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Microencapsulation of a cyclodextrin complex of the UV filter, butyl methoxydibenzoylmethane: In vivo skin penetration studies

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ABSTRACT

Lipid microparticles loaded with the complex between hydroxypropyl-β-cyclodextrin (HP-β-CD) and the sunscreen agent, butyl methoxydibenzoylmethane (BMDBM) were evaluated for their effect on the UV filter percutaneous penetration. The microparticles were prepared by the melt emulsification technique using tristearin as lipidic material and hydrogenate phosphatidylcholine as the surfactant. Human skin penetration was investigated in vivo by the tape stripping technique, a minimal invasive procedure based on the progressive removal of the upper cutaneous layers (stratum corneum) with adhesive tape strips. The amount of sunscreen fixed to each strip was determined by HPLC after solvent extraction. The recovery of the UV filter from spiked adhesive tapes was >94.4% and the precision of the method was better than 7.6% relative standard deviation. Non-encapsulated BMDBM, its complex with HP-β-CD, the lipid microparticles loaded with the sunscreen alone or the BMDBM/HP-β-CD complex were introduced into oil-in-water emulsions and applied to human volunteers. Compared to the cream with the non-encapsulated sunscreen agent (percentage of the applied dose penetrated, 9.7% ± 2.5), the amount of BMDBM diffusing into the stratum corneum was increased by the formulations containing the BMDBM/HP-β-CD complex (17.1% ± 3.2 of the applied dose) or the microparticles loaded with BMDBM only (15.1% ± 2.7 of the applied dose). On the contrary, a significant decrease in the level of UV filter penetrated into the stratum corneum was achieved by the cream containing the microencapsulated BMDBM/HP-β-CD complex (percentage of the applied dose penetrated, 6.0% ± 1.5). The reduced BMDBM percutaneous penetration attained by the latter system should enhance the UV filter efficacy and limit potential toxicological risks.

1. Introduction

Topical sunscreen products are widely used for protecting human skin against the harmful effects of exposure to the solar UV radiation, including sunburn, cutaneous photoageing, immunosuppression and skin cancers [1,2]. The active ingredients of these preparations, referred to as sunscreen agents or UV filters, decrease the dose of UV rays reaching the skin by absorbing, reflecting or scattering the radiation [3], the most common sunscreen agents being organic chemicals [4]. Ideally, a sunscreen product should provide effective protection against both the UVB (290–320 nm) and UVA (320–400 nm) radiation of sunlight, while exhibiting high photostability [4]. In addition, minimal percutaneous penetration of the UV filters is essential, since they exert their effect on the skin surface [3,5].

Transdermal absorption is strongly affected by the physicochemical properties of the substance, such as molecular weight and octanol/water partition coefficient ($\log P_{ow}$), as well as by the nature and properties of the formulation vehicle in which it is applied. The relatively low molecular weight, together with the lipophilic characteristics of most organic sunscreen agents promotes their partition/dissolution into the surface of the stratum corneum and the diffusion through its lamellar lipid domains. In order to prevent these phenomena and the possible subsequent risk of toxic effects and failure in sun protection, sun-care formulations should be designed to guarantee the localisation of UV filters at the skin surface or in the uppermost part of the stratum corneum [5]. Approaches aiming to inhibit or minimize sunscreen permeation through the skin include the use of vehicles in which UV filters are highly soluble [6], chemical modifications to increase their molecular weight [4], complexation with cyclodextrins [7] or incorporation in microparticles [8].

Butyl methoxydibenzoylmethane (BMDBM; Fig. 1) is the most widely used UVA absorber [4,9], being included in the list of authorized sunscreen agents in Europe, USA, Australia and Japan

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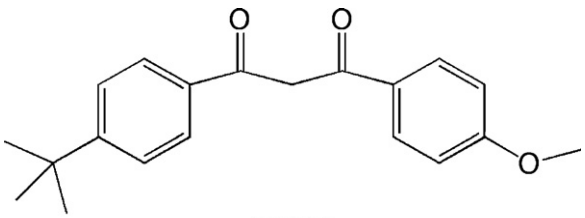

BMDBM

Fig. 1. Chemical structure of BMDBM.

[10]. However, this sunscreen agent has been shown to be photounstable [4] and to exhibit appreciable permeation into human stratum corneum and viable epidermis [5,11,12]. In earlier investigations we demonstrated that complexation of BMDBM with hydroxypropyl-β-cyclodextrin (HP-β-CD) reduced the sunscreen degradation under simulated sunlight [13,14], although no significant influence on the in vitro percutaneous penetration of the UVA filter was observed [7]. Cyclodextrins are cyclic oligosaccharides which can incorporate appropriately sized lipophilic compounds into their hydrophobic cavities, forming non-covalent inclusion complexes. They can enhance the aqueous solubility and chemical stability of the included active substance, control the release rate and increase or decrease their permeability into and through the skin [15,16].

