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Advanced Drug Delivery Reviews 59 (2007) 1152–1161
Advanced DRUG DELIVERY Reviews
www.elsevier.com/locate/addr

Transdermal skin delivery: Predictions for humans from *in vivo*, *ex vivo* and animal models ☆

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Received 10 May 2007; accepted 20 July 2007
Available online 16 August 2007

Abstract

The assessment of percutaneous permeation of molecules is one of the main steps in the initial design and later in the evaluation of dermal or transdermal drug delivery systems. The literature reports numerous *ex vivo*, *in vitro* and *in vivo* models used to determine drug skin permeation profiles and kinetic parameters, some studies focusing on the correlation of the data obtained using these models with the dermal/transdermal absorption in humans. This paper reviews work from *in vitro* permeation studies to clinical performance, presenting various experimental models used in dermal/transdermal research, including the use of excised human or animal skin, cultured skin equivalents and animals. Studies focusing on transdermal absorption of a series of drug molecules and various delivery systems as well as mathematical models for skin absorption are reviewed.

Keywords: Transdermal absorption; *In vitro-in vivo* correlation; Animal skin; Studies in humans; Skin equivalents; Percutaneous permeation

Contents

1. Introduction

The assessment of percutaneous absorption of molecules is a very important step in the evaluation of any dermal or transdermal drug delivery system. A key goal in the design and optimization of dermal or transdermal dosage forms lies in understanding the

☆ This review is part of the *Advanced Drug Delivery Reviews* theme issue on "Prediction of Therapeutic and Drug Delivery Outcomes Using Animal Models".

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0169-409X/$ - see front matter © 2007 Published by Elsevier B.V.
doi:10.1016/j.addr.2007.07.004

factors that determine a good *in vivo* performance. Certainly, the most reliable skin absorption data are collected in human studies; however, such studies are generally not feasible during the initial development of a novel pharmaceutical dosage form or consideration of a new drug candidate. Thus, one of the main challenges of biopharmaceutical research is finding a correlation between *ex vivo*, animal and human studies for prediction of percutaneous absorption in humans. It is practically impossible to assess the skin permeability of materials using *in vivo* experiments alone. Consequently, numerous *ex vivo* and *in vitro* models are frequently employed to assess drug skin permeation profiles and kinetic parameters. Hence, a method that can consistently correlate *ex vivo* and *in vivo* data to shorten and economize the process of drug development and minimize the number of human studies is critically needed.

This article begins with a short overview of various aspects as well as pros and cons of *in vitro* and *in vivo* animal models for skin permeation. Further, studies evaluating percutaneous absorption of various drugs with or without permeation enhancement techniques are covered. And finally, the use of data from experiments in skin cultures and mathematical/pharmacokinetic models for predicting transdermal absorption are critically discussed.

2. Issues related to *in vitro* and *in vivo* skin permeation studies

Despite ethical concerns, the use of animals or isolated animal skin models to assess percutaneous absorption of molecules is frequently reported. These models, generally more available than human skin, are of prime importance in basic research to improve our understanding of the processes, pathways and driving forces of various agents across the skin barrier. However, due to the large number of animal species described in the literature, it is quite difficult to compare the data in the field of dermal and transdermal drug delivery. Variations in methodology used with a specific skin model, such as type of diffusion cells, skin temperature, receiver media, application dose and diffusion area, can all significantly affect data [1]. Yet, it is important to emphasize that *in vitro* and animal models provide important tools for screening a series of drug formulations, evaluation of skin permeation enhancing properties and mechanism of action of the carrier systems and estimation of rank of skin transport for a series of drug molecules.

3. Skin structure: human vs. animal models

Skin is the largest body organ, weighing approximately 5 kg with a surface area of about two square meters in adult humans [2–4]. This multilayered organ has an essential function of protecting the body from the surrounding environment, thus being an efficient permeation obstacle for exogenous molecules. The barrier properties of the skin lie mainly within its uppermost strata, the stratum corneum (SC). This highly hydrophobic layer is composed of differentiated non-nucleated cells, corneocytes, which are filled with keratins and embedded in the lipid domain. meation of molecules is believed to be governed by diffusion laws [2]. The extent of skin permeation of a compound may depend on the route of absorption. There are three pathways which can be involved in the transdermal permeation of chemicals: (1) through the intercellular lipid domains in SC; (2) through the skin appendages; and (3) through the keratin bundles in SC [2,5].

