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Monoacyl-phospatidylcholine nanostructured lipid carriers: Influence of lipid and surfactant content on in vitro skin permeation of flufenamic acid and fluconazole

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ABSTRACT

Monoacyl-phosphatidylcholine (MAPL) is a skin-friendly phospholipid emulsifier that has successfully been employed for development of microemulsions. Its potential for stabilising particulate lipid drug carriers has not been explored so far. Thus, the aim of the present study was the development of MAPL-based nanostructured lipid carriers (NLC) as dermal drug delivery system with optimised physical stability. To this end, extensive comparative studies were performed and the role of crucial formulation parameters such as total lipid or surfactant content was investigated. Both blank placebo NLC and drug-loaded systems were developed using flufenamic acid and fluconazole as model drugs of different polarity. The resulting systems were characterised using photon correlation spectroscopy, laser Doppler electrophoresis, rheological measurements and cryo transmission electron microscopy. In vitro skin diffusion studies revealed that interestingly, both a low surfactant content and a low total lipid content led to increased skin permeation of flufenamic acid in vitro. According to its different polarity, a different trend was observed for fluconazole where higher surfactant contents in combination with different total lipid contents yielded the highest skin permeation. Satisfying long-term stability of the NLC formulations with optimised physical parameters was observed for a period of 30 weeks.

1. Introduction

Solid lipid nanoparticles (SLN) as developed at the end of the last century [3,19] consist of a solid lipid phase surrounded by a surfactant monolayer and the outer water phase. They can be prepared by high pressure homogenisation or ultrasound techniques. Nanostructured lipid carriers (NLC) represent the second generation of SLN which possess a more complex matrix: by incorporation of oil in the solid lipid matrix, drug crystallisation is prevented thanks to imperfections in the lattice. Thus, higher drug loading can be achieved. The matrix of NLC decreases the risk for drug expulsion and exhibits higher physical stability. A solid amount of research deals with the development of NLC formulations for dermal application [4,10,12,17,23] and emphasises their importance as modern carrier systems [14].

The choice of surfactant is a crucial point in the development of new carrier systems such as NLC. Due to growing interest in biodegradable and renewable raw materials the focus of formulation development lies with eudermic compounds such as phospholipids and carbohydrate-based surfactants. Minimal dermal toxicity is an essential requirement in modern dermopharmaceutics [16] and phospholipid-based carrier systems offer distinct advantages in this respect. In NLC development, soybean lecithin and phosphatidylcholine have been investigated as surfactants [18,28].

However, the use of monoacyl-phosphatidylcholine (MAPL) has

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Abbreviations

SLN	solid lipid nanoparticles
NLC	nanostructured lipid carriers
MAPL	monoacyl-phosphatidylcholine
PC	diacyl phosphatidylcholine
IPM	isopropyl myristate
PDI	polydispersity index
PCS	photon correlation spectroscopy
ZP	zeta potential
TEM	transmission electron microscopy

not been explored for the development of NLC so far. Recent studies have shown that phospholipid mixtures containing MAPL are useful compounds for the development of dermal microemulsions [8]. Taking this work a step further, the aim of the present study was the systematic development of NLC while optimising formulation parameters. In particular the role of the total lipid content and amount of surfactant should be elucidated. The physico-chemical stability in terms of particle size, polydispersity index, zeta potential and rheological behaviour of the systems was evaluated and drug release from the developed formulations was investigated in skin permeation studies. To this end, two model drugs of different log P value, flufenamic acid and fluconazole, were incorporated and their skin permeation from NLC was evaluated in diffusion cell studies.

2. Material and methods

2.1. Materials

A rapeseed-derived phospholipid mixture (20% w/w MAPL content, 20% w/w diacyl phosphatidylcholine (PC), Lipoid R LPC 20, briefly *LPC20*) and two soybean-derived phospholipid mixtures (64.9% or 82.7% w/w MAPL content with 26.4% w/w and 12.8% w/w of PC, respectively, Lipoid S LPC 65 and Lipoid S LPC 80, briefly *LPC65* and *LPC80*) as well as purified olive oil (PhEur), medium-chain triglycerides Lipoid MCT (PhEur/USP), Soybean (PhEur), Purified Fish Oil (PhEur Type I) and omega-3-acid triglyceride (PhEur) were kindly provided by Lipoid GmbH (Ludwigshafen, Germany). Precirol®-ATO5 (glyceryl distearate/glyceryl palmitostearate, 3092PPD) was provided by Gattefosse (Nanterre Cedex, France). Isopropyl myristate (IPM) was purchased from Herba Chemosan AG (Vienna, Austria). Tegosoft® liquid (cetearyl octanoate) was provided by Evonik Industries (Essen, Germany).

