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Research paper

Method to determine the impact of substantivity on ex vivo skin-permeation

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ABSTRACT

Topical formulations are the most common therapeutic agents in the treatment of skin diseases. They contain one or more active pharmaceutical ingredients (API) which need to penetrate or permeate the skin in order to exert their effect. However, after application a part of the formulation is removed from the skin due to contact with the environment. Therefore, a part of the active is then not available for penetration and thus, a loss in therapeutic effect will result. To achieve the desired therapeutic outcome a sufficient fraction of the formulation must remain on the skin. The extent to which the loss of preparation affects penetration and permeation is less investigated. This work presents a method to examine the influence of mechanical stress and formulation loss on skin permeation. A movable punch with a defined weight simulated contact between clothing or skin and the applied formulation. Weight of the tool, number of contacts and speed settings were variable and were investigated. Ex vivo permeation experiments were performed in Franz diffusion cells using porcine skin. Three preparations with nonivamide as active ingredient were chosen as model formulations: A semisolid cream, an oil-in-oil emulsion and a film-forming formulation. The last two show sustained permeation profiles. The method uses skin-to-formulation and clothing-to-formulation contact to simulate the removal of the formulations from the skin.

1. Introduction

Skin substantivity describes the adherence of a topical formulation on skin surface [1]. Li et al. [2] gave numerous examples where substantivity plays an important role. Protective characteristics of sunscreens, antimicrobial agents or insect repellents require adequate substantivity. On the other hand high retention rate can result in local reactions or toxic effects. Various types of topical dosage forms show different quality in skin substantivity [3]. Consequently a part of the applied formulation is removed from the skin surface. This in turn will cause a loss in therapeutic efficacy as the available amount of active ingredient is likewise reduced. In the worst case scenario the remaining part of the formulation does not suffice to achieve the intended therapeutic effect. In any case contamination of patient's environment e.g. clothing or bedding with the API will happen. Undesired contact and thus undesired effects such as skin or eye irritation will be the consequence. Additionally, more and more drug substances are found as contaminant in surface water. This does not only include APIs which are eliminated from the body after application but also active substances which are used in topical formulations e.g. Ketoconazole or Erythromycin are found [4]. This indicates that substantial amounts of formulation are removed from the skin and are unintendedly transferred to other sites. Improved substantivity can reduce the required amount of API in topical formulations to achieve comparable therapeutic outcome. Furthermore, application intervals and thus consumption of preparation and API can be affected by enhanced substantivity. Environmental and economical benefits are the consequence.

Substantivity against environmental influences, especially water (and sand) have already been investigated in the case of sun screen products. For the commercial claim support of sunscreen products as "water resistant" or "very water resistant" standardized in vivo methods are available [5,6]. Greiter et al. [7] published an alternative in vitro laboratory method where they apply the test product on pig ear skin and then flush it with a defined amount of water. Afterwards, the remaining amount of sunscreen was quantified. Rub resistance can be investigated by comparing the spectral transmission of epidermis samples. To this end, a baseline is recorded and then the sunscreen is applied and the spectral transmittance of the formulation is determined. After agitating the epidermis samples in a vessel that contains sand and subsequent cleaning of the surface from adhering grains of sand the spectral transmission can be recorded again. Sun protection factor prior and after rub of the formulation can be compared. [8]. Other methods

Abbreviations: API, active pharmaceutical ingredient; HNC, Hydrophilic Nonivamide Cream; NVA, nonivamide; PBE, phosphate buffered saline pH 7.4 and ethanol in equal parts; TRPV1, transient receptor vanilloid type 1 ion channel

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concentrate on adhesion properties. Thereby detachment force between formulation and the probe of a texture analyser is measured [9,10]. Furthermore, two methods to test substantivity have already been described by our group. To test substantivity against skin-to-formulation contact two skin discs are fixed to two metal cylinders. The formulation is applied to the lower one. Then the two cylinders are pressed together with a defined force. The formulation is washed off the skin and the amount of API is analysed. To test clothing-to-formulation contact a piece of fabric is fixed to a foamed plastic roll. This roll moves with a defined force over a strip of skin on which the formulation is applied. The amount of API on the skin and the piece of cloth is quantified [3]. But at the moment it is not possible to quantify the effects of substantivity on skin permeation and penetration. Also changes in permeation rate cannot be determined with the available methods. The purpose of this work was thus to investigate the extent to which skin permeation is affected by a lack of substantivity.

After application a formulation has contact with various surfaces and materials. A certain amount of human skin is covered with clothes and some areas are touched intimate by other skin surfaces e.g. crook of the arm or armpit. Therefore we chose skin-to-formulation and clothing-to-formulation contact to simulate mechanical stress to the formulation. We simulated this contact during a skin permeation experiment in Franz diffusion cells to study the impact of removal the formulation on skin permeation rate and permeated amount.

This work concentrates on formulations containing nonivamide as API. Nonivamide and other capsaicinoids are potent agonists on the transient receptor vanilloid type 1 ion channel (TRPV1). It is a non-specific ion channel, which is expressed in sensory nerve fibres in human skin [11]. The short-term result of the activation of TRPV1 in C- and Aδ-fibres is warming, burning, stinging and itching. Long lasting activation of TRPV1 leads to high intracellular calcium levels and thus cellular processes are disturbed [12]. Capsaicinoids are used in the therapy of neuralgic pain [13] and chronic pruritus [14] which is often associated with skin diseases [15].

