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Qualitative and Quantitative Study of the Potential of Lipid Nanocapsules of One Hundred Twenty Nanometers for the Topical Administration of Hydrophobic Molecules

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ABSTRACT

In this study, we evaluated the potential of lipid nanocapsules (LNC) of 120 nm as drug nanocarriers to treat skin diseases. As a model molecule, we encapsulated the fluorescent dye curcumin, which also is an antioxidant. Curcumin-loaded LNC showed interesting antioxidant properties and a low toxicity on human skin cells. The penetration of curcumin in the skin was determined by 2 complementary methods: high performance liquid chromatography was used to measure total curcumin accumulation in the skin, whereas fluorescence confocal spectral imaging of skin sections showed that curcumin preferentially accumulates in the *stratum corneum* and the viable epidermis. These results confirm that LNC of a size above 100 nm can vectorize hydrophobic compounds to the keratinocytes without transdermal delivery. They also demonstrate the interest of combining 2 analytical methods when studying the skin penetration of nanovectorized molecules.

Introduction

Thanks to its structure, the skin is an efficient physiological barrier against foreign material including active cosmetic or pharmaceutical ingredients. The penetration depth of active molecules depends mainly on their physicochemical properties.[1-3] The poor solubility of hydrophobic molecules in water limits their penetration in the viable layers of the skin as they have great affinity for the upper layer of the skin, the *stratum corneum* (SC), made of keratinized dead cells held together by a lipid matrix.[4] This poor solubility in water will also condition the formulation of the final pharmaceutical or cosmetic product, which will consequently be a lipophilic form, like ointments. These forms show good stability and remanence on the skin. However, they are not comfortable for patients who often stop their treatment. Moreover, they can lead to transdermal delivery through occlusion mechanisms. That is why the recourse to hydrophilic pharmaceutical forms like hydrogels would be a real improvement in the patients' everyday life.

The encapsulation of hydrophobic molecules in nanocarriers could be a way to achieve good penetration of active hydrophobic molecules in the skin while dispersing them in hydrophilic forms as gels.[5,6] Many drug delivery nanosystems were recently developed for skin application such as polymeric or lipid-based nanosystems.[1,5] Lipid-based nanosystems are the more explored systems, with a large variety of types of objects, like nanoemulsions, lipid-based nanocapsules, solid lipid nanoparticles, or nanostructured lipid carriers. It has been reported that lipid-based nanocarriers could improve the dermal or transdermal penetration of active molecules, depending on their size, their composition, and the properties of the encapsulated compound.[3,7]

Lipid nanocapsules (LNC), first described by Heurtault et al.,[8] are lipid-based nanocapsules that could be interesting nanosystems to help to increase the quantity of hydrophobic active molecules reaching the viable layers of the epidermis.[8] They present uncontestable qualities as nanovectors for skin delivery. Indeed, they are more stable than nanoemulsions thanks to their crystallized shell but they stay deformable,[9] which theoretically permit 2 mechanisms of skin penetration: the penetration of the whole LNC if its size is sufficiently small or its adsorption to the SC, modifying the organization of this superficial layer and leading to a penetration enhancement.[1,3] They present supplementary properties that can enhance their usefulness. First, LNC

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are prepared by a solvent-free, low-energy, and easily scaled up method,[8] making their industrialization possible in the short term. This method also provides high encapsulation efficiencies of hydrophobic molecules in the oily core made of medium chain triglycerides,[10] which is a promise of concentrated pharmaceutical or cosmetic final product. This method also permits to obtain monodisperse populations of LNC of different sizes without modifying the conditions or the nature of the components, but only by changing the concentrations.[11] This can allow us to prepare LNC adapted to the specific delivery of hydrophobic active molecules in the viable layers of the skin. Finally, their stabilization with PEGylated nonionic surfactants can make them stealth and functionalizable for further drug targeting investigations.

Mainly LNC of 20-50 nm has been studied, principally as nanocarriers for the intravenous or oral route of administration.[10,12] LNC of the same range of sizes have also been studied as transdermal delivery systems.[1,13] LNC showed a better permeation-enhanced effect than polymeric nanoparticles, and their drug loading and stability are higher than those of other lipid-based nanocarriers.[14] The aim of this study is to evaluate if specially prepared LNC of 120 nm, which has never been studied before, can deliver hydrophobic molecules to the viable epidermis through an intact SC, the final aim being the treatment of superficial skin diseases. Curcumin, a natural hydrophobic molecule with antioxidant activity,[15,16] was chosen as a model of encapsulated hydrophobic molecule because of its intrinsic fluorescence, permitting its monitoring even at small concentrations. The stability of LNC of 120 nm was explored, and their toxicity was determined on human skin cell lines. The antioxidant activity of curcumin-loaded LNC (LNCc) was measured to detect any decrease in the activity of the drug due to the encapsulation process. Curcumin penetration in the skin was evaluated by 2 complementary techniques giving quantitative and qualitative information: high performance liquid chromatography was used to determine the quantity of curcumin penetrated in the skin[17,18] and fluorescence confocal spectral imaging was used to visualize curcumin distribution in the different layers of the skin.

