European Journal of Pharmaceutics and Biopharmaceutics 124 (2018) 28–33

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Research paper

Dermal peptide delivery using enhancer moleculs and colloidal carrier systems. Part II: Tetrapeptide PKEK☆

Reinhard H.H. Neubert[a,*], Elfi Sommer[b], Melanie Schölzel[c], Benjamin Tuchscherer[c], Yahja Mrestani[a], Johannes Wohlrab[a,d]

[a] Institute of Dermatopharmacy, Martin Luther University Halle-Wittenberg, Weinbergweg 23, 06120 Halle (Saale), Germany
[b] Skinomics GmbH, Weinbergweg 23, 06120 Halle (Saale), Germany
[c] Evonik Industries AG, Goldschmidtstr., Essen, Germany
[d] Department of Dermatology and Venereology, Martin Luther University Halle-Wittenberg, Ernst-Grube-Straße 40, 06120 Halle (Saale), Germany

ARTICLE INFO

Keywords:
Tetrapeptide
PKEK
Nano-sized carrier system
Microemulsion
Skin penetration

ABSTRACT

Due to the lipophilic properties of the uppermost skin layer of the stratum corneum (SC) it is highly challenging to reach therapeutic concentrations of cosmetic actives and drugs. Particularly, the hydrophilic ones penetrate poorly across the SC. The purpose of this study was to improve the topical bioavailability of the hydrophilic, polar tetrapeptide PKEK (amino acid sequence in one-letter notation). A nano-sized carrier system (microemulsion, ME) was therefore developed since MEs provide excellent penetration enhancing properties. The penetration of PKEK from the ME was compared to the penetration from a standard formulation. For the two preparations the penetration of the tetrapeptide in ex vivo human skin was investigated. This allows to make statements regarding dermal penetration, localization and distribution of the active substances in each skin layer as well as the influence of vehicle variations, in this case the incorporation of PKEK into a ME system.

Relatively high amounts between 40 and 58% of the tetrapeptide PKEK penetrated from the standard cream into the skin. The major proportion of PKEK, which penetrated from the standard cream, remained in the SC and did not reach the target compartment within the skin. Penetration of PKEK from the ME was comparable with the cream for the shortest test time. However, very high PKEK amounts penetrated form the nano-sized carrier system (ME) into the human skin after 100 min (94%) and after 300 min (88%). The largest proportion did not remain in the skin, but permeated into the acceptor compartment. Therefore, the relative peptide content in the viable skin layers was predominantly comparable for the cream and the ME. For some samples a tendency could be observed that slightly higher amounts of PKEK were detected after the application of the standard cream. The absolute peptide concentrations gave a similar conclusion.

The results indicate that liquid nano-sized systems are very effective carriers for extremely hydrophilic peptides used in cosmetics and also in therapeutics.

1. Introduction

Since the skin is the largest organ of the human body it is an important target site for the application of drugs. However, in order to reach therapeutic drug concentrations the uppermost barrier, the stratum corneum (SC), has to be penetrated [1]. Due to the lipophilic properties of the SC, particularly hydrophilic drugs and cosmetic actives penetrate poorly. Therefore, it is highly challenging to attain topical bioavailability of hydrophilic actives such as peptides or peptide-like substances. The use of peptide drugs is a growing field in therapeutics as well as cosmetics since they provide high biological activity and specific efficacy with low risk for side effects [2]. Dermal application is preferred because extensive proteolytic degradation and poor permeability through the intestinal mucosa in the gastrointestinal tract exclude oral administration [3,4]. The aim of this study was to attain bioavailability of the ***model peptide PKEK*** (amino acid sequence of the tetrapeptide in one-letter notation) via dermal application.

PKEK (INCI: Tetrapeptide-30, CAS: 1036207-61-0) is a cosmeceutical peptide, which is commercially available in the product TEGO® Pep 4-Even. It is applied to the skin in order to reduce UVB-induced effects by inhibiting the pigmentation process. Furthermore lightening effects on aged skin are aspired and therefore an even skin appearance.

