International Journal of Pharmaceutics 434 (2012) 420–428

Contents lists available at SciVerse ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Pharmaceutical nanotechnology

Decylglucoside-based microemulsions for cutaneous localization of lycopene and ascorbic acid

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ARTICLE INFO

Article history:
Received 29 February 2012
Received in revised form 29 May 2012
Accepted 3 June 2012
Available online xxx

Keywords:
Lycopene
Ascorbic acid
Skin
Microemulsion
Topical delivery
Monoglycerides

ABSTRACT

Cutaneous delivery of combinations of antioxidants offers the possibility of enhanced protection against UV-radiation. In this study, we investigated the potential of sugar-based microemulsions containing monoglycerides to promote simultaneous cutaneous delivery of lycopene and ascorbic acid, and increase tissue antioxidant activity. Lycopene and ascorbic acid were incorporated (0.04% and 0.2% (w/w), respectively) in decylglucoside-based microemulsions containing isopropyl myristate mixed with monocaprylin (ME-MC), monolaurin (ME-ML) or monoolein (ME-MO) as oil phase. The microemulsions increased lycopene delivery into porcine ear skin by 3.3- to 8-fold compared to a drug solution. The effect of microemulsions on ascorbic acid cutaneous delivery was more modest (1.5–3-fold), and associated with an approximately 2-fold increase in transdermal delivery. According to their penetration-enhancing ability, the microemulsions were ranked ME-MC > ME-MO > ME-ML. This superiority of ME-MC coincided with a stronger effect in decreasing skin electrical resistance. After 18 h of treatment, the viability of bioengineered skin treated with ME-MC was 2.2-times higher compared to Triton-X100 (moderate irritant), demonstrating that ME-MC is less cytotoxic. Skin treatment with ME-MC containing both antioxidants increased the tissue antioxidant activity by 10.2-fold, but no synergism between the antioxidants was observed.

1. Introduction

Lycopene, a carotenoid found in red-colored fruits, has aroused great interest because of its strong *in vitro* antioxidant activity (Andreassi et al., 2004) and ability to inhibit UVB-induced ornithine decarboxylase and myeloperoxidase, reducing inflammation and skin thickness, and providing protection against photodamage (Cesarini et al., 2003; Fazekas et al., 2003; Mein et al., 2008). Because lycopene content in the skin seems to be inversely proportional to skin roughness, it may also be able to reduce furrows and wrinkles formation (Darvin et al., 2008). Based on all of these properties, cutaneous delivery of lycopene would be very attractive (Gonzalez et al., 2008).

In a previous study, we developed topical microemulsions in an attempt to address formulation and delivery challenges resulting from lycopene's strong lipophilicity, which makes it difficult to dissolve lycopene in aqueous systems as well as in oils used in food and cosmetics (Spernath et al., 2002), and prone to be retained within the stratum corneum (Lopes et al., 2010). Microemulsions containing mono and diglycerides of capric and caprylic acids as oil phase were most effective at increasing lycopene delivery into viable skin layers compared to those containing triglycerides of the same fatty acids (Lopes et al., 2010). In addition, incorporation of ascorbic acid in lycopene-containing microemulsions increased lycopene content in the skin by 25%, probably due to inhibition of its degradation (Biacs and Daood, 2000; Lopes et al., 2010).

Since ascorbic acid offered some protection against lycopene degradation, and a concomitant increase in lycopene and ascorbic acid plasma concentration improved protection from DNA oxidative damage (Lopes et al., 2010; Riso et al., 2004), development of a topical formulation that provides cutaneous localization of these two antioxidants could potentially maximize their benefits to the skin. However, lycopene and ascorbic acid have distinct structures, molecular weight (176 and 536.8 for ascorbic acid and lycopene respectively) and calculated log*P* values (approximately −1.64 and 17 for ascorbic acid and lycopene respectively), making their simultaneous delivery into viable skin layers challenging (Cotelle et al., 2003; Spernath et al., 2002; Vertzoni et al., 2006).

The ability of formulations to improve the release and transport of therapeutic agents into the skin may be influenced by the type of delivery system, its composition and concentration of components (Hosmer et al., 2011; Phelps et al., 2011; Savic et al., 2006).

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0378-5173/$ – see front matter © 2012 Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.ijpharm.2012.06.016

Thus, this study aimed at evaluating whether and how microemulsion composition affects skin penetration of lycopene and ascorbic acid. More specifically, we developed microemulsions containing sugar-based surfactants and various monoglycerides as oil phases to assess the influence of the acyl chain length of monoglycerides on skin permeability and cutaneous drug delivery. We used monoglycerides of caprylic acid (monocaprylin, 8 carbons, MC), lauric acid (monolaurin, 12 carbons, ML) and oleic acid (monoolein, 18 carbons, MO). While the penetration-enhancing ability of monocaprylin and monoolein has been described (Lopes et al., 2005, 2009), there is very little information on monolaurin, except that its antimicrobial properties can be advantageous in topical treatment (Fu et al., 2006). Our results demonstrate that the type of monoglyceride plays a major role on the ability of formulations to concomitantly maximize the cutaneous delivery of ascorbic acid and lycopene.

2. Material and methods

2.1. Material

Propylene glycol (PG), ascorbic acid, isopropyl myristate, neocuproine, ammonium acetate and copper chloride were obtained from Sigma (St. Louis, MO). Monoglycerides of caprylic acid (MC), lauric acid (ML) and oleic acid (MO) were kindly supplied by Abitec Corporation (Janesville, WI) or purchased from Nu-Check Prep (Elysian, MN). Decylglucoside was kindly supplied by Cognis (BASF, Cincinnati, OH). Lycopene standard was purchased from Wako Chemicals (Richmond, VA). Acetonitrile, methanol, ethanol and dichloromethane were purchased from Mallinckrodt Baker (Phillipsburg, NJ).

