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Research paper

Impact of lipid dynamic behavior on physical stability, *in vitro* release and skin permeation of genistein-loaded lipid nanoparticles

Lígia Marquez Andrade [a], Carolina de Fátima Reis [a], Lorena Maione-Silva [a], Jorge Luiz V. Anjos [b], Antonio Alonso [c], Rafael Caixeta Serpa [a], Ricardo Neves Marreto [a], Eliana Martins Lima [a], Stephânia Fleury Taveira [a,*]

[a] Pharmaceutical Technology Laboratory, Universidade Federal de Goiás (UFG), Goiânia, Brazil
[b] Physics Department, Universidade Federal de Goiás (UFG), Catalão, Brazil
[c] Biophysics Laboratory, Universidade Federal de Goiás (UFG), Goiânia, Brazil

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ABSTRACT

The aim of this study was to develop lipid nanoparticles to deliver genistein (GEN) to deeper skin layers. To do so, the impact of lipid dynamic behavior (nanoparticle flexibility) on stability, release and skin permeation studies was verified. GEN-loaded solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) were obtained and characterization was undertaken. Freshly prepared nanoparticles were produced with similar features (i.e., drug loading). However, a higher level of crystallization in GEN-SLN formulation was observed in differential scanning calorimetry experiments. Electron paramagnetic resonance measurements showed a lower mobility of the spin labels in the SLN, which would indicate that NLC could be more flexible than SLN. Despite the fact that NLC demonstrated more fluidity, GEN was released more slowly from NLC than from SLN. Skin permeation studies demonstrated that lipid nanoparticles increased GEN skin retention. More flexible particles (NLC) also favored drug penetration into deeper skin layers. GEN-NLC would seem to be a promising formulation for GEN topical delivery.

1. Introduction

Genistein (GEN), the main isoflavone found in soybeans [1], has great potential as an anticancer, antioxidant and antiproliferative agent [2]. Studies have shown the GEN inhibition of *in vivo* photocarcinogenesis in skin cancers [3,4]. In addition, topical administration of the drug to mouse skin has demonstrated acceptable safety levels [5], without carcinogenicity or genotoxicity [6]. Thus, the topical application of GEN seems to be an interesting alternative for the prevention and treatment of pre-malignant skin lesions. However, studies have reported that GEN has difficulty in permeating the skin when administered in conventional creams [7]. These drawbacks emphasize the need for better formulations if GEN skin penetration is to be improved.

Lipid nanoparticles are drug delivery systems comprised of a solid lipid matrix [8]. They have demonstrated a remarkable ability to improve drug skin penetration due to the formation of an occlusive film on the skin surface, which prevents transepidermal water loss [9]. Moreover, cationic nanoparticles can interact with negative charges of the skin and increase drug retention in the epidermis [10]. These advantages, along with their ability to accumulate in skin imperfections [11,12], make these carriers very promising for topical drug delivery.

The most widely studied lipid nanoparticles are solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC). Both present a solid lipid matrix, but NLC are prepared with a mixture of solid lipid and oil [8]. It has been suggested that NLC could increase drug loading and stability more than SLN formulations, probably due to the creation of a less ordered lipid matrix, by mixing solid lipid and oil. The mixed lipid matrix could prevent or reduce drug expulsion during storage [13].

Abbreviations: 5-DSA, 5-doxyl stearic acid; 16-DSA, 16-doxyl stearic acid; AFM, atomic force microscopy; CPC, cetylpyridinium chloride; DSC, differential scanning calorimetry; REC, drug recovery; EPR, electron paramagnetic resonance; EE, entrapment efficiency; GEN, genistein; NLC, nanostructured lipid carriers; RS, remaining skin; τ_c, rotational correlation time; SDS, sodium dodecyl sulfate; SLN, solid lipid nanoparticles; SC, stratum corneum; $2A_{//}$, static parameter associated with the orientational distribution of the spin label in the lipid domain.

* Corresponding author. Pharmaceutical Technology Laboratory, School of Pharmacy – Universidade Federal de Goiás (UFG), Praça Universitária c/1ª. Avenida, Qd. 62, 74.605-220 Goiânia, GO, Brazil. Tel./fax: +55 62 3209 6039.
E-mail address: stephaniafleury@ufg.com.br (S.F. Taveira).

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Despite these statements, controversial results have been found in the literature about the differences between SLN and NLC [14]. Some studies have been conducted comparing these formulations [15,16]; but no comparison has yet been made between the lipid dynamics of nanoparticle matrixes and the influence of these different matrixes on skin permeation of drugs encapsulated in these systems. Therefore, in an attempt to increase GEN penetration in the skin and verify the influence of the lipid matrixes on skin permeation of GEN, this study set out to develop and characterize cationic lipid nanoparticles (SLN and NLC) containing GEN for topical delivery.

In this study, the electron paramagnetic resonance (EPR) spectroscopy of fatty acid spin labels was used as a complementary technique to investigate the lipid dynamic behavior of both SLN and NLC dispersions as well as the influence of GEN on the fluidity of these nanoparticles. Other comparisons involving SLN and NLC were performed through stability studies of GEN-loaded lipid nanoparticles (180 days at 4 °C), differential scanning calorimetry (DSC), *in vitro* release and *in vitro* permeation studies.

