International Journal of Pharmaceutics xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Enhancement of the *in vitro* penetration of quercetin through pig skin by combined microneedles and lipid microparticles

Roberto Paleco [a], Sonja R. Vučen [b], Abina M. Crean [b], Anne Moore [b,c], Santo Scalia [a,*]

[a] Dipartimento di Scienze Chimiche e Farmaceutiche, Università di Ferrara, Ferrara, Italy
[b] School of Pharmacy, University College Cork, Cork, Ireland
[c] Department of Pharmacology and Therapeutics, University College Cork, Ireland

ARTICLE INFO

Article history:
Received 16 April 2014
Received in revised form 6 June 2014
Accepted 8 June 2014
Available online xxx

Keywords:
Quercetin
In vitro skin penetration
Microneedles
Lipid microparticles

ABSTRACT

Silicon microneedle patches were investigated, alone or in combination with lipid microparticles (LMs), as a system to improve the *in vitro* skin penetration of the antioxidant flavonoid, quercetin. LMs loaded with quercetin were prepared by melt emulsification and sonication. The flavonoid content of LMs was 11.7 ± 0.3% and their mean diameter and polydispersity index were 8.1 μm and 0.66, respectively. Emulsions containing quercetin, free or microencapsulated, were applied to untreated- or microneedle-treated pig skin mounted in Franz diffusion cells. The amount of flavonoid penetrated into the stratum corneum and viable epidermis were measured by HPLC, after validated tape-stripping and bead mill homogenization procedures, respectively. Compared to intact skin, a marked increase in quercetin levels permeated into the stratum corneum (from 1.19 ± 0.12 μg/cm^2 to 2.23 ± 0.54 μg/cm^2) and viable epidermis (from 0.10 ± 0.01 μg/cm^2 to 0.56 ± 0.27 μg/cm^2) was achieved when skin was treated with the flavonoid-loaded LMs in combination with microneedle arrays. Conversely, perforation of the cutaneous surface by microneedles did not produce any significant improvement in the skin penetration of non-encapsulated quercetin. The enhanced (5.5-fold) intra-epidermal delivery of quercetin attained by the LM/microneedle strategy described here, is particularly relevant since the main quercetin site of action is in the epidermis.

1. Introduction

The flavonoid quercetin (3,3′,4′,5,7-pentahydroxyflavone) has been shown to possess anti-inflammatory, anti-carcinogenic and anti-microbial properties (Cushnie and Lamb, 2005; Vijayababu et al., 2005; Ruiz et al., 2007; Boots et al., 2008). Among the flavonoid class, quercetin exhibits the highest antioxidant potency (Morel et al., 1993; Cao et al., 1997; Teixera et al., 2005), including free radical scavenging, inhibition of lipid peroxidation, metal ion chelation and modulation of cell antioxidant responses (Morel et al., 1993; Cao et al., 1997; Granado-Serrano et al., 2012). Focusing on the effect of quercetin on the skin, a survey of the literature indicates that its topical application reduces the cutaneous oxidative damage caused by increased formation of free radicals and other reactive species (*e.g.*, non-radical oxygen species) induced by sunlight exposure (Casagrande et al., 2006; Vicentini et al., 2010). In particular, experiments on animal skin have demonstrated that this flavonoid inhibits the inflammatory response, histological damage and the expression of matrix metallo-proteinases triggered by the solar UV radiation (Casagrande et al., 2006; Vicentini et al., 2008; Vicentini et al., 2010). Moreover, quercetin has been reported to diminish human melanoma cell growth (Piantelli et al., 1995) and to exhibit anti-inflammatory activity on human keratinocytes (Jung et al., 2010) and activated neutrophils (Lin et al., 2012).

Although the application of quercetin preparations to the skin provides direct delivery to the cutaneous tissue, the activity of this flavonoid following topical administration is greatly hampered by its poor percutaneous penetration (Vicentini et al., 2008; Kitagawa et al., 2009; Lin et al., 2012; Bose et al., 2013), which limits quercetin bioavailability at the site of action, in viable skin (Vicentini et al., 2008; Kitagawa et al., 2009; Tan et al., 2011; Belo et al., 2009; Liu et al., 2013). The scarce solubility of this flavonoid in water and simultaneously in oils has been advocated as one of the reasons contributing to its inefficient skin permeation (Kitagawa et al., 2009; Chen-yu et al., 2012; Bose et al., 2013).

