da, Sigma-Aldrich, St. Louis, USA) were all used for the preparation of the NLC. The spin labels (5-doxyl stearic acid (5-DSA) and 16-doxyl stearic acid (16-DSA)) were purchased from Sigma Aldrich (St. Louis, Missouri). Water was purified using a Milli-Q system (Millipore, Billerica, USA) with a 0.22 μm pore end filter. All other chemicals were of analytical grade.

2.2. Analytical procedure

The HPLC system consisted of an isocratic pump (ProStar 210), autosampler (ProStar 400), and UV detector (ProStar 325) all from Varian, Palo Alto, USA. Separation was achieved using an Agilent ZORBAX Eclipse SB-C18 column (250 × 4.6 mm, 5 μm). The mobile phase was a 73:27 (v/v) mixture of acetonitrile and water. The flow rate was 1.0 mL/min and the injection volume 50 μL. UV detection was carried out at 240 nm for the first 7 min, and then at 210 nm from the 7th to the 12th min. Data acquisition was performed using Galaxie® Chromatography Data System software (Varian, Palo Alto, USA).

CLO retention time was 4.8 min and 10.4 min for TAC. A linear calibration curve was obtained for CLO ($y = 85.604x + 6.2171$; $r = 1$) and TAC ($y = 32.962x - 0.6962$; $r = 1$) based on the analysis of different concentrations (0.1–60 μg/mL for CLO and TAC) in triplicate. The limit of quantitation (LOQ) of the method was 0.3 μg/mL for CLO and 1 μg/mL for TAC. Selectivity was investigated (formulation components and skin homogenate) and no interference was observed in drug retention time. The method was validated in accordance with FDA [24] guidelines.

2.3. Production of nanostructured lipid carriers (NLC) containing clobetasol (CLO) and tacrolimus (TAC)

Clobetasol-loaded NLC (CLO-NLC), tacrolimus-loaded NLC (TAC-NLC) and the co-encapsulated particles (TAC+CLO-NLC) were prepared using a microemulsion technique described by Gasco [25]. A mixture of lecithin, taurodeoxycholate, stearic acid, and oleic acid was heated, and the drugs added to the melted material. After that, 250 μL of distilled water were added to the mixture under magnetic stirring, and a microemulsion system was formed. This microemulsion was dispersed into cold HEPES buffer (pH 5.5; 25 mM; 2–4 °C) under vigorous stirring (13,400 rpm for 10 min, T25 ULTRA-TURRAX®, IKA, Staufen, Germany) using a 1:20 ratio (microemulsion:water) to form NLC dispersion. The dispersion had a total lipid content of 2% (w/v), stabilized by 1.25% (w/v) of surfactant (lecithin: taurodeoxycholate, 4:1 ratio). The stearic acid:oleic acid ratio in the NLC was 3:1 while the final volume of