2.2. Methods

2.2.1. Lycopene extraction and purification

Lycopene was extracted from commercial tomato paste as previously described by ours and other groups (Lopes et al., 2010; Periago et al., 2004; Ronman, 1985). Briefly, tomato paste (100 g) was dehydrated with 95% ethanol, filtered and the residue was dissolved in dichloromethane and dried with anhydrous sodium sulfate. After solvent removal *in vacuo*, the extract was purified by flash column chromatography (silica gel, petroleum ether:dichloromethane 75:25, v/v) (Periago et al., 2004). The identity of the purified lycopene was confirmed by comparison to a commercially available standard. As previously described, purified and standard lycopene presented similar R_f values in thin layer chromatography, similar UV–vis spectra (characteristic peaks at 504, 472, and 446 nm), similar retention times when analyzed by HPLC (9.4 min, even though peak areas were generally 25% smaller for the extracted compound, suggesting the occurrence of some degradation during extraction), and presented a molecular ion of m/z 537 when analyzed by mass spectrometry (positive ionization mode, Waters TQD tandem quadrupole detector, Milford, MA) (Lopes et al., 2010). The purified lycopene was used for formulation development.

2.2.2. Phase diagram construction and sample preparation

Ternary phase diagrams were constructed using the water titration method at room temperature. Decylglucoside was used as surfactant and propylene glycol as co-surfactant. They were chosen due to their low irritation potential (Graf et al., 2008; Savic et al., 2006). Propylene glycol, as other short and medium chain length alcohols, is commonly added as co-surfactant to further reduce the interfacial tension and aid microemulsion formation (Fan et al., 2011; Lawrence and Rees, 2000). It is miscible with water and the oil used, and thus, able to partition into these phases (Lawrence and Rees, 2000). For consistency with previous studies, propylene glycol was considered a co-surfactant, and its weight fraction was included in the surfactant-co-surfactant blend (El Maghraby, 2008; Fan et al., 2011; Li et al., 2012; Peira et al., 2008). Surfactant and co-surfactant were mixed at 3:1, 1:1 and 1:3 (w/w). The oil phase consisted of mixtures at 1:9 (w/w) of isopropyl myristate and monoglycerides. We used monocaprylin (8 carbons, MC), monolaurin (12 carbons, ML) or monoolein (18 carbons, MO) as monoglycerides; isopropyl myristate was included to aid melting and mixing of monolaurin and monoolein with the surfactant blend. Mixtures of surfactant-co-surfactant and oil phase at 1:9 to 9:1 (w/w) were titrated with water under vortexing, and the systems were characterized by visual inspection. Phase diagrams were plotted to show the relationship between formulation composition and phase behavior. Formulations that were fluid, clear, and did not undergo phase separation were classified as microemulsions, and assigned to a monophasic region (black-shaded) in the phase diagrams (Hathout et al., 2010).

Based on the phase behavior of mixtures and dimensions of the monophasic region in phase diagrams (Fig. 1), surfactant–co-surfactant blends composed of decylglucoside:propylene glycol at 1:1 (w/w) were selected for the preparation of three microemulsions, all containing the same ratio of components (surfactant:oil:water at 50:20:30, w/w/w), but each with one type of monoglyceride: monocaprylin (ME-MC), monolaurin (ME-ML) or monoolein (ME-MO). By keeping the ratio between components constant, a comparison of the influence of monoglycerides can be made. The oil phase was set at 20% to increase cutaneous over transdermal delivery (Lopes et al., 2009). Lycopene was incorporated in the oil phases of these microemulsions at a final concentration of 0.04% (w/w), while ascorbic acid was incorporated in the aqueous phase at 0.2% (w/w).

2.2.3. Microemulsion characterization

Polarized light microscopy (Axiotop, Zeiss, Thornwood, NY) was used to verify the isotropicity of the selected microemulsions. The internal structure of the systems (water-in-oil, oil-in-water or bicontinuous) was assessed by electrical conductivity at $25 \pm 0.5^\circ$ C using a Jenway 4520 Conductivity/TDS meter (Techne Inc., Burlington, NJ). The ratio between surfactant:co-surfactant and oil was kept constant (2.5:1, w/w, surfactant blend:oil), while water was added at small increments along a dilution line (gray lines shown in the diagrams in Fig. 2A–C). Water was added until the microemulsions were transformed into turbid or gel-like systems (borderline of the monophasic region). Conductivity was measured after each water addition.

2.2.4. Effect of microemulsions containing various monoglycerides on cutaneous delivery of antioxidants

Skin penetration of lycopene and ascorbic acid was studied using Franz diffusion cells. Briefly, the skin from the outer surface of a freshly excised porcine ear was carefully dissected, stored at -20° C, and used within a month. On the day of the experiment, the skin was thawed and mounted in a Franz diffusion cell (diffusion area of 1 cm^2; Laboratory Glass Apparatus, Inc., Berkeley, CA). The receptor compartment was filled with 100 mM phosphate buffer (pH 7.4, containing 10% ethanol) and maintained at 37°C under constant stirring (Lopes et al., 2010). Microemulsions (100 mg) containing lycopene and ascorbic acid were placed in the donor compartment of diffusion cells for 6 or 12 h; solutions of 0.04% (w/w) lycopene and 0.2% ascorbic acid in isopropyl myristate/propylene glycol were used as control formulations.

After the abovementioned periods of time, skin samples were rinsed, blotted dry, and the stratum corneum (SC) was separated from the epidermis and dermis (ED) by tape stripping. Fifteen pieces of tape were used, and the pieces were placed