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Research Paper

A strategy for in-silico prediction of skin absorption in man

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ABSTRACT

For some time, in-silico models to address substance transport into and through the skin are gaining more and more importance in different fields of science and industry. In particular, the mathematical prediction of in-vivo skin absorption is of great interest to overcome ethical and economical issues. The presented work outlines a strategy to address this problem and in particular, investigates in-vitro and in-vivo skin penetration experiments of the model compound flufenamic acid solved in an ointment by means of a mathematical model. Experimental stratum corneum concentration–depth profiles (SC–CDP) for various time intervals using two different in-vitro systems (Franz diffusion cell, Saarbruecken penetration model) were examined and simulated with the help of a highly optimized three compartment numerical diffusion model and compared to the findings of SC–CDPs of the in-vivo scenario. Fitted model input parameters (diffusion coefficient and partition coefficient with respect to the stratum corneum) for the in-vitro infinite dose case could be used to predict the in-use conditions in-vitro. Despite apparent differences in calculated partition coefficients between in-vivo and in-vitro studies, prediction of in-vivo scenarios from input parameters calculated from the in-vitro case yielded reasonable results.

1. Introduction

The importance of substance transport into and through the skin has grown consistently in different fields of science and industry over the past decade. Not only pharmaceutical applications for the local or systemic administration of drugs or applications in the cosmetic industries rely on careful investigations of the underlying transport kinetics, especially regulation authorities such as REACH (Regulation, Evaluation, Authorisation and Restriction of Chemicals) [1] request information about characteristics of potential harmful xenobiotic exposure to the human skin [2,3].

In-vivo experiments in the field of skin absorption are considered the gold standard but are difficult to perform and evaluate due to the complexity of processes involved and obviously face ethical concerns [4,5]. Hence, experiments with in-vitro test systems are used frequently to provide this information [6–8]. In the past, it has been shown repeatedly that these test systems are capable of mimicking the in-vivo situation reasonably [9–11].

Many investigations in dermal research are performed under infinite dose conditions. These studies are done by applying a sufficiently large amount of the test formulation to the surface of the skin, so that neither the drug nor any of the vehicular components are appreciably reduced during the course of the experiment. The advantage of this procedure of the application of the vehicle is very reproducible and quasi steady-state conditions may be reached in the *stratum corneum* (SC) [12]. Effects caused by rubbing are also avoided. On the other hand, this procedure does not mimic applications as they are done in practice, where typically small quantity of drug preparation (finite dose) is applied on a relatively large area of the skin by massaging.

Abbreviations: SC, *Stratum corneum*; SB-M, Saarbruecken penetration model; FD-C, franz diffusion cell; FFA, flufenamic acid; Q, released amount per area; T, time; C_0, initial concentration at time $t = 0$; k_p, permeability; D, diffusion coefficient; K, partition coefficient; h, height of the membrane or barrier; c, concentration; DSL, deeper skin layers (viable epidermis and parts of the dermis); RSE, residual standard error.

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Today, considerable information about drug distribution within the skin, following infinite dosing, is available. In contrast, the data for finite dosing are rather limited [13]. Data for semisolid drug preparation are also less common than permeation data.

Based on available data [14] it was decided to carry out a study gathering input parameters (SC diffusivity and partition coefficient) with respect to a mathematical model based on experimental results of two different in-vitro test systems, the Saarbruecken penetration model (SB-M) and the Franz diffusion cell (FD-C), using the tape stripping technique [15]. With the help of an in-silico model these input parameters were used to predict the situation in-vivo. Experimental data were examined under infinite dose and finite dose conditions for the aforementioned test systems and in-vivo studies. Finite dosing was carried out on the basis of different guidance documents, which state that finite dosing is given when the amount of ointment applied to the skin is between 1 and 10 mg/cm^2 [16–18]. Consequently, infinite dose conditions were implemented by application of more than 10 mg/cm^2 of formulation. These procedures were performed with a semisolid drug preparation.

Mathematical models predicting skin absorption have, for some time, been considered as alternatives to experimental investigations by different regulatory agencies [19–21]. For complex mechanistic models it could be shown that in-vitro infinite and finite dose concentration–depth profiles could be predicted reasonably but require a complex set of input parameters [22–24]. In contrast, in the current work a sparse parameter one-dimensional diffusion model was used not only to investigate and predict the in-vitro situation but also to predict the in-vivo situation based on input parameters derived from an in-vitro based model. For this purpose, the underlying diffusion equation was solved numerically. First of all, the model was fitted to in-vitro concentration–depth profiles to obtain information about diffusivity and partition coefficient with respect to the SC. Subsequently, in-vitro infinite dose fitting results were used to predict the in-vitro finite dose scenario as conducted successfully before for aqueous formulations [22,25]. Finally, the model was used to predict in-vivo concentration–depth profiles for the finite and infinite dose case.