However, the photostabilizing effect on BMDBM produced by its complexation with HP-β-CD decreased following its incorporation in an emulsion vehicle, probably due to the competitive displacement of the UV filter from the cyclodextrin cavity by the formulation excipients [17]. This is a major disadvantage, since emulsions represent the most common type of sunscreen preparations and hence simulate the actual conditions of use of sun-protective products [9]. This drawback was overcome by incorporation of the BMDBM/HP-β-CD complex in lipid microparticles, particles in the micrometer size-range consisting of a solid fat core stabilized by a layer of surfactant molecules at the surface [8,18]. In fact, after introduction in a cream preparation, the microparticle-entrapped BMDBM/HP-β-CD complex exhibited higher photostabilization efficacy than the non-encapsulated form [17], the observed effect being traced to the particle matrix limiting the formulation excipient interference on complexation.

The purpose of this study was to evaluate whether the encapsulation of the BMDBM/HP-β-CD complex into lipid microparticles could also affect the skin permeation of the sunscreen agent. The lipoparticles loaded with the complex between BMDBM and the cyclodextrin were incorporated in a model emulsion formulation, applied to human volunteers and their influence on the UV filter percutaneous penetration was assessed by the tape-stripping technique, a non-invasive in vivo procedure based on the selective removal of the stratum corneum with adhesive tapes. The amount of BMDBM fixed to each strip was determined by high-performance liquid chromatography, after solvent extraction. For comparison purposes, formulations containing the non-encaspulated BMDBM/HP-β-CD complex or lipoparticles loaded with uncomplexed BMDBM were also prepared and examined.

2. Materials and methods

2.1. Materials

Butyl methoxydibenzoylmethane was supplied by Merck (Darmstadt, Germany). Tristearin was purchased from Fluka Chemie (Buchs, Switzerland). Hydrogenated soybean phosphatidylcholine was a gift by Cargill (Hamburg, Germany). Hydroxypropyl-β-cyclodextrin (average molar substitution 0.6) was purchased from Sigma–Aldrich (Milan, Italy). Caprylic/capric triglyceride (Miglyol 812) was obtained from Polichimica (Bologna, Italy). Adhesive tapes (Scotch Crystal 600, 19 mm width) for the in vivo tape stripping were purchased from 3M France (Cergy-Pontoise Cedex, France). Methanol, acetonitrile and water of HPLC grade were from Merck. All other chemicals were of analytical grade (Sigma, St. Louis, MO, USA).

2.2. High-performance liquid chromatography

The high-performance liquid chromatographic (HPLC) apparatus consisted of a Model LabFlow 3000 pump (LabService Analytica, Bologna, Italy), a Model 7125 injection valve with a 10 μl sample loop (Rheodyne, Cotati, CA, USA) and a Model 975-UV variable wavelength UV–vis detector (Jasco, Tokyo, Japan) set at 350 nm. Data acquisition and processing were accomplished with a personal computer using Borwin software (JBMS Developments, Le Fontanil, France). Sample injections were effected with a Model 701 syringe (10 μl; Hamilton, Bonaduz, Switzerland). Separations were performed according to the method of Scalia et al. [19], with minor modifications. A 5 μm Zorbax SB-CN column (150 mm × 3.0 mm i.d.; Agilent Technologies, Waldbronn, Germany), eluted isocratically at a flow-rate of 0.4 ml/min with methanol–acetonitrile–water (55:25:20, v/v/v), was used at ambient temperature. The identity of the BMDBM 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.

2.3. Preparation and characterization of the BMDBM/HP-β-CD complex

The complex was prepared at a 1:2 molar ratio of BMDBM to HP-β-CD by the co-evaporation method, as described previously [17]. The complex was characterized by powder X-ray diffraction analysis (D5000 powder diffractometer; Siemens, Munich, Germany). The BMDBM content in the complex was determined by HPLC after proper dilution.

2.4. Microparticle preparation and characterization

Lipid microparticles were prepared according to Scalia et al. [17], by emulsifying at 70 °C melted tristearin (3.6 g) containing BMDBM (1.2 g) or the BMDBM/HP-β-CD complex (2.4 g), respectively, with 2% (w/v) hydrogenated soybean phosphatidylcholine in phosphate buffer (50 ml; 0.1 M, pH 7.4). The sample was mixed (13,500 rpm for 3 min) with an Ultra-Turrax T25 (IKA-Werk, Staufen, Germany) and then rapidly cooled at room temperature under magnetic stirring. The formed particles were recovered by centrifugation (6000 rpm for 15 min), freeze-dried and characterized by scanning electron microscopy (SEM, Cambridge Stereoscan 360, Cambridge Instruments, Bar Hill, UK) and optical microscopy (Nikon Diaphot inverted microscope, Tokyo, Japan) coupled with a computerized image analysis system (Micrometrics™ camera 122CU and software vision 1.0). In vitro release studies were performed by adding samples containing equivalent amounts of BMDBM (5 mg) to propylene glycol (100 ml) under mechanical stirring (50 rpm) at 37° C. At appropriate time intervals, 1-ml aliquots of the release medium were withdrawn and replaced with an equal volume of fresh medium. The samples were filtered (0.45 μm) and assayed for BMDBM by UV spectrophotometry at 330 nm (Lambda 3B, Perkin-Elmer, Norwalk, USA). The amount of BMDBM entrapped in the lipid microparticles was determined by HPLC after dissolution of the particles (30–40 mg) in ethanol (10 ml) under sonication (15 min).