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Enhanced antioxidation *via* encapsulation of isooctyl p-methoxycinnamate with sodium deoxycholate-mediated liposome endocytosis

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ABSTRACT

Isooctyl *p*-methoxycinnamate(OMC) is a commonly used chemical ultraviolet B sunscreen that suffers rapid degradation with current delivery systems following sun exposure. In this study, deoxycholate-mediated liposome (DOC-LS) endocytosis was employed to improve the antioxidation effects of OMC following topical administration, and the *in vitro* cell uptake was investigated to understand the enhanced cutaneous absorption of the drug *via* this nanocarrier. Following topical application, structural changes in the stratum corneum were identified. With the increase of DOC content, the drug deposition in skin decreased; from this, a DOC-LS formulation was selected that showed significantly more drug delivery in skin than did the other preparations ($P<0.05$). DOC-LS decreased skin resistance, suggesting its ability to induce skin barrier disruption. *In vitro* HaCaT keratinocyte cell uptake of coumarin-6 incorporated in the two types of phosphatidylcholine (PC) vesicles (*i.e.*, LS or DOC-LS) yielded similar fluorescence intensities following incubation for different periods ($P<0.05$). However, CCC-ESF-1 embryonic fibroblast cell uptake of the fluorescence revealed time-dependence, and the emitted light from DOC-LS incubated cells was stronger than that from cells incubated with LS ($P<0.05$). These findings might be associated with the endocytic pathway of HaCaT, which mainly exhibited adsorption or physical adhesion of the fluorescent vesicles, whereas CCC-ESF-1 markedly internalized the PC vesicles *via* the lysosomes, as shown by intracellular fluorescence co-location studies. Following loading with the same amount of OMC, the DOC-LS vesicles exhibited superior skin tissue antioxidative capacity among the preparations tested, corroborating the *in vivo* skin drug deposition results. Thus, our results suggest that DOC-LS is a promising system for OMC dermal delivery without promoting skin irritation, which is quite advantageous for therapeutic purposes.

1. Introduction

Liposomes (LS) have been used for topical preparations for decades (Allen and Cullis, 2013); these offer a wide range of advantages including prominent biocompatibility, biodegradability, effective extended drug delivery into skin, and increased moisturization (Rahimpour and Hamishehkar, 2012). The lipid components that constitute LS, *i.e.*, phosphatidylcholine (PC) and cholesterol, are similar to the lipid compositions of the stratum corneum (SC). Following topical application, the PC vesicles can fuse with the lipids in the SC, transforming the structure of the SC lipid membrane; in addition, components of the SC can be exchanged with those of the LS (Shim et al., 2010). Eventually, the well-assembled structure of the SC lipid lamellar region is disturbed, which is conducive to transporting the payload of drug molecules into the skin. However, the rigid LS conventionally utilized aggregate primarily on the superficial layer of the skin, and have difficultly permeating into deep skin. LS with poor deformability can barely pass through the normal small pores of the SC, which have smaller dimension than the PC vesicles themselves. Furthermore, LS with particle sizes over 1.7-fold that of the physiological pores are completely unable to enter into skin with intact form; instead, they are detained on the skin surface where they dehydrate and are unable to fuse together (Lasch et al., 1992).

Abbreviations: BS, bile salt; DOC, deoxycholate; DMEM, Dulbecco's modified Eagle's medium; DOC-LS, deoxycholate-mediated liposomes; EDTA, ethylene diamine tetraacetic acid; EE, entrapment efficiency; ELISA, enzyme linked immune sorbent assay; FBS, fetal bovine serum; HPLC, high-performance liquid chromatography; LS, liposomes; MDA, methane dicarboxylic aldehyde; MTT, methyl thiazolyl tetrazolium; OD, optical density; OMC, isooctyl *p*-methoxycinnamate; PBS, phosphate-buffered saline; PC, phosphatidylcholine; SC, stratum corneum; SOD, superoxide dismutase; UVB, ultraviolet B.

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Therefore, standard LS carrying drug cargo exhibit a confined permeability that is only able to achieve drug delivery in the SC.

To increase the fluidity of the liposomal lipid bilayer, specific substances entitled edge activators have been added during the process of PC vesicle formation, resulting in upgraded LS with preferable deformability that were defined as elastic or deformable LS, and also named transfersomes™ by their earliest inventor, (Cevc et al., 1995). Bile salt (BS) is widely applied as an edge activator for establishing elastic LS, as it can effectively soften the PC membrane to achieve prominent deformability (Cui et al., 2015). The BS embedded in the lipid bilayer structure provides elasticity to promote the ability of PC vesicles to squeeze into minute cracks on the SC with aperture sizes many times lower than the size of the applied nanocarriers (Paolino et al., 2012). The skin permeability of BS-containing LS has been reported to result primarily from the evaporation of water in the application vehicle that can lead to an osmotic gradient between the surface and inner components of the skin, thus promoting the permeation of nanovesicles into the skin (Zheng et al., 2012). The BS-mediated deformable liposomes are therefore applied non-occluded to the skin, as occlusion leads to an absence of osmotic pressure difference and is thus adversive toward permeation of the elastic nanovesicles into skin (Geusens et al., 2011). This type of PC vesicle has been investigated as a drug vehicle for a range of small molecules, peptides, proteins, and vaccines, both *in vitro* and *in vivo*, for delivery into and *via* the skin (Benson, 2010; El Maghraby et al., 2008). For example, a sustained and controlled drug release was obtained following topical administration using ultradeformable vesicles for the dermal delivery of tretinoin (Ascenso et al., 2014), lycopene (Ascenso et al., 2013), meloxicam (Duangjit et al., 2013), and diclofenac (Cevc and Blume, 2001), as well as for cutaneous gene delivery for the treatment of a variety of skin disorders (Geusens et al., 2011), and for non-invasive transtympanic ototopical delivery of ciprofloxacin (Al-Mahallawi et al., 2014).

