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Idebenone-loaded solid lipid nanoparticles for drug delivery to the skin: *In vitro* evaluation

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ABSTRACT

Idebenone (IDE), a synthetic derivative of ubiquinone, shows a potent antioxidant activity that could be beneficial in the treatment of skin oxidative damages. In this work, the feasibility of targeting IDE into the upper layers of the skin by topical application of IDE-loaded solid lipid nanoparticles (SLN) was evaluated. SLN loading different amounts of IDE were prepared by the phase inversion temperature method using cetyl palmitate as solid lipid and three different non-ionic surfactants: ceteth-20, isoceteth-20 and oleth-20. All IDE loaded SLN showed a mean particle size in the range of 30–49 nm and a single peak in size distribution. *In vitro* permeation/penetration experiments were performed on pig skin using Franz-type diffusion cells. IDE penetration into the different skin layers depended on the type of SLN used while no IDE permeation occurred from all the SLN under investigation. The highest IDE content was found in the epidermis when SLN contained ceteth-20 or isoceteth-20 as surfactant while IDE distribution into the upper skin layers depended on the amount of IDE loaded when oleth-20 was used as surfactant. These results suggest that the SLN tested could be an interesting carrier for IDE targeting to the upper skin layers.

1. Introduction

In recent years, great interest has been focused on the use of antioxidants for topical administration. Being the outermost barrier of the body, the skin is exposed to various exogenous sources of oxidative stress, including ultraviolet radiation and pollutants. As response to these oxidative attacks, reactive oxygen species (ROS) and other free radicals are generated in the skin (Dreher and Maibach, 2001). To counteract the deleterious effects of ROS, an antioxidant network consisting of a variety of lipophilic (*e.g.* vitamin E, ubiquinones, carotenoids) and hydrophilic (*e.g.* vitamin C, uric acid and glutathione) antioxidants is present in the skin and is responsible for the balance between pro-oxidants and antioxidant (Thiele et al., 2000). An impairment of this balance, due to an increased exposure to exogenous sources of ROS, has been defined as "oxidative stress" and involves oxidative damages of lipids, proteins and DNA (Sies, 1985). Generally, the epidermis contains higher concentrations of antioxidants compared to the dermis while the horny layer lacks of co-antioxidants such as ubiquinol 10 that, on the contrary, is the most abundant ubiquinone contained in human skin. Topical administration of antioxidants is regarded as an interesting strategy in reducing ROS induced skin damages since it may improve skin antioxidant capacity (Dreher and Maibach, 2001). A topical supplementation with antioxidants could be particularly beneficial for the stratum corneum due to its high susceptibility for UV and ozone-induced depletion of antioxidants (Thiele et al., 1998).

In the last decades, many colloidal carriers have been proposed for drug targeting to the skin, such as liposomes (Bernard et al., 1997; Mezei et al., 1994) and solid lipid nanoparticles (SLN) (Papakostas et al., 2011; Pardeike et al., 2009; Zhang and Smith, 2011). The latter show several advantages compared to other drug delivery systems: good local tolerability, improved drug stability, drug targeting, increased bioavailability, ability to incorporate drugs with different physico-chemical properties, high inclusion rate for lipophilic substances and small particle size allowing close contact to the stratum corneum (Müller et al., 2000; Mehnert and Mäeder, 2001).

Recently, we have developed a novel technique to prepare SLN using low amounts of surfactants by means of the phase inversion temperature (PIT) method, that allowed us to obtain SLN with promising physico-chemical and technological properties such as good stability, small particle size, narrow size distribution and good loading capacity (Montenegro et al., 2011, 2012). Such SLN were loaded with idebenone (IDE, Fig. 1), a synthetic derivative of ubiquinone with a shorter carbon side chain and a subsequent increased solubility (Wieland et al., 1995). IDE anti-oxidant activity is due to its structural analogy with coenzyme Q_{10}, a natural

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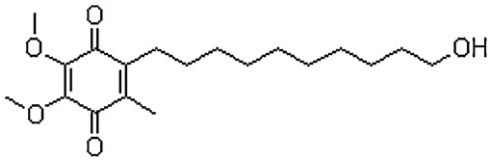

Fig. 1. Chemical structure of IDE.

antioxidant of cell membranes involved in the mitochondrial electronic transport chain (Crane, 2001; Dallner and Sindelar, 2000). IDE potent antioxidant activity has been mainly attributed to its ability to inhibit lipid peroxidation (LPO), and to protect cell and mitochondrial membranes from oxidative damage (Imada et al., 1989). IDE antioxidant activity has been proposed to be beneficial in preventing skin aging and to protect the skin from oxidative damages due to its exposure to environmental oxidative agents (Hoppe et al., 1999), other than in the treatment of neurodegenerative diseases (Schols et al., 2004).

In recent years, nanostructured lipid carriers have been reported to be effective in increasing skin permeation of Coenzyme Q_{10} (Junyaprasert et al., 2009) and of idebenone (Li and Ge, 2012), thus suggesting that nanoparticles containing these antioxidants could have significant potential use as topical formulations for reducing skin oxidative damages.

Therefore, in this work we assessed the feasibility of targeting IDE into the upper layers of the skin (stratum corneum and epidermis) by topical application of IDE-loaded SLN prepared by the PIT method. With this aim, *in vitro* permeation and penetration studies were performed on newborn pig skin using SLN loaded with different amounts of IDE, consisting of cetyl palmitate as lipid core and various non-ionic surfactants. IDE loaded SLN investigated in this paper had a composition similar to that of IDE loaded SLN described for drug delivery to the brain (Montenegro et al., 2011, 2012). Cetyl palmitate was chosen as solid lipid because of its good tolerability both after topical and systemic administration (Wang et al., 2009; Lukowski et al., 2000). After *in vitro* application on the skin surface of IDE-loaded SLN, IDE penetration into the different skin layers together with its permeation through the skin were evaluated.

