International Journal of Pharmaceutics 491 (2015) 162–169

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Importance of a suitable working protocol for tape stripping experiments on porcine ear skin: Influence of lipophilic formulations and strip adhesion impairment

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ARTICLE INFO

Article history:
Received 23 April 2015
Received in revised form 16 June 2015
Accepted 18 June 2015
Available online 24 June 2015

Keywords:
Dermal formulations
Tape stripping
Protein quantification

ABSTRACT

The tape stripping method is a very important tool for dermopharmacokinetic experiments in vitro and the accurate measurement of the removed corneocytes is key for a reliable calculation of a drug's skin penetration behavior. Therefore, various methods to quantify the amount of corneocytes removed with each tape strip have been employed, ranging from gravimetric approaches to protein assays and recently near infrared densitometry (NIR) has become very widely used. As this method is based on a reduction of light intensity, interference of formulation components seems conceivable, as they could scatter light and change the results. In this study, NIR measurements were compared to a protein assay and in addition, the influence of highly lipophilic formulations on the results of tape stripping experiments was investigated as impairment of the adherence of strips has been reported. To this end, different tape stripping protocols were employed. The obtained results ensure suitability of the NIR method and moreover suggest a more pronounced influence on adherence with increasing lipophilicity in applied formulations. The results show that adaptation of the tape stripping protocol to the specifications of envisioned experiments is important for reliable results. Two protocols were found favorable and are presented in this work.

1. Introduction

The tape stripping method has become a very important tool for dermopharmacokinetic experiments and has been widely used for example as in vitro models for in vivo studies and in order to assess in vivo bioavailability and bioequivalence of topically applied substances (Alberti et al., 2001; Klang et al., 2011b; Pelchrzim et al., 2004; Reddy et al., 2002; Van der Molen et al., 1997; Wiedersberg et al., 2008, 2009). Various methods to quantify the amount of corneocytes removed with each tape strip are reported in the literature from gravimetric approaches to protein assays (Klang et al., 2011a; Lademann et al., 2009; Mohammed et al., 2012), as an accurate calculation of the skin penetration depth of a substance is only possible if the amount of stratum corneum (SC) removed can be reliably measured. In recent years, quantification of corneocytes via near infrared densitometry (NIR) has become very widely used as it is a quick quantification method that allows sample handling and measurement with minimal manipulation of the samples, thereby rendering it a very attractive method as both SC removal and drug content can be quantified for each strip in parallel (Hahn et al., 2010; Voegeli et al., 2007). However, the operating mode of corneocyte quantification via NIR is based on measuring a reduction of light intensity due to the pseudo-absorption of corneocyte, which is a combined effect of light being scattered, reflected and diffracted by the corneocytes and it is thereby an optical method (Voegeli et al., 2007). This makes interference of formulation components conceivable, as light scattering because of lipophilic components could possibly change the result of NIR measurements (Hahn et al., 2010).

Following this consideration, the aim of the present study was to evaluate the influence of various formulations on the quantification of removed corneocytes via NIR. To differentiate between the amount of removed corneocytes measured and calculated via NIR densitometry and the amount of corneocytes actually removed, the results of NIR measurements were compared to results from the microBCA protein assay. Moreover, previously published works suggest a problematic influence of highly lipophilic formulations on the results of tape stripping experiments (Jacobi et al., 2003). So in another aim of this study, we

Abbreviations: SC, stratum corneum; WO, water-in-oil; NIR, near-infrared; BCA, bicinchoninic acid.

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http://dx.doi.org/10.1016/j.ijpharm.2015.06.031
0378-5173/

investigated the impairment of tape adherence due to vehicles with varying lipophilicity and the influence of the exact specifications of the tape stripping protocol on the calculation of the SC penetration depth as a suitable protocol is key for reliable results.

2. Materials and methods

2.1. Materials

Standard Corneofix F 20 adhesive tapes with a surface area of approximately 4.0 cm^2 were purchased from Courage + Khazaka GmbH (Cologne, Germany).

The model drugs fludrocortisone acetate (CAS: 0000514363), and diclofenac sodium (CAS: 15307-79-6) were purchased from Sigma Aldrich (St. Louis, USA) and used as received. Buffer substances as potassium dihydrogen phosphate (KH_2PO_4) and sodium hydrogen phosphate (Na_2HPO_4) were purchased from ACROS Organics (Geel, Belgium).

Lecithin S75 was kindly donated by Lipoid GmbH (Switzerland), isopropyl myristate (IPM) and sodium chloride were purchased from Herba Chemosan (Vienna, Austria).

Sodium hydroxide (CAS: 1310-73-2) and hydrochloric acid (CAS: 7647-01-0) were purchased from Sigma Aldrich as well as the solvents methanol (CAS: 0000067561), acetonitrile (CAS: 75-05-8), chloroform (CAS: 67-66-3), and 2-propanol (IPA; CAS: 67-63-0) that were of analytical grade and used as obtained.