In this study, we present the development of LS containing deoxycholate (DOC) as an edge activator (DOC-LS), to attain the enhanced dermal delivery of isooctyl *p*-methoxycinnamate (OMC, Fig. 1). OMC is most widely used as a chemical sunscreen for absorbing ultraviolet B (UVB) radiation (Kirkland et al., 2005). OMC possesses photochemical activities, and shows good actinic stability in a purified state. However, its sunlight resistance, *i.e.*, actinic stability, is compromised by exposure to ultraviolet radiation when it is dispersed in water-containing systems such as conventional emulsions or short-chain alcohol solutions (Monteiro et al., 2012). In general, over half of the OMC molecules will suffer light degradation after sunlight irradiation representing 10 minimal erythema dosages (Pattanaargson and Limphong, 2001). Therefore, a high concentration of OMC needs to be applied in the conventional formulation to maintain an effective dose (Reeve et al., 2001; Schauder and Ippen, 1997). In addition, the photodegradation products might generate a stimulative effect on the skin (Xu et al., 2001). However, by incorporation of lipophilic OMC into the PC bilayers, the drug stability can be heightened efficaciously. In particular, an ultradeformable LS-DOC can penetrate further into the skin and deposit drugs into the deep skin layers; this might reduce the photodegradation of OMC compared with conventional topical preparations, which cannot achieve drug delivery into the dermis but allows accumulation on the superficial skin and direct exposure to sunlight (Wolf et al., 1995).

In the present work, we evaluated the *in vivo* skin deposition of OMC delivered by DOC-LS using standard LS and a tincture as the comparative preparations for topical administration. In addition, the *in vivo* skin tissue antioxidant efficacies as well as the HaCaT and CCC-ESF-1 cell uptakes of OMC-loaded nanocarriers were investigated, in order to optimize and determine the value of drug dermal delivery *via* DOC-LS.

2. Materials and methods

2.1. Materials

Lipoid S 100 containing 95.8% PC was kindly provided by Lipoid GmbH (Ludwigshafen, Germany). OMC (purity ≥98%) was supplied by Hubei Yuan-Cheng Pharmaceutical Co., Ltd. (Wuhan, China). LysoTracker Red and Hoechst 33342 were purchased from Santa Cruz Biotechnology, Inc. (Dallas, TX, USA). Methyl thiazolyl tetrazolium (MTT), high-glucose Dulbecco's modified Eagle's medium (DMEM/High, HyClone Corporation, Logan City, UT, USA), 0.25% trypsin-0.02% ethylene diamine tetraacetic acid (EDTA) (Gibco, Thermo Fisher Scientific Inc., Grand Island, NY, USA), penicillin-streptomycin solution (Gibco), fetal bovine serum (FBS, BioInd, Biological Industries, Kibbutz Beit Haemek, Israel), phosphate-buffered saline (PBS, HyClone), and enzyme linked immune sorbent assay (ELISA) kits for superoxide dismutase (SOD) and methane dicarboxylic aldehyde (MDA) were obtained from Shanghai Usen Biotechnology (Shanghai, China). Sodium DOC and others chemicals were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China) and were of high-performance liquid chromatography (HPLC) or analytical grade.

2.2. Animals and cell lines

Male Sprague–Dawley rats that weighed 180–220 g, nude mice weighing 20–30 g, and KM mice weighing 20–22 g were used for this study. The animal study was approved by the Animal Ethical Committee of The Shanghai University of Traditional Chinese Medicine and conducted in accordance with the committee's guidelines. Animals were kept in a standard environment with free access to rodent chow and water, and were acclimatized for at least one week before the start of the study.

The human keratinocyte HaCaT cell line was purchased from the American Type Culture Collection (Manassas, VA, USA) and the human embryonic skin fibroblast CCC-ESF-1cell line was obtained from the Cell Culture Center of the Chinese Academy of Medical Sciences (Beijing, China).

2.3. HPLC analysis

OMC concentrations were analyzed using a LC-2010A HT Liquid Chromatograph system (Shimadzu Corporation, Kyoto, Japan) using a Diamonsil C18 reversed-phase column (5 μm, 4.6 mm inner diameter × 25 cm; Dikma Technologies, Inc., Beijing, China). The mobile phase was methanol:water (75:25, v/v) with a flow rate of 1 mL/min. The column temperature was kept constant at 30 °C and the detection wavelength used was 309 nm. The intra-day and inter-day relative standard deviation values for OMC were 2.30% and 1.88%, respectively. The samples were filtered through a disposable nylon 0.45 μm pore size syringe filter (diameter: 13 mm, Shanghai Anpel Scientific Instrument Inc., Shanghai, China) before automatic injection into the HPLC apparatus.

2.4. Preparation of OMC-loaded DOC-LS and standard LS

The film dispersion method was used to prepare the different DOC-LS formulations. As shown in Table 1, OMC, Lipoid S 100, and DOC for DOC-LS or cholesterol for LS were dissolved respectively in an organic mixture of chloroform and methanol (1:1,v/v). The solution was then placed in a pear-shaped, single-neck flask and incubated in a water bath maintained at 37 °C. To remove the organic solvent, a rotary evaporator (Shanghai Jingtian Electronic