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Research paper

Comparison of stratum corneum penetration and localization of a lipophilic model drug applied in an o/w microemulsion and an amphiphilic cream

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Abstract

Vehicle dependent effects on the penetration behavior of drugs following topical application are well known from the literature. In this context, many reports concerning the enhancing activities for hydrophilic as well as lipophilic substances by colloidal drug carrier systems, particularly microemulsions, are available. However, there is little knowledge about the localization of the drugs within the skin and the stratum corneum, respectively. In the present study, the lipophilic dye curcumin incorporated in an oil-in-water microemulsion and in an amphiphilic cream was applied onto the skin of human volunteers. Using the method of tape stripping to remove the stratum corneum (SC), the depth profiles of the dye within the horny layer were compared. Applying the microemulsion, a deeper part of the SC was accessible by a number of 20 tapes removed and significantly smaller amounts of curcumin were found on the skin surface. Also differences in the distribution and localization of the dye within the stratum corneum were observed by laser scanning microscopy. Furthermore, curcumin was detected in hair follicles. It was obvious that the microemulsion led to a penetration into the complete follicular infundibula, whereas, following application of the cream, a fluorescence signal was only received from the follicular orifices.

Keywords: Microemulsion; Penetration; Curcumin; Tape stripping; Stratum corneum; Follicle

1. Introduction

Human skin is an important target site for the application of drugs. Especially in the treatment of local diseases, a topical drug delivery is an appropriate strategy to restrict the therapeutic effect on the affected area and to reduce systemic incrimination. In order to reach therapeutic drug concentrations in certain skin layers, the uppermost barrier, the stratum corneum (SC), has to be overcome. This process is affected by various factors, e.g., the physicochemical properties of the drug and the vehicle used for application [1–10]. The focus of our research work is to increase dermal availability of lipophilic drugs. From in vivo studies it is known that they are preferably localized on the skin surface and in the superficial SC after topical administration [1,7]. However, using the optimal vehicle, even for lipophilic substances it is possible to minimize this accumulation.

Modern drug carrier systems are microemulsions (MEs). These are thermodynamically stable, low viscous, transparent and optical isotropic formulations with a dynamic microstructure that form spontaneously by combining appropriate amounts of a lipophilic and a hydrophilic ingredient, as well as a surfactant and a co-surfactant. During the last years, a number of investigations have been carried out which demonstrated that drugs incorporated into

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microemulsions penetrate efficiently into the skin and through the SC-barrier [11,12]. Important features are their high drug solubilization capacity, which leads to high concentration gradients towards the skin and a microstructure that allows free and fast drug diffusion [12,13]. Dependent on the physicochemical properties of the active substance, different types of microemulsions can be the optimal carrier. Lipophilic drugs are preferably solubilized in o/w microemulsions whereas w/o (water-in-oil) systems seem to be the better choice for hydrophilic drugs [14].

In the present study, the in vivo penetration of the model drug curcumin was investigated in volunteers. Curcumin is a lipophilic fluorescent food dye, which has already been used to examine the reservoir function of the SC [15] and the distribution of topically applied substances within this layer [3,16].

Vehicle dependent effects on amount and distribution of curcumin within the stratum corneum were studied by means of tape stripping. Therefore, the dye was incorporated into an o/w microemulsion (ME) and an amphiphilic cream (Cremor basalis DAC). The latter does not show a typical droplet-like microstructure. According to Junginger, it represents an amphiphilic system of three coherent phases which are water, lipophilic ingredients and lamellar ordered surfactants [17]. In order to obtain reliable depth profiles also the amount of removed corneocytes was determined.

Additionally, differences in the localization of curcumin within the stratum corneum due to the vehicles were investigated by laser scanning microscopy (LSM). Since the follicular pathway might play a more significant role in dermal drug uptake [15,18–22], LSM was also applied to visualize a possible participation of the appendages, i.e., the follicles, on the penetration process.

2. Materials and methods

2.1. Volunteers

The study was performed on the flexor forearms of 6 healthy volunteers, 4 male and 2 female, aged between 21 and 31 years (mean age: 24.7 ± 3.8 years), with skin photo-types I–III [23]. Approval for these experiments had been obtained from the Ethics Committee of the Charité and the volunteers had signed informed consent forms.

2.2. Applied formulations

Curcumin (Merck-Schuchardt, Hohenbrunn, Germany) was the lipophilic model drug. As its pK_a is in the region of 8 [24], the protonated, sparingly soluble state of curcumin dominates in all chemical environments occurring in the study, e.g., in the formulations used and the SC (pH 4.5–6.9 [25]). The partition coefficient within the relevant pH-range is given by $\log D \geqslant 2.9$ [24].

0.5% of this fluorescent dye was incorporated in an amphiphilic cream (Cremor basalis according to *Deutscher Arzneimittel Codex 2003* [26], purchased from Synopharm, Barsbüttel, Germany) as well as in an o/w microemulsion.

The amphiphilic cream contains 4% glycerol monostearate, 6% cetyl alcohol, 7% polyoxyethylene glycerol monostearate, 7.5% medium-chain triglycerides, 10% propylene glycol, 25.5% white soft paraffin, and 40% distilled water.

The microemulsion consisted of 8% Tagat® O2 (polyoxyethylene glycerol mono-oleate, kindly provided by Frankenchemie, Wendelstein, Germany), 12% Synperonic® PE/L 101 (poloxamer 331, kindly provided by C.H. Erbslöh KG, Krefeld, Germany), 5% Pelemol® BIP (eutectic mixture of *N*-butylphthalimide and *N*-isopropyl-phthalimide, kindly provided by Phoenix Chemical Inc., Somerville, NJ, USA), 50% propylene glycol (Caesar & Loretz GmbH, Hilden, Germany) and 25% distilled water. The formulation was obtained simply by stirring the prepared surfactant blend and the lipophilic component. Subsequently, the mixture of propylene glycol/water was added up to 100%. The percentage values given represent % (w/w).

2.3. Application

The study was performed under controlled conditions, which means constant temperature and humidity. Both formulations were tested on the same volunteer on the same day.

The flexor forearms of the volunteers were prepared by rinsing with hand-warm water and drying using soft tissue. Then, two application areas of 5 cm × 6 cm were marked with a permanent marker and a silicon barrier was applied around these areas in order to avoid lateral spreading of the formulations [7]. Afterwards, 2 mg/cm^2 of either the ME or the amphiphilic cream was applied to these areas and distributed homogeneously using a glove-finger saturated with the formulation [27]. During the penetration time of 1 h, which is a common period for standard tape stripping procedures, the volunteers remained seated without covering the skin areas with textiles.

2.4. Tape stripping

One hour after application, the tape stripping procedure was performed utilizing an adhesive film (*Tesa* film No. 5529, Beiersdorf, Hamburg, Germany) with a width of 19 mm. A piece of this adhesive film of approx. Five centimeters in length was applied onto the treated skin and pressed with a roller to stretch the skin surface [27]. Thus, the influence of the furrows and wrinkles on the tape stripping procedure is negligible [28]. Each tape strip was removed with a quick movement and fixed onto a slide frame for handling. For the first volunteer, the horny layer had been removed completely using 80 tape strips [29]. After the removal of 20 tape strips for both formulations, only small amounts of curcumin were detected in the deeper layers of the SC. Therefore, only 20 tape strips were taken in the further experiments performed on 5 additional