2. Materials and methods

2.1. Materials

Polyoxyethylene-20-cetyl ether (Brij 58®, Ceteth-20) was supplied by Fluka (Milan, Italy). Polyoxyethylene-20-isohexadecyl ether (Arlasolve 200 L®, Isoceteth-20) was a kind gift of Bregaglio (Milan, Italy). Polyoxyethylene-20-oleyl ether (Brij 98®, Oleth-20, was bought from Sigma–Aldrich (Milan, Italy). Glyceryl oleate (Tegin O®, GO) was obtained from Th. Goldschmidt Ag (Milan, Italy). Cetyl Palmitate (Cutina CP®, CP) was purchased from Cognis S.p.a. Care Chemicals (Como, Italy). Idebenone (IDE) was a kind gift of Wyeth Lederle (Catania, Italy). Methylchloroisothiazolinone and methylisothiazolinone (Kathon CG®), and imidazolidinyl urea were kindly supplied by Sinerga (Milan, Italy). Poloxamer 188 (Lutrol® F68) was a gift of BASF (Ludwigshafen, Germany). Regenerated cellulose membranes (Spectra/Por CE; Mol. Wt. Cut off 3000) were supplied by Spectrum (Los Angeles, CA, USA). Methanol and water used in the HPLC procedures were of LC grade and were bought from Merck (Darmstadt, Germany). All other reagents were of analytical grade and used as supplied.

2.2. Preparation of SLN

IDE-loaded SLN, whose composition is reported in Table 1, were prepared using the phase inversion temperature (PIT) method, as previously reported (Montenegro et al., 2011). Briefly, the aqueous phase and the oil phase (cetyl palmitate, the selected emulsifiers and different percentages w/w of IDE) were separately heated at ~90 °C; then the aqueous phase was added drop by drop, at constant temperature and under agitation, to the oil phase. The mixture was then cooled to room temperature under slow and continuous stirring. At the phase inversion temperature (PIT), the turbid mixture turned into clear. PIT values were determined using a conductivity meter mod. 525 (Crison, Modena, Italy) which measured an electric conductivity change when the phase inversion from a W/O to an O/W system occurred. Water contained 0.35% (w/w) imidazolidinyl urea and 0.05% (w/w) methylchloroisothiazolinone and methylisothiazolinone as preservatives. A TLC analysis confirmed that no degradation of IDE occurred under these conditions.

Table 1
Composition (%, w/w) of IDE-loaded SLN.

SLN	Ceteth	Isoceteth	Oleth	GO	CP	IDE	Water[a]
C1	8.7	–	–	4.4	7.0	0.5	q b 100
C2	8.7	–	–	4.4	7.0	0.7	q b 100
C3	8.7	–	–	4.4	7.0	1.1	q b 100
I1	–	10.6	–	3.5	7.0	0.5	q b 100
I2	–	10.6	–	3.5	7.0	0.7	q b 100
O1	–	–	7.5	3.7	7.0	0.5	q b 100
O2	–	–	7.5	3.7	7.0	0.7	q b 100
O3	–	–	7.5	3.7	7.0	1.1	q b 100

[a] Water containing 0.35% (w/w) imidazolidinyl urea and 0.05% (w/w) Kathon CG.

2.3. Transmission electron microscopy (TEM)

For negative-staining electron microscopy, 5 μl of SLN dispersions were placed on a 200-mesh formvar copper grid (TAAB Laboratories Equipment, Berks, UK), and allowed to be adsorbed. Then the surplus was removed by filter paper. A drop of 2% (w/v) aqueous solution of uranyl acetate was added over 2 min. After the removal of the surplus, the sample was dried at room condition before imaging the SLN with a transmission electron microscope (model JEM 2010, Jeol, Peabody, MA, USA) operating at an acceleration voltage of 200 kV.

2.4. Photon correlation spectroscopy (PCS)

SLN particle sizes were determined at room temperature using a Zetamaster S (Malvern Instruments, Malvern, UK), by scattering light at 90°. The instrument performed particle sizing by means of a 4 mW laser diode operating at 670 nm. The values of the mean diameter and polydispersity index were the averages of results obtained for three replicates of two separate preparations.

2.5. Differential scanning calorimetry (DSC) analyses

DSC analyses were performed using a Mettler TA STAR^e System equipped with a DSC 822^e cell and a Mettler STAR^e V8.10 software. The reference pan was filled with 100 μl of water containing 0.35% (w/w) imidazolidinyl urea and 0.05% (w/w) methylchloroisothiazolinone and methylisothiazolinone. Indium and palmitic acid (purity ≥99.95% and ≥99.5%, respectively; Fluka, Switzerland) were used to calibrate the calorimetric system in transition temperature and enthalpy changes, following the procedure of the Mettler STAR^e software. 100 μl of each SLN sample (unloaded SLN prepared using the same procedures but without the addition of IDE) was transferred into a 160 μl calorimetric pan, hermetically sealed and submitted to DSC analysis as follows: (i) a heating scan from 5 to 65 °C, at the rate of 2 °C/min; (ii) a cooling scan from 65 to 5 °C, at the rate of 4 °C/min, for at least three times. Each experiment was carried out in triplicate.