The industrial WO cream base Ultrabas® was a kind gift from Bayer Austria GesmbH (Vienna, Austria). According to the manufacturer, the ingredients are the following: purified water, white petrolatum, liquid paraffin, Dehymuls E (dicocoyl pentaerythrityl distearyl citrate, sorbitan sesquioleate, cera alba, aluminum stearate), white wax, perfume oil. Water content is approximately 30% (w/w).

The industrial water-free ointment Ultralip® was a kind gift from Bayer Austria GesmbH (Vienna, Austria). According to the manufacturer, the ingredients are the following: solid paraffin, white petrolatum, microcrystalline wax, jojoba oil. This water-free ointment contains no water.

The microBCA protein assay kit together with standard solutions of bovine gamma globulin (BGG) was purchased from Fisher Scientific (Vienna, Austria) and used according to the instructions provided by the manufacturer. Greiner Bio-One microplates were purchased from Dinkelberg Analytics (Gablingen, Germany).

2.2. Skin material

Excised porcine ears were used for the tape stripping experiments as they are established as a reliable model for tape stripping experiments (Klang et al., 2011b). The ears were obtained from a local abattoir (EU-Schlachthof Gantner, Hollabrunn, Austria) and the pigs were approximately six months old at the time of slaughter. Care was taken to have the ears removed before the carcass was exposed to high-temperatue cleaning procedures as these would have a negative impact on the skin barrier and would therefore impair the envisioned experiments (Herkenne et al., 2006). The excised ears were cooled during transport, rinsed with cold water and carefully blotted dry with soft tissues before storage at −18 °C until use. The ears were stored frozen for up to six months which was reported as suitable storage conditions that do not interfere with the envisioned experiments (Klang et al., 2011b; Stracke et al., 2006). Ears were thawed prior to use for experiments at room temperature and the skin was left on the cartilage to allow for easier handling and prevent skin contraction that would interfere with the homogeneity of SC removal (Breternitz et al., 2007; Lademann et al., 2009).

2.3. Production of test formulations

2.3.1. WO cream and water-free ointment

The WO cream Ultrabas® and the water-free ointment Ultralip® were used for experiments as received. The model drugs fludrocortisone or diclofenac sodium, respectively, were incorporated into the mixture to a final concentration of 0.5% by means of the automated mixing system TopiTec® (WEPA Apothekenbedarf, Hillscheid, Germany).

2.3.2. Microemulsion

Microemulsions containing two different model drugs were produced according to an already published method (Mahrhauser et al., 2014) Briefly, the surfactant lecithin S 75 was mixed with the co-solvent isopropanol in a ratio 1:1. This mixture was then added to the lipophilic isopropylmyristate (IPM), serving as the oily component, and distilled water to the ratio of 20:60:20 corresponding to IPM:surfactant:water. The mixture was stirred until a transparent and homogeneous formulation was achieved. The model drug was added to the microemulsion in a concentration of 0.5% (w/w) and stirred until dissolution of the drug. Microscopic evaluation with a light microscope Nikon Labphot-2 microscope (Nikon, Japan) showed an isotropic liquid and thereby confirmed the formation of microemulsions.

2.4. Tape stripping experiments

After allowing the ears to thaw, the area intended for tape stripping was marked with a permanent marker and the visible hairs were clipped with scissors. Care was taken not to damage the skin barrier. To assess the condition of the skin barrier, TEWL (transepidermal water loss) was measured on the areas designated for tape stripping. TEWL values below 20 g/m^2/h were considered as indication of an intact barrier. On the marked areas, the formulations were applied as the carefully weighed amount of 2 mg/cm^2 onto the marked areas and distributed for 1 min with the saturated tip of a latex glove. For this purpose, one finger of a latex glove was removed, dipped into the formulation to fill the crevices und furrows on the latex and before distribution of the formulation on the ear, the glove was wiped clean. This was done in order to minimise the amount of formulation removed with the glove so as to achieve an accurate amount of the formulation on the tape stripping sites. This step was performed by the same experimenter to foreclose the influence of different application techniques. For tape stripping experiments investigating the influence of formulations on the removal of SC proteins on either untreated skin or with blank WO cream, water-free ointment, and a microemulsion were chosen. For investigation of the protocol's influence on calculation of drug recovery and SC penetration depth, tape stripping experiments were performed with 0.5% of model drug in WO cream, water-free ointment and microemulsions. After a 1 hour incubation period, tape stripping was started; however, different protocols were followed to investigate the effect of the exact protocol specifications. For each key point of the envisioned results, at least 4 tape stripping sites were examined and the results combined to be presented as means and standard deviations in the results section ($n \geq 4$).

2.4.1. Protocol A – excess of formulation removed

After penetration time of 1 hour, the remaining excess of formulation was removed from the skin surface by means of a soft tissue (Klang et al., 2011b). This tissue was approximately 2 by 2 cm in size and later extracted in the same fashion as the tape