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Influence of the composition of monoacyl phosphatidylcholine based microemulsions on the dermal delivery of flufenamic acid

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ABSTRACT

Although microemulsions are one of the most promising dermal carrier systems, their clinical use is limited due to their skin irritation potential. Therefore, microemulsions based on naturally derived monoacyl phosphatidylcholine (MAPL) were developed. The influence of the water, oil and surfactant content on dermal delivery of flufenamic acid was systematically investigated for the first time. A water-rich microemulsion led to significantly higher in vitro skin penetration of flufenamic acid compared to other microemulsions. The superiority of the water-rich microemulsion over a marketed flufenamic acid containing formulation was additionally confirmed. Differences in drug delivery could be explained by alterations of the microemulsions after application. Evaporation of isopropanol led to crystal-like structures of MAPL on the skin surface from the surfactant- or oleic acid-rich microemulsions. In contrast, the formation of this additional barrier was hindered in case of the water-rich microemulsion. The skin penetration of MAPL was additionally analyzed by combined ATR-FTIR and tape stripping experiments, where MAPL itself penetrated only into the initial layers of the stratum corneum, independent of the microemulsion composition. Since a surfactant must penetrate the skin to cause irritation, MAPL can be presumed as a skin-friendly emulsifier with the ability to stabilize pharmaceutically acceptable microemulsions.

1. Introduction

Drug delivery of NSAIDs via the skin has captured attention in order to minimize and avoid the gastrointestinal problems of the peroral route of administration (Wagner et al., 2004). The major challenge of overcoming the stratum corneum can be achieved by an appropriate dermal drug delivery system (Hadgraft and Lane, 2011). Microemulsions are one of the most promising systems for dermal drug delivery, not least because of their easy preparation, excellent long-term stability and solubilization properties (Santos et al., 2008). However, the clinical use of microemulsions is limited due to their skin irritation potential mainly caused by the type and amount of the surfactant (Heuschkel et al., 2008). The need of skin-friendly surfactants and an increased attention to the environment has produced a growing interest in the field of natural surfactants. Especially phospholipids are recognized as both, ecofriendly surfactants and skin permeation enhancers with low skin irritation potential (Santos et al., 2008; Elnaggar et al., 2014; Dreher et al., 1996). This led to extensive studies on the ability of lecithin to stabilize microemulsions over the last years (Aboofazeli and Lawrence, 1993; Schwarz et al., 2012; Hoeller et al., 2008; Paolino et al., 2002; Sahle et al., 2013).

Diacyl phosphatidylcholines are the predominant group of substances found in commercially available lecithin from soybean or rape seed (van Nieuwenhuyzen and Szuhaj, 1998). From these natural raw materials, monoacyl phosphatidylcholines (MAPL) can be manufactured by enzymatic hydrolysis and purified to different levels of MAPL content. MAPL contains one fatty acid chain less compared to diacyl phosphatidylcholine and is hence more polar, soluble in water and exhibits greater emulsification power (Kim et al., 2001; Trotta et al., 1996).

Therefore, the aim of this work was to investigate the dependency of dermal flufenamic acid delivery on the composition of the novel MAPL based microemulsions. For this purpose, Franz-type diffusion cell and in vitro tape stripping experiments on porcine ear skin were performed. Since flufenamic acid is a topically used and marketed NSAID, the obtained results were

Abbreviation: MAPL, monoacyl phosphatidylcholine.

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additionally related to the dermal delivery of flufenamic acid from a marketed formulation. Moreover, alterations of the microemulsions after application on the skin surfaces were monitored by light microscopic investigations.

In order to address one of the major disadvantages of microemulsions, namely their skin irritation potential caused by the high surfactant content, recently validated combined ATR-FTIR and tape stripping experiments were performed to investigate the skin penetration of MAPL (Hoppel et al., 2014a). Besides determination of the spatial distribution of the surfactant, molecular effects on stratum corneum constituents and hydration were investigated by this method.

2. Materials and methods

2.1. Materials

A soybean monoacyl phosphatidylcholine fraction with 80% MAPL content (Lipoid S LPC 80) was kindly provided by Lipoid GmbH (Ludwigshafen, Germany). Oleic acid was procured from Herba Chemosan (Vienna, Austria) and flufenamic acid (log P 5.62, pK_a 3.65 (Hadgraft et al., 2000)) from Kemprotec Limited (Cumbria, United Kingdom). Isopropanol, methanol and glacial acetic acid were obtained from Sigma–Aldrich (Vienna, Austria). The marketed formulation Mobilisin® STADA (registration No. 15.361, lot No. 1703), containing 3% flufenamic acid, was used as a reference.

2.2. Methods

2.2.1. Construction of pseudoternary phase diagrams

Pseudoternary phase diagrams were constructed in order to determine the ability of MAPL to stabilize microemulsions. Oleic acid, water and a 1:1 (w/w) mixture of S LPC 80 as surfactant and isopropanol as co-solvent were weighed into vials and stirred at room temperature for 12 h. After equilibration, the mixtures were characterized by macroscopic observation. All mixtures with a transparent appearance were then observed microscopically.