A positive outcome of the presented study should not only give a better hint on the theory of the drug's diffusion into the SC but most likely be a starting point to reduce experimental work in-vitro and in-vivo. It should therefore reduce costs and time required for the development of semisolid drug formulations and other formulations for dermal application.

2. Materials and methods

The following materials and equipment were used: Flufenamic acid (Kali-Chemie Pharma, Hannover, D); wool alcohols ointment and Multifilm kristall-klar (Beiersdorf, Hamburg, D); Ringer solution, McIlvaine citric acid-phosphate buffer pH 2.2, NaOH (Merck, Darmstadt, D); Plastibase® (Heyden GmbH, Muenchen, D); methanol (Baker, Deventer, NL); Franz diffusion cells type 4G-01-00-20-15, area = 3.142 cm^2, acceptor volume = 15 ml (Perme Gear, Riegelsville, PA, USA) teflon filter Minisart – pore size 0.2 µm (Sartorius, Goettingen, D); isocratic HPLC consisting of a 655 A 40 autosampler, L 4250 detector, L 6220 pump, 6000 K data interface and 5 µm LiChrospher® 100/RP-18 column/12.5 cm × 4 mm (Merck-Hitachi, Darmstadt, D); Dialysis membrane, cut off 10,000 (Dianorm GmbH, Munich, Germany); Cellulose membrane, cut off 10,000 (Medicell International Ltd, London, GB).

The non-steroidal drug flufenamic acid (FFA), solved in a concentration of 0.9% in wool alcohols ointment (German Pharmacopoeia 1999), was used as drug preparation under infinite and finite dose conditions. Prior to the application, the drug preparation was stored at 32 °C for one week to allow complete dissolution of the drug in the ointment base. The absence of drug particles was checked by light microscopic investigations.

2.1. Skin samples for in-vitro experiments

Skin samples were taken from Caucasian female donors undergoing abdominal surgery with the approval of the ethic committee of the Caritas-Hospital Lebach, Germany. Immediately after excision the subcutaneous fatty tissue was removed using a scalpel. The skin was cut into 10 × 10 cm pieces, wrapped in aluminium foil and stored in polyethylene bags at −26 °C until use. The maximum storage time was three months.

2.2. Saarbruecken penetration model (SB-M) experiments

Details of the experiments are given in (10). Briefly, for infinite dosing an ointment layer of at least 2 mm was applied whereas for finite dosing 4–6 mg ointment per cm^2 was evenly spread on the skin surface. All experiments were carried out at a skin surface temperature of 32 ± 1 °C for different time intervals (0.5, 1, 3 and 6 h) at occlusive conditions.

2.3. Franz diffusion cell (FD-C) experiments

Details of the experiments are given in [10]. In short, application of the ointment was conducted similar to the procedure described previously (SB-M). As a receptor fluid Sorensen phosphate buffer pH 7.4 was used and mixed with a magnetic stirring bar at 500 rpm. To allow equilibration the skin specimen was pre-equilibrated for 30 min prior to the application of the formulation. All experiments were carried out as described in the previous section (SB-M).

2.4. Horizontal segmentation of the stratum corneum (SC)

To compare the results of both models, the skin was always treated in exactly the same manner at the end of all experiments [10]. First, the remaining ointment was removed by wiping the skin with cotton. Second, the skin was successively stripped with 20 pieces of adhesive tape. In a standardized procedure (pressure: 2 kg for 10 s), tapes were removed rapidly and combined in 6 pools of 1, 1, 3, 4, 5 and 6 strips for analytical purposes. Due to this procedure, each of the removed cell layers had nearly the same thickness, which had been shown in previous studies in different laboratories [26–28]. The first tape strip was always discarded because of potential contamination.

2.5. In-vivo experiments

Six human volunteers (3 males, 3 females), aged 23–29 years, from whom informed consent was obtained, participated in the study [10]. They were in good health and had no history of any dermatological disease.

A template of Fixomull® with 4 holes was fixed on the volar left and right forearm of each volunteer. Each hole released an area of 15 mm in diameter and represented one experimental area. The drug preparation was applied according to the in-vitro experiments. The administration areas were not covered during the incubation time (0.25, 0.5, 1 and 3 h), but the volunteers were asked to reduce their movements to avoid any loss of drug preparation.

The tape-stripping procedure (see the previous section) was performed by exerting the pressure just with the forefinger. To increase reproducibility, the procedure was carried out by the same person according to the AAPS/FDA Workshop report [29] and the Guidance for Industry [30].