In order to overcome this drawback, several strategies have been developed, including use of permeation enhancers

* Corresponding author at: Dipartimento di Scienze Chimiche e Farmaceutiche Via Fossato di Mortara, 17 44121 Ferrara, Italy. Tel.:+39 532 455919; fax: +39 532 455953.
E-mail address: sls@unife.it (S. Scalia).

http://dx.doi.org/10.1016/j.ijpharm.2014.06.010
0378-5173/

(Saija etal., 1998; Scalia et al., 2013a,b) , synthesis of prodrugs (Lin et al., 2012), development of microemulsion-based vehicles (Kitagawa et al., 2009; Vicentini et al., 2008), incorporation in liposomes (Chessa et al., 2011; Liu et al., 2013) and encapsulation in polymeric (Tan et al., 2011) or lipid nanoparticles (Chen-yu et al., 2012; Bose et al., 2013).

A different approach is presented in this study involving the use of silicon microneedle arrays to produce transient pores in the outer skin layers (Coulman et al., 2009; Zhang et al., 2010; Carey et al., 2011; Vučen et al., 2013), which should facilitate the permeation of quercetin through the principal barrier, the stratum corneum (Lin et al., 2012), into deeper skin tissues. The potential of the microneedle technology to enhance the flavonoid topical delivery was investigated *in vitro* on excised pig skin mounted in Franz diffusion cells, using quercetin in the free form or encapsulated in lipid microparticles (LMs), which consist of a solid lipid core stabilized by surfactants (Jaspart et al., 2005). Moreover, because of the high instability of quercetin in physiological media (Boulton et al., 1999; Scalia et al., 2013a,b), particular attention was focused on the ability to recover this drug for analysis from skin samples.

2. Materials and methods

2.1. Materials

Quercetin and tristearin were obtained from Sigma–Aldrich (Steinheim, Germany). Hydrogenated phosphatidylcholine was a gift from Cargill (Hamburg, Germany). The excipients for the cream preparations were supplied by Fagron Italia (Bologna, Italy) and Croda (Snaith, UK). Methanol, acetonitrile and water were high-performance liquid chromatography (HPLC)-grade from Fisher Scientific (Leicester, UK). Adhesive tapes (Scotch Pop-up, 19 × 50 mm tapes) for *in vitro* tape stripping were from 3M (St. Paul, MN, USA). Silicon microneedle arrays (36 microneedles over 1 cm^2; microneedle height, 200 μm) were fabricated using a previously described method (Wilke et al., 2005) and provided by the Tyndall National Institute (Cork, Ireland). All other reagents and solvents were of analytical grade (Sigma).

2.2. High-performance liquid chromatography

HPLC was performed using an Agilent 1200 series HPLC system with a UV/VIS detector (Agilent Technologies, Santa Cara, CA, USA). Quercetin analysis was carried out on a reversed-phase column (5 μm Luna C18, 150 × 4.6 mm i.d.; Phenomenex, Torrance, CA) fitted with a guard column (5 μm particles, 4 × 2 mm i.d.) and eluted isocratically, at a flow-rate of 0.8 ml/min, with sodium acetate buffer (pH 3.8; 0.04 M)-acetonitrile (60:40, v/v) containing EDTA (0.5 mM). Injection volume of 10 μl was employed. Quercetin was detected at 370 nm. The identity of the quercetin peak was assigned by co-chromatography with the authentic quercetin standard. Quantification was carried out by integration of the peak areas using the external standardization method. Calibration curves were linear over the range 0.2–100 μg/ml, with correlation coefficients greater than 0.998. The detection and quantification limits (ICH Guidelines Q2(R1), 1997) were 0.04 μg/ml and 0.18 μg/ml, respectively. The precision of the method, evaluated by repeated analyzes ($n = 10$) of sample solutions containing quercetin at levels of 0.5 and 10 μg/ml, was demonstrated by relative standard deviation values <2.0%.

