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Research paper

Effect of nanostructured lipid vehicles on percutaneous absorption of curcumin

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ABSTRACT

The present study describes the production and characterization of monoolein aqueous dispersions (MAD) and lecithin organogels (ORG) as percutaneous delivery systems for curcumin (CUR).

In particular, MAD stabilized by sodium cholate/poloxamer and w_0 3 ORG lipid carriers, both in the presence and absence of CUR, have been considered: MAD morphology and dimensional distribution have been investigated by Cryogenic Transmission Electron Microscopy (cryo-TEM) and Photon Correlation Spectroscopy (PCS), while the inner structure of MAD and ORG has been studied by X-ray scattering techniques. As a general result, CUR chemical stability has been found to be better controlled by MAD, probably because CUR is more protected in the case of CUR-MAD with respect to CUR-ORG.

To investigate the performance of differently composed lipid formulations as CUR delivery system, *in vitro* studies, based on Franz cell and stratum corneum–epidermis (SCE) membranes, and *in vivo* studies, based on skin reflectance spectrophotometry and tape stripping, were then performed. The results indicated that ORG induces a rapid and intense initial penetration of CUR probably due to a strong interaction between the peculiar supramolecular aggregation structure of phospholipids in the vehicle and the lipids present in the stratum corneum. Conversely, CUR incorporated into MAD can be released in a controlled fashion possibly because of the formation of a CUR depot in the stratum corneum. In this respect ORG could be employed in pathologies requiring rapid CUR action, while MAD could be proposed for assuring a prolonged CUR activity.

1. Introduction

A wide variety of disorders and diseases affect the skin, e.g. acne, warts, multiple inflammatory dermatoses, skin cancers, autoimmune diseases, occupational dermatoses and contact dermatitis, requiring different investigations and therapies. Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer worldwide [1]. Treatment protocols include disfiguring surgery, platinum-based chemotherapy and radiation, all of which may result in tremendous patient morbidity [2–4]. As a result, there is significant interest in developing adjuvant chemotherapies to augment currently available treatment protocols, which may allow decreased side effects and toxicity without compromising therapeutic efficacy. Curcumin (CUR) is one such potential candidate, in fact it has been recently demonstrated that CUR has a beneficial role in skin diseases, in particular it can be employed as a single agent in the treatment of HNSCC and used as an adjuvant agent in combination with standard platinum-based chemotherapy [5,6].

Despite the efficacy of CUR, its scarce water solubility may limit its administration. On the matter a number of research work has been done in order to develop delivery strategies for CUR, e.g. liposomes, solid lipid nanoparticles, and cyclodextrins have been investigated [7].

Abbreviations: MAD, monoolein aqueous dispersions; ORG, organogel; CUR, curcumin; x-gum, xanthan gum.

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Recently we have proposed the solubilization of CUR in monoolein aqueous dispersions (MAD) stabilized by different emulsifiers, resulting in the formation of complex lyotropic liquid crystalline phases, depending on the emulsifier used [8].

In order to treat cutaneous pathologies such as HNSCC, a semisolid formulation constituted of biocompatible materials able to assure targeted delivery of actives, minimizing at the same time toxic systemic effects appears the best strategy. A non-aqueous microemulsion system based on lecithin could be a good solution at this aim [9,10].

It is well known that lecithin, thanks to its chemico-physical properties, is one of the most promising and useful agents able to increase the skin permeation. In fact lecithin is a non-toxic, naturally occurring biocompatible surfactant able to form poly-molecular structures such as direct and inverted micelles or hexagonal, cubic and lamellar phases, offering the chance to produce innovative topical forms [10–13].

When a precise amount of water is added to an oil lecithin solution, initially the lecithin molecules form spherical reversed micelles, then the micellar aggregates entangle, forming a three-dimensional network in the bulk phase [14,15]. This peculiar w/o microemulsion is called lecithin organogel (ORG) since it consists of a gel-like reverse micellar system in which the external phase is an organic solvent [14]. By adjusting the amount of added water it is possible to modulate the system viscosity [11,14,16].

ORG are particularly suitable as cutaneous delivery systems for many reasons. Indeed, they are thermodynamically stable, they are able to solubilize hydrophilic and lipophilic molecules and eventually they can be produced with biocompatible solvents [17–21].

In the present study, we investigate the performances of different lipid based formulations for CUR cutaneous administration (i.e. MAD and ORG).

In particular, the first part of the present study describes the production and characterization of liquid MAD stabilized by poloxamer in mixture with sodium cholate and ORG. The MAD viscosity was then adjusted by the use of xanthan gum (x-gum).

The second part concerns an *in vitro* and *in vivo* investigation aimed to compare the release modalities of CUR after the administration of the semisolid lipid formulations on the skin.