Experimental

Chemicals

Linoleic acid, dextran, curcumin, 2,2-diphenyl-1-picrylhydrazyl (DPPH·), sodium chloride, benzoic acid, and butylhydroxytoluene were provided by Sigma-Aldrich (Saint-Quentin-Fallavier, France). Polysorbates 40 and 80 were provided by Seppic (Puteaux, France). Labrafac® WL1349 was purchased from Gattefossé (Nanterre, France). Lipoid® 75-3 was kindly provided by Lipoid (Duisburg, Germany). Solutol® HS15 was purchased from BASF (Levallois-Perret Cedex, France). Phosphoric acid, disodium hydrogen phosphate dihydrate, potassium dihydrogen phosphate, hydrochloric acid, methanol, acetonitrile, dimethylsulfoxide, and ethanol were purchased from Carlo Erba (Val de Reuil, France). Ammonium thiocyanate was purchased from Labosi (Paris, France). Ferrous chloride tetrahydrate was purchased from Acros (Geel, Belgium). Hydroxypropylcellulose (HPC; Klucel®) was provided by Brenntag (Vitrolles, France). Dulbecco's modified Eagle's medium, fetal bovine serum, Hank's balanced salt solution, penicillin-streptomycin blend, and tetrazolium dye methylthiazolyldiphenyltetrazolium bromide (MTT) were purchased from Fisher Bioblock (Illkirch-Graffenstaden, France).

LNC Preparation and Characterization

Preparation

Aqueous suspensions of LNC were prepared by the phase inversion temperature method previously described by Heurtault et al[8] with modifications.[8] Labrafac® WL1349 (12.09 g), Lipoid S75-3 (0.75 g), Solutol HS15 (4.84 g), NaCl (0.89 g), and ultrapure water (31.43 g) were mixed under magnetic stirring for 1 h, then submitted to 3 temperature cycles from 90°C to 60°C at 4°C/min. During the last cooling phase, cold ultrapure water was added to the mixture, leading to the formation of nanocapsules. Slow stirring was continued for 10 min, and then the volume was adjusted to 200 mL with ultrapure water. To obtain LNCc, curcumin was dissolved in Labrafac® WL1349 prior to the process (4.6 mg/g), resulting in 55 mg of curcumin for a suspension volume of 200 mL. These LNCc were concentrated by dialysis (MWCO = 25 kD; Biovalley, Nanterre, France) against a solution of dextran 10% wt/wt for 48 h to obtain a volume close to 50 mL.[19] As this concentration is not exactly reproducible, the actual curcumin concentration was determined as follows and the suspension was then diluted to obtain a 1 g curcumin/L suspension.[19]

Size and Zeta Potential

The hydrodynamic diameter was determined by dynamic light scattering (HPPS; Malvern Instruments, Ile-de-France, France) on a 1:100 dilution of the suspensions of LNC. The zeta potential of the particles was determined on the same sample by using Malvern NanoZ. Measurements were carried out in triplicate on at least 3 different batches.

Curcumin Content

LNCc suspensions were dissolved in ethanol (1:100) and curcumin was determined at 426 nm by ultraviolet-visible spectrometry (Genesys 10S UV-VIS; Thermo Scientific, Villebon-sur-Yvette, France). Measurements were carried out on at least 3 different batches.

Antioxidant Activity

The antioxidant activity of LNCc (0.1 g curcumin/L) was first determined according to the ferric thiocyanate method, based on the peroxidation of lipids.[20,21] The antioxidant activity was calculated after 7 days, when the absorbance of the negative control reached its maximum value. Additionally, it was measured by the radical scavenging method based on DPPH· transformation.[22,23] The reaction kinetics profile was plotted for each antioxidant concentration tested and the percentage of residual DPPH· at the steady state was determined as a function of the molar ratio of antioxidant. Curcumin and butylhydroxytoluene solutions in ethanol at 0.1 g/L served as positive controls, and ethanol as a negative control.

Fluorescence Stability Study

The fluorescence of curcumin in LNCc was followed up to 6 months after preparation at 4°C to study the stability of curcumin encapsulation. A LabRAM laser scanning confocal microspectrometer (Horiba SA, Villeneuve d'Ascq, France) was used in this experiment. In total, 500 µL samples of LNCc were scanned with the 488 nm line of an Ar^+ laser (laser power at the sample was approximately 170 µW) under a 10× microscope objective (Olympus, Tokyo, Japan), with a confocal hole of 1000 µm, for statistically averaged analysis of a large sample volume. Acquisition and data analysis were carried out with Labspec 4.18 software (Horiba SA). The position of the center of mass of the fluorescence spectra was calculated as follows:

$$\lambda_{CM}(nm) = \frac{\sum \lambda_i \cdot I(\lambda_i)}{\sum I(\lambda_i)}$$

where $I(\lambda_i)$ is the fluorescence intensity at emission wavelength λ_i.