☆ ☆Part I: Skin Pharmacol. Physiol. 25(6) (2012) 281–287.

* Corresponding author.
E-mail addresses: reinhard.neubert@pharmazie.uni-halle.de (R.H.H. Neubert), johannes.wohlrab@medizin.uni-halle.de (J. Wohlrab).

https://doi.org/10.1016/j.ejpb.2017.12.004
Received 20 October 2017; Received in revised form 11 December 2017; Accepted 13 December 2017
Available online 14 December 2017
0939-6411/

Studies on skin epithelial cells and human keratinocytes show, that PKEK reduces UVB-induced mRNA expression of interleukins (IL-6, IL-8), TNF-α and proopiomelanocorticotropin (POMC) [5]. In vivo studies confirmed these findings and also demonstrated skin-lightening efficacy [5,6]. Consequently, PKEK has to reach its target compartment in the basal layer of the epidermis to influence the keratinocyte-induced activation of the melanocytes at the cellular level. Unfortunately, it possesses disadvantageous properties for dermal penetration (see "Physicochemical Characterization of PKEK").

The use of nano-sized carrier systems such as MEs is an appropriate strategy for improved dermal delivery of cosmetic actives as well of drugs. MEs offer benefits such as high solubilization capacity, non-invasive administration or easy preparation and application. Due to their excellent penetration enhancing properties, these systems are able to increase therapeutic levels of drugs with unfavorable physicochemical properties. MEs are thermodynamically stable systems of oil and water stabilized by surfactants and co-surfactants. MEs usually appear in different microstructures. According to the major compound of the system, they can be categorized as water-in-oil (w/o) or oil-in-water (o/w) MEs with a droplet-like structure embedded in a mono-continuous main phase. Bicontinuous structures can occur, if the system contains equal amounts of oil and water. However, the common feature of all appearing microstructures in MEs is that they are highly dynamic, undergoing continuous and spontaneous fluctuations. Despite the research of MEs as carrier for biologically active peptides being at an early stage, it has proven to be of interest [1,3,4,7–13]

The objective of this project was to investigate the penetration of PKEK into human skin from a standard preparation and from a ME of the water-in-oil (w/o) type. To evaluate the topical applicability of the test preparations, ex vivo penetration tests in human skin were carried out. This allows to make statements regarding dermal penetration, localization and distribution of the active substances in each skin layer on a certain time scale (concentration-time-profile). Furthermore, the influence of vehicle variations, in this case the incorporation of PKEK in an ME system can be monitored.

2. Materials and methods

2.1. Materials

The tetrapeptide PKEK, the standard formulation, TEGO® Care PL4 (polyglyceryl-4 laurate), TEGO® SMO V (sorbitan oleate) and TEGOSOFT® P (isopropyl palmitate) were kindly provided by Evonik Industries (Essen, Germany). The radiolabeled peptide [^{3}H]-PKEK-OH was produced by RC Tritec AG (Teufen, Switzerland). Ammonium acetate was supplied by Sigma-Aldrich Chemie GmbH (Steinheim, Germany) and 1,3-butylene glycol by Fagron GmbH (Barsbüttel, Germany). Hionic Fluor and Soluene were purchased from Canberra-Packard GmbH (Dreieich, Germany), 1,4-bis-2-(5-phenyloxazol-2-yl)-benzene (POPOP), 2,5-Diphenyloxazole (PPO) and toluene and methanol for the penetration experiment from Carl Roth GmbH + Co. KG (Karlsruhe, Germany). HPLC grade methanol for stability experiments and acetontrile were obtained from VWR International, (Darmstadt, Germany). Water was of bidistilled quality. Human skin samples (breast, ear, navel and foreskin) were kindly offered by the Department of Dermatology and Venereology with approval of the independent ethics committee of Medical Faculty at the Martin Luther University Halle-Wittenberg.