The lack of correlation in transdermal permeation of molecules across species or from different application sites in the same animal model is due mainly to variations in skin (or SC) thickness, in the composition of intercellular SC lipids and in the number of skin shafts. Netzlaff et al. [6] have shown that the amount of free fatty acids and triglycerides and the density of hair follicles are important factors causing differences between the skin barriers among species. As the majority of molecules applied onto the skin permeate along the SC lipid domain, the organization of these regions is very important for the barrier function of the skin. The SC lipid composition and organization differ from that of other biological membranes, with long chain ceramides, free fatty acids, cholesterol and cholesteryl esters being the main lipid classes [2–4,7,8].

To evaluate transdermal absorption of a molecule, the most relevant membrane is human skin. Skin from various sources, including cosmetic surgery and amputations, has been used for the *in vitro* assessment of percutaneous penetration [9,10]. However, its availability is limited and animal skin is therefore frequently used. A wide range of animal models has been suggested as a suitable replacement for human skin and has been used to evaluate percutaneous permeation of molecules. These include primates, porcine, mouse, rat, guinea pig and snake models.

Since the use of primates in research is highly restricted, the most relevant animal model for human skin is the pig. Porcine skin is readily obtainable from abattoirs and its histological and biochemical properties have been repeatedly shown to be similar to human skin [11–15]. Porcine ear skin is particularly well-suited for permeation studies and gives comparable results to human skin. Studies examining thickness of various skin layers have shown that the SC thickness in pigs is 21–26 μm [10,12] which is comparable to human skin [10,16]. The viable epidermis in porcine ear skin is 66–72 μm thick [10,12], which is very similar to the human epidermal thickness of 70 μm (shoulder) [17]. The follicular structure of pig skin also resembles that of humans, with hairs and infundibula extending deeply into the dermis. An average of 20 hairs are present per 1 cm^2 of porcine ear skin as compared to 14–32 hairs (except the forehead area) in humans [12]. Moreover, the vascular anatomy and collagen fiber arrangement in the dermis, as well as the contents of SC glycosphingolipids and ceramides are similar in man and in the domestic pig [18].

Due to its availability, skin of rodents (mice, rats and guinea pigs) is the most commonly used in *in vitro* and *in vivo* percutaneous permeation studies. The advantages of these animals are their small size, uncomplicated handling and relatively low cost. There are a number of hairless species (nude mice, hairless rats) in which the absence of hair coat mimics the human skin

avoiding the risk of injury to cutaneous tissue. Other models have a disadvantage of an extremely high density of hair follicles and require hair removal. Since both issues may affect percutaneous absorption of molecules, hairy rodent skin is usually not used in *in vitro* permeation studies, although *in vivo* studies are still performed on these species. Among rodents, rat skin has more structural similarities to human tissue (Table 1).

Except for rat skin, rodent skin generally shows higher permeation rates than human skin [20–21]. Regarding the rat skin, permeation kinetic parameters are frequently comparable with human skin.

Snake skin was also proposed as a membrane in skin permeation experiments. Differential scanning calorimetry (DSC) thermograms and infra-red (IR) spectra showed that the SC of snake, porcine and human skins have some similarities in structure and components [22]. The distinguishing feature of the shed snake membrane is its lack of follicles.

4. *In vitro* permeation across human skin vs. animal models

Various studies have been carried out in an attempt to correlate *in vitro* permeation data in animal and human skin. Some of them are reviewed here. Most of reports substantiate the value of the pig as an animal model for man in skin permeation studies. Singh et al. [23] evaluated skin permeability coefficients (Kp) and SC reservoir of three hydrocarbons in porcine ear compared to human skin. They reported that pig skin was slightly more permeable to the substances with the ratios Kp porcine skin/Kp human skin of 1.71, 1.28 and 1.16 for heptane, hexadecane and xylene, respectively. The permeation profiles of heptane across human and porcine skin are presented in Fig. 1. SC binding of the hydrocarbons to porcine and human skins was also comparable. The skin permeability (Kp) of nicorandil was investigated by Sato and co-authors [21] using excised skin samples from hairless mouse, hairless rat, guinea-pig, dog, pig, and human. Among the tested skins, the Kp values of nicorandil in pigs and humans were in good agreement. The authors also found that comparable porcine and human skin permeation could be attributed to similar surface lipids, barrier thickness, and morphological aspects of the excised pig skin samples and human tissue. In another series of experiments, the *in vitro* permeability of pig ear skin was compared with human (abdominal) skin and rat (dorsal) skin using both hydrophilic (water, mannitol, paraquat) and lipophilic (aldrin, carbaryl, fluazifop-butyl) penetrants [13]. Pig skin was found to have a closer permeability character than rat skin to human skin, particularly for lipophilic penetrants. The authors suggested that electrical conductivity measurements across pig skin membranes could be a valuable tool for evaluating the integrity of