2.2.2. Drug loaded microemulsions

Three microemulsions with different water, oil and surfactant content were chosen for further in vitro skin studies. The composition of these mixtures is given in Table 1. The position of the chosen microemulsions is marked by dots in the pseudoternary phase diagram (Fig. 1). 3% (w/w) flufenamic acid were added to the microemulsions and stirred until a transparent system was obtained. Isotropy of the drug loaded microemulsions was confirmed at room temperature as well as after equilibration at 32 °C. The pH values of the microemulsions were determined with a pH meter (Orion 420A, Bartelt, Vienna, Austria).

2.2.3. Optical light microscopy

A light microscope (Optiphot-2, Nikon GmbH, Düsseldorf, Germany) equipped with a cross-polarizer was used to characterize the formed microemulsions. To confirm isotropy, an appropriate amount of the microemulsions was applied on a slide and covered with a cover glass to prevent evaporation of isopropanol. To monitor the formation of crystalline phases after evaporation of isopropanol, microemulsions were spread on a slide without covering.

Table 1
Composition of the microemulsions in % (w/w).

Formulation code	S LPC 80	Isopropanol	Oleic acid	Distilled water
ME-oil	25	25	40	10
ME-aqua	25	25	10	40
ME-surf	40	40	10	10

2.2.4. In vitro skin permeation: Franz-type diffusion cells

In vitro skin permeation studies were performed using Franz-type diffusion cells (PermeGear, Hellertown, USA). Porcine skin was cut with a dermatome (GB 228R, Aesculap, Center Valley, USA) set at 700 μm. Appropriately cut skin pieces were clamped between the donor and the receptor chamber of the diffusion cells having a permeation area of 0.95 cm^2. The receptor compartment was filled with 2 ml of phosphate buffer (0.012 M, pH 7.4) and the diffusion cells were kept at 32 °C. An infinite dose of 100 mg cm^{-2} of formulation was applied onto the skin in the donor chamber. The donor chamber was covered with a piece of Parafilm®. Samples for the analysis of the permeated drug by HPLC were taken after 2, 4, 6, 8, and 24 h by replacing the whole receptor medium with fresh phosphate buffer, in order to provide sink conditions. At least five parallel experiments were performed for each formulation ($n \geq 5$).

2.2.5. In vitro skin penetration: tape stripping

In vitro tape stripping experiments on full-thickness porcine ear skin were performed as previously reported (Hoppel et al., 2014b). Pig ears were procured from a local abattoir (Hollabrunn, Austria). At the beginning of the tape stripping procedure the pig ears were defrosted, cleaned with cold water and blotted dry with a soft tissue. After carefully clipping the hair with a scissor, the transepidermal water loss (TEWL) of the skin was measured (AquaFlux®, Biox Ltd., London, UK), in order to confirm an intact skin barrier function and to monitor the defrosting process. Afterwards, 5 mg cm^{-2} of the respective formulation were applied and distributed with a saturated glove finger. After a residence time of 1 h, the skin surface was cleaned with a dry tissue before the tape stripping procedure and 20 sequential strips (Corneofix®, Courage + Khazaka electronic GmbH, Cologne, Germany) were removed afterwards. Each tape strip was at first analyzed for its amount of removed corneocytes by NIR densitometry (SquameScan® 850A, Heiland electronic GmbH, Wetzlar, Germany) and for its drug content by HPLC, after extracting the drug from the strip with methanol. At least four parallel experiments for each formulation were performed ($n \geq 4$).

2.2.6. ATR-FTIR studies

A recently validated combination of ATR-FTIR and tape stripping experiments was used to track the penetration of microemulsion components in different layers of the stratum corneum (Hoppel et al., 2014a).

In short, full-thickness porcine ear skin samples (2 cm × 7.5 cm) were incubated with the respective microemulsion for 2 h at the skin surface temperature of 32 °C. Afterwards, 20 sequential tape strips were removed and ATR-FTIR spectra were recorded from the skin surface prior to the tape stripping procedure as well as after each removed tape strip. The amount of corneocytes removed with the individual adhesive films after incubation with the different microemulsions was analyzed using the infrared densitometer SquameScan® 850 A (Heiland electronic GmbH, Wetzlar, Germany). Experiments were performed in triplicate ($n = 3$). Samples incubated with water served as control.

Porcine ear skin samples were placed at the stratum corneum facing down on the ZnSe ATR crystal of an ATR-FTIR spectrometer (Tensor 27, Bio-ATR I tool, Bruker Optics, Ettlingen, Germany) equipped with a liquid nitrogen cooled mercury cadmium telluride (MCT) detector. Additionally, a scroll-type compressor (Atlas Copco SF1, Atlas Copco GmbH, Vienna, Austria) was used to maintain stable conditions. Spectra were recorded at the skin surface temperature of 32 °C at the average of 60 scans in the frequency