Flufenamic acid (CAS 530-78-9, Lot BCBL8705V), Fluconazole (CAS 86386-73-4, Lot LRAA6502) and potassium sorbate (CAS 24634-61-5) were purchased from Sigma Aldrich (St. Louis, USA). Aqueous phosphate buffer pH 7.4 (PhEur) was composed of 2.38 g of Na_2HPO_4 x 12 H_20, 0.19 g of KH_2PO_4 and 8.0 g of sodium chloride per 1000 ml of purified water. Porcine abdominal skin was obtained from a local abattoir (Johann Gantner GmbH, Hollabrunn, Austria). All further chemicals were of analytical reagent grade and used without further purification.

2.2. Production of nanostructured lipid carriers

Nanostructured lipid carriers were produced as recently described [21]. Formulations were produced in triplicate using a batch size of 20 g. The aqueous phase and the lipid phase were prepared separately by stirring them at 70 °C. Next, the phases were mixed rapidly. After 1 min of pre-homogenisation with a rotor-stator homogeniser at 4000 rpm (Omni International 5000, USA) they were subjected to ultrasound treatment for 10 min with a Bandelin Sonopuls MS 73 (Bandelin, Germany) to achieve a mean energy input of 15 kJ at 70 °C with a thermometer attached to a magnetic stirrer (Heidolph EKT3000 + MR3001K, Germany).

This temperature was chosen since lower temperatures, as employed in previous work, did not lead to satisfying drug dissolution. In order to achieve controllable process parameters, the temperature was automatically monitored with a Heidolph EKT3000 temperature control unit and a MR3001K magnetic stirrer device; care was taken not to exceed the envisioned production temperature; the heating temperature was automatically lowered accordingly if required.

In case of drug-loaded NLC, the drug was either dissolved in the oil phase (flufenamic acid, 1% w/w) or the aqueous phase (fluconazole, 1% w/w). Both blank placebo NLC and drug-loaded NLC were stored in airtight containers at 8 °C after cooling down to room temperature on a magnetic stirrer (IKA RO10, Germany).

2.3. Preliminary studies: selection of surfactant and liquid lipid phase

Three commercially available phospholipid mixtures of variable MAPL content were evaluated for production of NLC, namely *LPC20*, *LPC65* and *LPC80*, containing roughly 20%, 65% and 80% w/w of MAPL, respectively. The lipid phase was kept constant with 2.0 g of medium-chain triglycerides and 7.0 g of Precirol®-ATO5 (ratio liquid to solid lipid of 2:7) per 100.0 g of distilled water. Each surfactant blend was employed at different concentrations (1.5, 2.5, 5.0 and 7.0% w/w, respectively). The resulting blank formulations were characterised as described in section 2.5. The parameters of interest were monitored over 24 weeks during storage for a preliminary stability assessment (data not shown).

After these preliminary studies, which are detailed in the results section, the soybean-derived *LPC65* with 65% w/w of MAPL was found to be the most promising surfactant blend for NLC development and was thus chosen for all further studies. Furthermore, different oils were evaluated for their suitability to produce MAPL-stabilised NLC formulations to replace medium-chain triglycerides: olive oil, soybean oil, IPM, Tegosoft® liquid, purified fish oil and omega-3-acid triglyceride. Olive oil was found to be the most promising choice as liquid oil compound and was employed for all further studies.

2.4. Final NLC formulation composition

The composition of the final *LPC65*-based NLC formulations with olive oil is given in Table 1. Both the amount of surfactant (*LPC65*) and total lipid content (*O*) were varied to assess the impact of each parameter. The ratio of liquid olive oil to solid oil compound Precirol®-ATO5 was kept constant at 2:7. The model drugs were incorporated at 1% w/w and the aqueous phase was respectively corrected for the incorporated drug amount.

2.5. Physico-chemical characterisation and stability evaluation

The parameters of interest were measured directly after NLC production and were monitored fortnightly over an observation period of 30 weeks. Since preliminary studies had shown that refrigerated storage led to improved physical stability of the produced NLC systems, all formulations were stored at 8 °C.

2.5.1. Mean particle size and PDI

The mean particle size and the polydispersity index (PDI) of all