Table 1
Thickness of skin strata in rat, mice and humans [10]

	SC, μm	Epidermis, μm	Whole skin, mm
Rat	18	32	2.09
Mouse	9	29	0.70

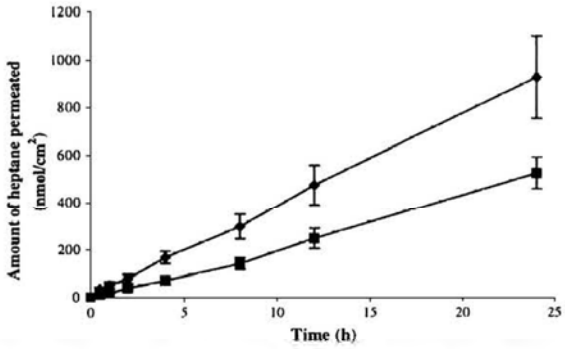

Fig. 1. *In vitro* permeation profiles of heptane across human (squares) and porcine (rhombs) skin (reproduced with permission from Ref. [23]).

membranes. Sekkat et al. [24] reported that differentially tape-stripped, porcine skin could serve as an *in vitro* model for the evaluation of transdermal drug delivery to premature neonates. In this study the passive permeation of caffeine, phenobarbital, and lidocaine and the iontophoretic delivery of lidocaine across tape-stripped porcine skin barriers were tested. The barrier function of the tissue was monitored by measuring the trans-epidermal water loss (TEWL). For all tested drugs, the permeation behavior correlated well with the skin barrier function [24]. The results were sustained by a study on diamorphine *in vivo* absorption in premature neonates [25]. Iontophoretic lidocaine delivery was precisely controlled, independent of the barrier capability. Lin et al [22] compared *in vitro* penetration of theophylline, sodium diclofenac and benzoic acid through artificial cellulose membrane, animal skin (frog, snake with or without scales, nude mice, Sprague–Dawley rat and porcine) and human skin. The fastest permeation of substances was observed through cellulose membrane and frog skin and the slowest through human skin, with benzoic acid being the fastest penetrant through all skin types. In the case of sodium diclofenac the transdermal permeation flux in porcine SC was 33 times higher than in intact skin, but in snake and human skin, the rate through SC was only 2.2 and 1.6 times higher than through intact ones.

A focus of several reports was to compare transdermal permeation kinetics between rodent- and human skin. In a study by Roy et al. [26] permeability coefficients of morphine, fentanyl, and sufentanil across full-thickness hairless mouse skin were in an order of magnitude higher than those found for human epidermis. There was no correlation between the enhancement in percutaneous transport caused by SC removal in hairless mice and human epidermis. Another study examined permeation characteristics of human skin from various sites compared to animal skins, and found that shed snake and hairless rat skin showed similar permeability to human breast and thigh skin, while Wistar rat and nude mouse performed similarly to human cheek, neck, and inguinal skin [27]. Ravenzwaay et al [28] evaluated transport of compounds with various lipophilicities across rat and human skins *in vitro* and *in vivo* in rats. In all cases the *in vitro* dermal penetration through rat skin was higher than *in vivo* and rat skin

(Eq. (1)) to estimate transdermal transport through human skin, based on the combined use of *in vivo* and *in vitro* data:

$$\%\,\text{Percutaneous absorption}_{\text{human}} = \%\,\text{Percutaneous absorption}_{\text{rat}} \times (J_{\text{human}}/J_{\text{rat}}) \quad (1)$$

where J is the percutaneous permeation flux.

In a separate study evaluating *in vitro* percutaneous absorption of four antihypertensive drugs in mice and human cadaver skin, Ghosh et al. reported that the permeation rate in mice skin was much higher than that in human skin [29]. Van de Sandt et al. [30] reported a multi-center skin permeation trial, comparing the *in vitro* absorption of benzoic acid, caffeine, and testosterone compounds through human skin (nine laboratories) and rat skin (one laboratory) in ten European laboratories. All laboratories ranked the absorption of benzoic acid through human skin as the highest of the three molecules (overall mean flux of 16.54±11.87 μg/cm^2×h), while the absorption of caffeine and testosterone through human skin was comparable (2.24±1.43 and 1.63±1.94 μg/cm^2×h, respectively). In this study, no differences were observed between the mean absorption through human skin and the one rat study for benzoic acid and testosterone, however for caffeine, the flux value and the total quantity permeated across the rat skin were higher than the correspondent values in human skin.

