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Enhancement of in vivo human skin penetration of resveratrol by chitosan-coated lipid microparticles

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ABSTRACT

In this study, lipid microparticles (LMs) uncoated or coated with chitosan, and containing the antioxidant polyphenol, resveratrol were developed in order to enhance its in vivo skin permeation. The LMs loaded with resveratrol were prepared by melt emulsification and sonication, using tristearin as lipidic material and hydrogenated phosphatidylcholine as the surfactant. Two different methods were examined for the coating of the LMs: chitosan addition during LM preparation or treatment of already formed LMs with a chitosan solution. The latter method achieved a better modulation of the in vitro release of resveratrol and hence was used for subsequent studies.

The resveratrol loading and mean diameter of the LMs were 4.1 ± 0.3% (w/w) and 5.7 μm and 3.8 ± 0.2 % (w/w) and 6.1 μm for the uncoated and the chitosan-coated LMs, respectively. Chitosan coating changed the LM surface charge, from a negative zeta potential value (−17.8 ± 4.8 mV) for the uncoated particles, to a higher positive values (+64.2 ± 4.4 mV) for the chitosan-coated ones.

Creams containing resveratrol free, encapsulated in the uncoated or chitosan-coated LMs were applied to the forearm of human volunteers and the penetration of the polyphenol in the stratum corneum was investigated in vivo by the tape stripping technique. Uncoated LMs did not produce any significant increase in the fraction of the applied resveratrol dose diffused in the stratum corneum (32.8 ± 8.9 %) compared to the control cream containing the non-encapsulated polyphenol (26.2 ± 5.6 % of the applied dose). On the other hand, application of the cream containing the chitosan-coated LMs produced a significant enhancement in the in vivo permeation of resveratrol to 49.3 ± 5.9% of the applied dose, the effect being more marked in the upper region of the horny layer. The observed improvement in the human stratum corneum penetration of resveratrol achieved by the LMs coated with chitosan should favour the efficiency of its topical application.

1. Introduction

Resveratrol (3,5,4′-trihydroxystilbene), a naturally occurring polyphenolic compound produced by a variety of plants [1,2], is a potent antioxidant which has been shown to exhibit anti-inflammatory, anti-carcinogenic and anti-microbial properties, together with cardioprotective and neuroprotective activities [1,3,4]. In addition to these array of beneficial pharmacological effects, the topical application of resveratrol has been reported to exert a therapeutic action against several cutaneous pathologies such as oxidative damage (e.g., oxidative DNA damage, lipid peroxidation) mediated by excessive formation of free radicals and other reactive species (non-radical oxygen species), inflammation, microbial infections, skin cancer and damage induced by the solar UV radiation [5–10]. In particular, in vitro and in vivo investigations on animal skin have demonstrated that resveratrol reduced tumorogenesis, inhibited inflammatory leukocyte infiltration as well as the increase in collagen degradation and lipid peroxidation triggered by exposure to the sun UV rays [5,7,9]. Moreover, resveratrol has been shown to possess antiproliferative properties against skin carcinogenesis on human epidermal cell lines [9].

For the treatment of skin disorders, the topical administration of resveratrol provides its direct delivery to the area of interest, circumventing the problem of poor bioavailability due to its rapid metabolism and limited aqueous solubility [9,11,12]. However, the activity of this polyphenol following skin application is hampered by its inefficient percutaneous penetration and chemical instability [4,11,13–15]. In order to circumvent the above limitations, incorporation of resveratrol in novel carrier systems including liposomes

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[4,10,13,16] and lipid nanoparticles [1,15,17] has been reported. The effect of these systems on the skin absorption of resveratrol has been evaluated in vitro on excised animal skin mounted in Franz diffusion cells [1,4,10,13,15,16], these conditions being not representative of the actual application of dermatological products in man. Hence, although the in vitro testing represents a useful model for the prediction of percutaneous penetration, for realistic and conclusive information, in vivo studies in humans are required [18,19].

Aim of this work was to evaluate whether the incorporation of resveratrol in lipid-based microparticles, prepared in the absence or presence of chitosan, could enhance the in vivo skin permeation of this polyphenol. Lipid microparticles (LMs), which consist of a solid hydrophobic core stabilized by a layer of surfactant molecules, represent an appropriate carrier for topical administration since their constituents are physiologically compatible and biodegradable. Compared to liposomes and lipid nanoparticles, LMs due to their micron dimensions have the advantage of higher stability and reduced amount of potentially toxic surfactants required for their preparation [20–22]. Moreover, LMs exhibit high loading capacity for lipophilic substances, such as resveratrol, and their solid matrix protects the encapsulated compound against degradation [20,21]. In order to improve the performance of the LMs as topical delivery system for resveratrol, their surface characteristics were modified by coating with chitosan, a biocompatible and non-toxic cationic polysaccharide of natural origin which possess absorption enhancing properties and has been shown to increase skin permeation [23–25].

LMs without or with chitosan coating and loaded with resveratrol were prepared, characterized, introduced in a model cream formulation and their effect on the polyphenol skin penetration was assessed in vivo on human volunteers using the non-invasive tape stripping technique.