2.2. Formulations

The ***standard formulation*** consists of 3.0% polyglyceryl-3-methylglucose distearate, 1.0% stearyl alcohol, 2.0% glyceryl stearate, 9.5% C12-15 alkyl benzoate, 9.5% caprylic/capric triglyceride, 0.8% Microcare MEM (methylisothiazolinone, methylparaben), 50% water and 25% mixture of active ingredient (2% PKEK, 6% butylene glycol, 82% water and 10% glycerol).

Colloidal carrier system (w/o ME): 0.5% PKEK were dissolved in 10% of a water-butylene glycol mixture (1:1 (w/w)) representing the hydrophilic phase. Subsequently, the emulsifiers TEGO® Care PL4 (16.7%) and TEGO® SMO V (8.3%) were added as well as 65% of the oil component (TEGOSOFT® P). Finally, the mixture was moved on a laboratory shaker until all components were dissolved. A clear and yellow to amber colored formulation was received. Comprehensive characterization by means of polarized light microscopy, conductivity measurements, differential scanning calorimetry, rheological measurements and dynamic light scattering ensured that the system is a stable ME of w/o type [14]. The pH of it was 6.56 ± 0.13. All given percentage values represent % (w/w).

2.3. Penetration studies ex vivo

The penetration experiments were carried under finite-dose conditions using Franz diffusion cells (Crown Glass Company, Somerville, New York, USA) [15]. Investigations were carried out in triplicate (n = 3) using excised human skin from reduction mammoplasty. The tissue sections were postoperatively cleaned with mull pads and isotonic NaCl solution. The subcutaneous adipose tissue was mechanically dissected and discarded. Circular pieces of skin (20 mm in diameter) were punched, hermetically sealed in tin foil, packed in an occlusive polyethylene bag and stored at −20 °C.

At the time of the experiment the pieces of skin were completely defrosted at room temperature and the surface was dried using cotton pads. 20 ml of phosphate buffered saline pH 7.4 were used as acceptor phase. In the following the specimens were applied on filter gauze and placed on the diffusion cell that was preheated to 32 °C. Direct contact between filter gauze and the continuously stirred acceptor fluid was ensured. The system was protected from water evaporation by a glass cover loosely applied during experiment. Occlusion effects can be excluded by this procedure. Skin specimens of three donors were used. Three pieces of skin from each donor were used per test preparation, one for each of the respective application period of 30, 100 and 300 min.

20 mg standard formulation or 20 µl ME were applied on each piece of skin. Previously, 40 µl of the radioactive peptide solution were added and solvent was evaporated at room temperature. The solvent should therefore influence neither the penetration behavior of the peptides nor the microstructure of the formulations, especially the colloidal structure of the ME. To determine the amounts of penetrated active substance at the respective application period, the skin specimens were removed from the diffusion cell and the remaining preparation (residues) were carefully eliminated from the surface using a cotton swap, which was placed in a Pico glass vial (6 ml). A template with a circular recess (1.6 cm in diameter) was placed on the skin and SC was removed by means of tape stripping with 20 adhesive strips. Two strips each were transferred together in an Econo glass vial (20 ml). Three punch biopsies (0.6 cm in diameter, 0.2827 cm^2 in size) were taken (Kromayer punch, Stiefel-Laboratorium, Offenbach, Germany) from the exposed area and the individual skin layers were separated horizontally using a cryo-microtome (Jung, Heidelberg, Germany). 10 slices of 20 µm were taken following 15 slices of 80 µm. Thickness of the epidermis was microscopically determined for each skin sample. The first three sections were accordingly assigned to the viable epidermis and the other samples to the dermis. The remaining skin piece is called stump. Each of the skin slices and stump were placed separately in Pico glass vials (6 ml). Acceptor medium was transferred in an Econo glass vial (20 ml). Sample preparation was carried out as indicated in Table 1. Scintillator solution for swap, adhesive strips, skin slices and stump consisted of 4 g PPO and 0.1 g POPOP in 1 l toluene.