To achieve the desired effect conventional formulations, as the Hydrophilic Nonivamide Cream (HNC), have to be applied up to five times a day [16]. This is quite uncomfortable for the patients and leads to poor compliance.

To improve patient's compliance topical formulations with sustained release were developed [10,17]. These formulations form an API reservoir on the skin. Due to the sustained delivery of the drug the application interval is prolonged. An improved patient compliance is desired [10]. The benefit of these therapeutics agents will not be achieved if the formulation does not show adequate substantivity on the skin. We thus used the two sustained release formulations that have previously been described by our group and the conventional cream (HNC) to investigate the impact of substantivity or lack thereof on skin permeation. The aim of this work was to assess how clothing-to-formulation and skin-to-formulation contacts affects the permeated amount of the model API nonivamide from different topical formulations.

2. Methods

2.1. Material

Syloid® XDP 3050 was kindly donated by Grace GmbH & Co. KG (DE-Worms), Eudragit® RS 30D was kindly provided by Evonik Industries AG (DE-Darmstadt) and Q7-9120 silicone fluid 20 cst and emulsifier BY 11–030 were kindly donated by Dow Corning GmbH (BEL-Seneffe). Nonivamide (NVA) and triethyl citrate (Sigma Aldrich Chemie GmbH, DE-Steinheim), medium-chain triglycerides (Myritol® 312) and propylene glycole (BASF SE, DE-Ludwigshafen). White soft paraffin (Hansen & Rosentahl KG, DE-Hamburg), cetyl alcohol and refined castor oil (Ceasar & Loretz, DE-Hilden). PEG-20-glyceryl stearate (Tagat S2®) and glyceryl stearate (Fagron GmbH & Co. KG, DE-Barsbüttel). Solvents ethanol and acetonitrile were HPLC gradient grade. Disodium phosphate, potassium dihydrogen phosphate and phosphoric acid were of European Pharmacopeia grade.

2.2. Preparation of formulations

Hydrohpilic Nonivamide Cream (HNC) was prepared in accordance with the monograph "Hydrophile Capsaicinoid Creme NRF 11.125" [18]. As a minor adaption capsaicin was replaced by nonivamide. Cetyl alcohol, medium chained triglycerides, white soft paraffin and glyceryl stearate were melt on a water bath. PEG-20-glyceryl stearate and propylene glycol were dissolved in hot water. Subsequently, the aqueous phase was added to the oily phase under constant stirring. The obtained amphiphilic cream was mixed with propylene glycol, purified water and a solution of nonivamide (10% w/w) in ethanol [19] by a syringe-to-syringe technique.

The oil-in-oil emulsion was prepared by weighing all ingredients, Q7-9120 silicone fluid 20 cst, emulsifier BY 11-030 and refined castor oil containing nonivamide, into a syringe. The syringe was connected to a second syringe by an adapter. By means of transferring the liquid from one syringe to the other 70 times, the formulation is prepared [17,20].

The film-forming formulation was prepared by dissolving nonivamide in refined castor oil and incorporating this oily solution into mesoporous silica (Syloid® XDP 3050). To reduce its glass transition temperature, Eudragit RS 30 D was mixed with 7.0% triethyl citrate as a plasticiser. One part oil loaded powder was then mixed with three parts Eudragit® RS 30 D containing triethyl citrate. The manufacturing is described in more detail by Heck et al. [10].

All formulations contained 0.9% (w/w) nonivamide as active ingredient. Compositions are given in Table 1.

2.3. Preparation of dermatomed pig ear skin

Porcine skin is a common and accepted material to predict in vivo experiments with human skin [21]. Fresh pig ears of German Land Race pigs (age: 15–30 weeks; weight: 40–65 kg) were washed with isotonic saline. Postauricular skin was excised. Skin samples were cleaned off blood with isotonic saline and cotton swabs, patted dry with tissue, wrapped in aluminum foil and stored at -30 °C. On the day of the experiment, the skin was thawed at room temperature, cut into strips of approximately 3 cm in width and fixed to a block of styrofoam with pins. The skin was dermatomed to a thickness of 1 mm (Dermatom GA 630; Aesculap AG & Co. KG, DE-Tuttlingen). Afterwards pieces with a diameter of 25 mm were punched out of the strips.

2.4. Ex vivo permeation experiments

Ex vivo permeation experiments were performed using modified

Table 1
Composition of the tested formulations.

Hydrohpilic Nonivamide Cream	Oil-in-oil emulsion	Film-forming formulation
0.90 g Nonivamide	0.90 g Nonivamide	0.90 g Nonivamide
2.00 g Glyceryl stearate	46.60 g Refined castor oil	14.10 g Refined castor oil
3.00 g Cetyl alcohol	47.50 g Q7-9120 silicone fluid 20 cst	10.00 g Syloid® XDP 3050
3.75 g Medium-chain triglycerides	5.00 g Emulsifier BY 11-030	69.75 g Eudragit® RS 30 D
12.75 g White soft paraffin		5.25 g Triethyl citrate
3.50 g PEG-20-glyceryl stearate		
15.00 g Propylene glycol		
8.10 g Ethanol		
51.00 g Purified water		