CUR applied on the skin was studied determining its *in vivo* topical anti-inflammatory activity after cutaneous application of MAD and ORG. The ultraviolet B (UVB)-induced erythema was chosen as inflammatory model on healthy human volunteers and was monitored by reflectance visible spectrophotometry. Moreover, tape-stripping experiments have been performed on skin after topical administration of MAD and ORG to quantify CUR presence in the stratum corneum.

2. Experimental methods

2.1. Materials

Glyceryl monooleate RYLO MG 19 (monoolein) was a gift from Danisco Cultor (Grindsted, Denmark). Pluronic F127 (Poloxamer 407, poloxamer) (PEO_{98}–POP_{67}–PEO_{98}) was obtained from BASF (Ludwigshafen, Germany). Curcumin (CUR), (1E,6E)-1,7-bis(4-Hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, sodium cholate (Na cholate) (3α,7α,12α-Trihydroxy-5β-cholan-24-oic acid sodium salt), Xanthan gum (x-gum) and isopropylpalmitate (IPP) were purchased from Sigma Chemical Company (St. Louis, MO, USA). The soybean lecithin (90% phosphatidyl choline) used for ORG preparation was Epikuron 200 from Lucas Meyer, Hamburg, Germany. Solvents were of HPLC grade and all other chemicals were of analytical grade.

2.2. MAD preparation

Production of dispersions was based on the emulsification of monoolein and emulsifier in water, as previously described [22]. MAD composition was monolein 4.5% w/w, Na cholate 0.15% w/w, poloxamer 0.5% w/w and water.

After emulsification, the dispersions were subjected to homogenization (15,000 rev min^{-1}, Ultra Turrax, Janke & Kunkel, IKA-Werk, Sardo, Italy) at 60 °C for 1 min, then cooled and maintained at room temperature in glass vials.

To produce CUR containing MAD (CUR-MAD), 7.5 mg of CUR (0.33% w/w with respect to the monoolein, 0.015% w/w with respect to the dispersion) was added to the molten monoolein/emulsifier mixture and dissolved before addition to the aqueous solution. During production the vial was protected from light with an aluminum foil to prevent photo-degradation of CUR.

2.3. Characterization of MAD

2.3.1. Cryo-Transmission Electron Microscopy (cryo-TEM)

Samples were vitrified as described in a previous study [22].

The vitrified specimen was transferred to a Zeiss EM922Omega (Carl Zeiss Microscopy, Oberkochen, Germany) transmission electron microscope using a cryoholder (CT3500, Gatan, Munich, Germany). Sample temperature was kept below 100 K throughout the examination. Specimens were examined with reduced doses of about 1000–2000 e/nm^2 at 200 kV. Images were recorded by a CCD digital camera (Ultrascan 1000, Gatan, Munich, Germany) and analyzed using a GMS 1.8 software (Gatan, Munich, Germany).

2.3.2. Photon Correlation Spectroscopy (PCS)

Submicron particle size analysis was performed using a Zetasizer 3000 PCS (Malvern Instr., Malvern, England) equipped with a 5 mW helium neon laser with a wavelength output of 633 nm. Glassware was cleaned of dust by washing with detergent and rinsing twice with sterile water. Measurements were made at 25 °C at an angle of 90° with a run time of at least 180 s. Samples were diluted with bidistilled water in a 1:10 v:v ratio. Data were analyzed using the "CONTIN" method [23]. Measurements were performed on MAD after production and after 6 months from production.

2.3.3. CUR content of MAD

The encapsulation efficiency (EE) of CUR in the MAD was determined as described by Nayak and colleagues [24]. 100 μl aliquot of MAD was loaded in a centrifugal filter (Microcon centrifugal filter unit YM-10 membrane, NMWCO 10 kDa, Sigma Aldrich, St. Louis, MO, USA) and centrifuged (Spectrafuge™ 24D Digital Microcentrifuge, Woodbridge NJ, USA) at 8000 rpm for 20 min. The amount of free and entrapped CUR was determined by dissolving the supernatant with a known amount of ethanol (1:10, v/v). The amount of CUR in the supernatant was determined by high performance liquid chromatography (HPLC) method, as below reported. The EE was determined as follows:

$$EE = T_{CUR} - S_{CUR}/T_{CUR} \times 100 \quad (1)$$

where T_{CUR} stands for the total amount of CUR added to the formulation and S_{CUR} for the amount of drug measured in the supernatant.

2.4. ORG preparation

ORG were prepared by dissolving lecithin (200 mM) in IPP.

From these reverse micellar solutions, microemulsion gels were prepared by adding water, under magnetic stirring, to obtain the