2. Materials and methods

2.1. Materials

Resveratrol was supplied by Fagron Italia (Bologna, Italy). Glyceryl behenate was a gift from Gattefosse' (Cedex, France). Stearic acid and Tween 20 were purchased from Fluka Chemie (Bucks, Switzerland). Hydrogenated phosphatidylcholine was received as a free sample from Cargill (Hamburg, Germany). Tristearin, low- and medium- molecular weight chitosan, high-performance liquid chromatographic (HPLC)-grade methanol and water were from Sigma–Aldrich (Steinheim, Germany). The excipients for the cream preparations were obtained from Croda (Snaith, UK), Seppic (Paris, France) and Fagron Italia (Bologna, Italy). Transparent adhesive tapes (Scotch Crystal 600, 19 mm width) were purchased from 3 M (Cergy-Pontoise, France). All other reagents and solvents were of analytical grade from Sigma.

2.2. High-performance liquid chromatography

The HPLC system comprised a Model LabFlow 3000 pump (LabService Analytica, Bologna, Italy), a Model 7125 injection valve with a 20 μl sample loop (Rheodyne, Cotati, CA, USA) and a Model 975-UV variable wavelength UV–vis detector (Jasco, Tokyo, Japan) set at 306 nm. Data acquisition and processing were performed with a personal computer using Borwin software (JBMS Developpements, Le Fontanil, France). Sample injections were performed with Model 80365 syringe (10 μl; Hamilton, Bonaduz, Switzerland). Separations were achieved on a 5-μm Zorbax SB-C18 column (150 mm × 4.6 mm i.d.; Agilent Technologies, Waldbronn, Germany) fitted with a guard column (5-μm particles, 4 mm × 2 mm i.d.; Phenomenex, Torrance, CA, USA) and eluted isocratically, at a flow-rate of 0.8 ml/min, with methanol–water (65:35, v/v) containing 0.4% (v/v) acetic acid. Chromatography was performed at ambient temperature. The identity of resveratrol peak was assigned by co-chromatography with the authentic standard. Quantification was carried out by integration of the peak areas using the external standardization method. Calibration curves were linear over the range 0.2–40.0 μg/ml, with correlation coefficients greater than 0.998. The precision of the method, evaluated by repeated analyses ($n = 6$) of the same sample solution containing resveratrol at levels of 0.2 and 40.0 μg/ml, was demonstrated by relative standard deviation values lower than 5.0%.

2.3. Microparticle preparation

LMs were prepared by adding pre-heated (75–85 °C) deionized water (40 ml) containing the surfactant (0.7–1.4 %, w/v) to the molten lipid phase (3.8 g), in which resveratrol (0.25–0.30 g) was dispersed. The hot aqueous phase was poured into the molten lipid (phase-inversion process) to avoid loss of excipient and drug during the preparation process. The mixture was then subjected to high-shear mixing (17500 rpm for 1–2 min) using an Ultra-Turrax T25 mixer (IKA-Werk, Staufen, Germany) at 75–85 °C. For the optimized preparations, the samples were also sonicated at 20 kHz for 5 min (power output, 10 W), using an ultrasonic probe (Model VCX130; Sonix, Newtown, CT, USA). The resulting oil-in-water emulsion was rapidly cooled at room temperature under magnetic stirring and the formed suspension was subjected to centrifugation (6000 rpm for 15 min) and lyophilization to obtain water-free microparticles.

For the preparation of the chitosan–coated LMs a 1% (w/v) chitosan solution (40 ml) in aqueous acetic acid (pH 4.2) including the surfactant was used as the water phase of the emulsion formulation. Alternatively, the chitosan solution was added to the LM suspension formed during the cooling phase of the emulsion. Unloaded particles were also prepared with the same procedures, by omitting resveratrol.

2.4. Microparticle characterization

2.4.1. Particle morphology

The morphological features of the LMs were observed by optical microscopy (B-500 TPL microscope, Optika Microscopes, Bergamo, Italy) and variable-pressure (ca. 90 Pa) scanning electron microscopy (VP-SEM; Zeiss EVO40XVP, Arese, Milan, Italy).

2.4.2. Particle size analysis

Preliminary estimation of particle dimensions was performed by computerized image analysis (Micrometrics™ camera 122CU and software vision 2.02) on a minimum of 100 particles using the B-500 TPL optical microscope (Optika Microscopes, Bergamo, Italy). The particle size (expressed as mean diameter), polydispersity index (PDI) and surface charge were determined by dynamic light scattering using a Zetasizer (Model 6.12; Malvern Instruments, Worcs, UK) equipped with a 4 mW He-Ne laser (633 nm) and a DTS software (Version 5.0) The particles were dispersed in Milli-Q water (18.2 MO, Millipore) at a concentration of ca. 5 mg/ml. Analyses were performed in triplicate.

2.4.3. Resveratrol loading of LMs

The amount of resveratrol entrapped in the LMs was determined by dissolving the microparticles (10 mg) in ethanol (5 ml) under heating (80 °C for 5 min) and sonication (10 min), in sealed glass vials. The obtained sample was diluted to volume (20 ml) with methanol, filtered (0.45 μm membrane filters) and assayed by HPLC. The encapsulation efficiency was calculated as the percentage ratio between the quantity of resveratrol entrapped in the