2.3. Lipid microparticle preparation

LMs were prepared by adding pre-heated (75 °C) deionized water (50 ml) containing 1.4% (w/v) phosphatidylcholine as surfactant, to the melted lipid phase (3.6 g tristearin), in which quercetin (0.7 g) has been dispersed. The mixture was subjected to high-speed stirring (21,500 rpm for 2 min at 75 °C) with an Ultra-Turrax T25 mixer (IKA-Werk, Staufen, Germany) and then sonicated at 22 kHz for 4 min (power output, 40 W) at 75 °C, using an ultrasonic probe (Microson XL2000 Ultrasonic Cell Disruptor; Misonix, Farmingdale, NY). The obtained emulsion was rapidly cooled at room temperature under magnetic stirring and the formed suspension was subjected to centrifugation (6000 rpm for 15 min) to recover the LMs which were then lyophilized. Unloaded microparticles were prepared with the same procedure, by omitting quercetin.

2.4. Lipid microparticle characterization

The microparticle morphological features were examined by variable-pressure (ca. 90 Pa) scanning electron microscopy (VP-SEM; Zeiss EVO40XVP, Arese, Milan, Italy).

The particle size distribution and surface charge were measured by dynamic light scattering (Zetasiser Nano ZS instrument; Malvern Instruments, Worcestershire, UK). The LMs were dispersed in HPLC-water and analyzed using a measurement angle of 173°. For data analysis, the viscosity (0.8872 mPa s) and refractive index (1.33) of water were used to determine the mean particle diameter and polydispersity index (PDI). The electrophoretic mobilities were measured in distilled water and the obtained values were converted to zeta potential using the Smoluchowski equation. Data were calculated from the average of three determinations.

The amount of quercetin entrapped in the LMs was determined by dissolving the microparticles (25 mg) in ethanol (20 ml) under heating (70 °C for 5 min) and sonication (10 min), in sealed glass vials. The obtained sample was diluted to volume (50 ml) with methanol, filtered (0.45 μm membrane filters) and assayed by HPLC, as outlined above. The encapsulation efficiency was calculated as the percentage ratio between the quantity of quercetin entrapped in the microparticles and the amount of flavonoid initially added to the melted lipid phase. The results were the average of at least three determinations.

Quercetin dissolution and release from the LMs were assessed by adding quercetin (2.0 mg) or LMs, containing an equivalent amount of flavonoid, to 100 ml of phosphate buffer (0.05 M, pH 7.4), containing polysorbate 20 (0.5%, w/w) as solubilizer to ensure sink conditions (Scalia and Mezzena, 2009). The samples were kept under mechanical stirring at 50 rpm and 37 °C. At appropriate time intervals, 1-ml aliquots of the medium were withdrawn and replaced with an equal volume of fresh fluid. The test samples were filtered (0.45 μm) and assayed for quercetin by HPLC, as outlined above. The flavonoid release (%) was calculated from the total quercetin content of the LM preparation. This was determined by extraction of the particles, after the release experiment, with ethanol under heating and sonication, as described above. A minimum of three replicates were performed for each sample.

2.5. Emulsion formulations

Creams (oil-in-water emulsions) containing non-encapsulated quercetin (1.0%, w/w) in conjunction with blank LMs or an equivalent amount of flavonoid incorporated in LMs were prepared. The emulsion excipients were: cetearyl isononanoate (7.5%), glyceryl stearate (0.5%), Phenonip® (0.8%; phenoxyethanol and parabens), benzyl alcohol (0.5%) and Montanov™ 82 (5.0%; cetearyl alcohol and coco-glucoside) for the internal phase and glycerin (2.0%), EDTA (0.1%), ethanol (3%) and deionized water (qs 100%) for the external phase. The creams were prepared according to the common procedure used in compounding

Please cite this article in press as: Paleco, R., et al., Enhancement of the *in vitro* penetration of quercetin through pig skin by combined microneedles and lipid microparticles, Int J Pharmaceut (2014), http://dx.doi.org/10.1016/j.ijpharm.2014.06.010