2. Materials and methods

2.1. Reagents

GEN (min. 99%) (Fig. 1) was purchased from LC Laboratories (Woburn, USA). HPLC grade acetonitrile, methanol, n-propanol and acetic acid were purchased from JT Baker (Phillipsburg, USA). Polysorbate 80 (Tween® 80), sorbitan trioleate (Span® 85), cetylpyridinium chloride (CPC) and the spin labels (5-doxyl stearic acid (5-DSA) and 16-doxyl stearic acid (16-DSA), shown in Fig. 1), were purchased from Sigma Aldrich (St. Louis, Missouri). Glyceryl behenate (Compritol® ATO 888) was purchased from Gattefossé (Weilam Rhein, Germany). Capric and caprylic triglycerides (Miglyol® 812 N) were purchased from Stallergenes SA (Antony, France). Distilled water used in all preparations was purified using a water purification system (Milli-Q Millipore Simplicity 185, Bedford, USA). All other chemicals were of analytical grade.

2.2. Analytical procedure

The amount of GEN was determined using a UV high-performance liquid chromatography method (HPLC) [17]. The HPLC system consisted of a quaternary pump (ProStar 240, Varian, Palo Alto, USA), autosampler (ProStar 410, Varian, Palo Alto, USA) and UV detector (ProStar 310, Varian, Palo Alto, USA). Separation was obtained in an Agilent ZORBAX® SB-C18 column (250 × 4.6 mm, 5 μm), which was protected with an Eclipse® XDB-C18 short guard column (12.5 × 4.6 mm). The mobile phase was 0.5% acetic acid in water (pH 3.0), acetonitrile and n-propanol 50:48:2 (v/v/v). Flow-rate was 1.0 mL/min, injection volume was 50 μL and UV detection was carried out at 270 nm. Data acquisition was performed using Galaxie® Chromatography Data System Software (Varian, Palo Alto, USA).

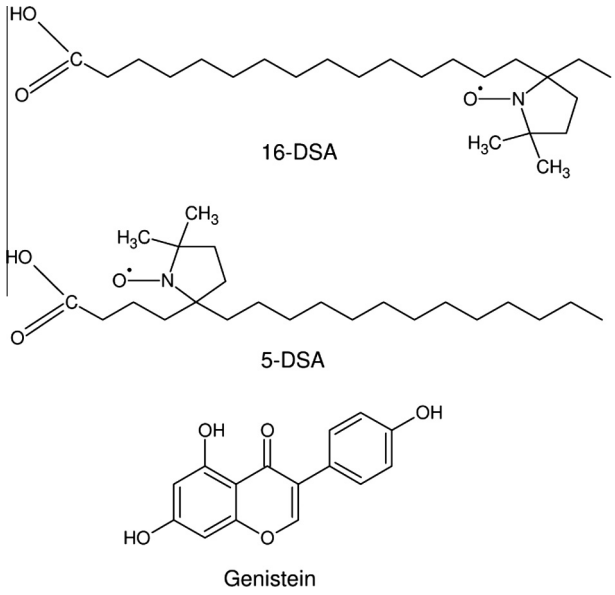

Fig. 1. Chemical structures of two spin labels, 5-DSA and 16-DSA, and the GEN.

The retention time of GEN was 4.65 min. A linear calibration curve ($y = 185.53 \times +38.6$; $r = 0.9997$) was obtained based on the analysis of different concentrations (0.1–60 μg/mL) in triplicate. The limit of quantitation (LOQ) of the method was 100 ng/mL. The selectivity was investigated (formulation components and skin homogenate) and no interference was observed in the GEN retention time. The method was validated in accordance with FDA (2001) [18] guidelines and published elsewhere [17].

2.3. Production of solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) containing genistein (GEN)

Lipid nanoparticles were prepared using a microemulsion technique [15]. In summary, lipid phase (glyceryl behenate for SLN or 7:1 glyceryl behenate/capric caprylic triglyceride for NLC), polysorbate 80, sorbitan trioleate and cetylpyridinium chloride (CPC) were heated, and GEN was added (1.6 mg/mL) to these melted materials. Then, 250 μL of distilled water was added to the mixture under magnetic stirring to obtain a microemulsion. The hot microemulsion was dispersed into cold water (2–4 °C) under vigorous stirring (13,400 rpm for 10 min, IKA® T25 Ultra-Turrax, Staufen, Germany) using a 1: 20 ratio (microemulsion: water) to form a GEN-SLN and GEN-NLC aqueous dispersion. This dispersion had a total lipid content of 2% (w/v), stabilized by 0.5% (w/v) of CPC and 1% (w/v) in a 3:1 ratio of polysorbate 80/sorbitan trioleate. In addition, nanoparticles without GEN were obtained.

2.4. Characterization of lipid nanoparticles (SLN and NLC)

The mean diameter of the nanoparticle dispersion was measured by photon correlation spectroscopy (PCS) using a Zetasizer Nano ZS (Malvern Instruments Ltd., Worcestershire, UK) with polystyrene cuvettes. Zeta potential was analyzed in folded capillary cells with the same instrument. The entrapment efficiency (EE) of GEN-loaded nanoparticles was determined by ultrafiltration as previously described [19]. Average diameter was also studied by atomic force microscopy (AFM) (AFM/STM, Agilent Technologies 5500, Santa Clara, USA). AFM was performed in a non-contact mode. The samples were prepared by depositing dilute particle dispersion in mica plates, followed by drying at 25 °C. The images were treated in Gwyddion® version 2.31 (Agilent, Santa Clara, USA).

2.5. Stability studies

GEN-SLN and GEN-NLC dispersions were stored at 4 ± 2 °C for 180 days. Stability was evaluated by comparing the initial particle size, PdI, EE and GEN initial concentration with those obtained from samples withdrawn after 8, 15, 30 and 180 days of storage.

2.6. Differential scanning calorimetry (DSC) analysis

DSC analysis was performed using a Shimadzu DSC-60 calorimeter (Maryland, USA). Pure GEN, bulk lipids, SLN and NLC were analyzed. The physical mixture of glyceryl behenate and capric/