5. The use of tissue culture-derived skin equivalents in transdermal research

A number of tissue culture derived skin equivalents such as living skin equivalent models (LSEs) and human reconstructed epidermis (HRE) have been used to measure percutaneous absorption. These models generally are comprised of human cells grown as tissue culture and matrix equivalents normally present in skin, and are utilized as alternatives to animal skins. LSEs resemble human skin, having a dermis, epidermis and partially-differentiated stratum corneum, but are deficient in skin appendages including pilosebaceous units, hair follicles and sweat glands [31]. These tissues provide much lower barrier properties than the whole skin due to their structure and lipid composition. For this reason, the kinetic parameters of skin permeation obtained when using LSEs usually highly overestimate flux across human skin. For example, in a study by Schmook et al., the permeation characteristics of human, porcine and rat skins with the Graftskin® LSE and the Skinethic ® HRE models were compared using four low molecular weight dermatological drugs with various hydrophilicities [32]. The permeation of more hydrophobic compounds (clotrimazole and terbinafine) through the skin equivalents resulted in an 800–900 fold higher flux than through split-thickness human skin. On the other hand, transdermal flux of a less hydrophobic compound, salicylic acid, was in the same order of magnitude as fluxes obtained with human skin. In this study porcine skin performed as the most appropriate model for human skin and they concluded that reconstituted skin models are not suitable for *in vitro* penetration studies [32]. A similar conclusion was drawn from results of another study in which Bronaugh et al. [33] evaluated LSE and human cadaver skin. In the case of cadaver skin, the permeability coefficient increased as the carbon chain length increased. However, this relationship was not observed in the permeability coefficients of these esters across LSE. Moreover, LSE showed very low resistance to flux compared to cadaver skin as the permeability coefficients of these esters through LSE were an order of magnitude higher than through cadaver skin.

On the other hand, numerous reports support the use of skin equivalents for evaluation of skin irritation [31,34]. In a study by Monteiro-Riviere and colleagues [35], EpiDerm LSE ® was found to be morphologically and biochemically comparable to normal human epidermis, providing a model in toxicological and skin metabolism studies. Ponec and Kempenaar [36] reported that architecture, homeostasis and lipid composition of reconstructed human skin models (EpiDerm ®, SkinEthic ®, Episkin ® and RE-DED ®) were comparable to native human tissue. It is noteworthy that Colipa, the European Trade Assocation for cosmetic and toiletry industry, recommends the use of *in vitro* reconstructed skin equivalents as the preferred testing model for skin irritation studies [34]. However, the overall use of skin cultures is likely to be limited due to questionable performance as a barrier in skin permeation studies, as well as due to their cost and data reproducibility.

6. *In vitro* skin permeation studies focusing on delivery systems

Correlation of permeation between animal and human skin studies from drug delivery systems and pharmaceutical dosage forms has attracted significant attention from the pharmaceutical industry, academia, and regulatory sectors. Design and optimization of carriers for active agents is a time- and resource-consuming process that is an integral part of the development of any drug delivery system. *In vitro* tests reflecting bioavailability data are required to prove that a new delivery carrier is bioequivalent with or superior to the standard. Mechanistic studies with sophisticated carriers are performed in animal and human skin to try to predict the future performance of the drug delivery systems in humans from *in vitro* data.

Among the drug delivery systems tested were carriers based on chemical skin permeation enhancers, specially designed vesicles, physical and microinvasive techniques. Touitou et al. [37] tested transport of tetrahydrocannabinol from an enhancing carrier containing 10% w/w oleic acid/propylene glycol/polyethylene glycol 4000/ethanol mixture. In this study drug permeation across Sabra-strain rat skin was found to be about 12.8-fold higher than across human skin. Differing lag times, 11.5 vs 8.5 h for the rat and human skin, respectively, may point toward different diffusion pathways for this drug across the skin of these two species. Priborsky and Muhlbachova [38] assessed the effect of chemical permeation enhancers on the *in-vitro* transport across human skin as compared to animal models. Rat skin was ~3.3–4 times more permeable than human tissue. Using rat skin, the least potent enhancer was dimethylsulphoxide and the maximum permeation enhancement was observed

guinea-pig skins were not significantly different in the permeation of N-methyl-2-pyrrolidone. In another study, transdermal delivery of 6-beta-naltrexol, the active metabolite of naltrexone, across human skin and guinea pig skin *in vitro* and in hairless guinea pigs *in vivo* was assessed from a propylene glycol/ buffer mixture [39]. *In vitro* flux of naltrexone was about 2.3 and 5.6 times higher than 6-beta-naltrexol across guinea pig and human skin, respectively, and 6-beta-naltrexol lag times were longer in both skin types (Fig. 2). *In vivo* studies in guinea pigs showed that the steady-state plasma level of naltrexone was twofold greater than 6-beta-naltrexol, which correlated well with *in vitro* data in guinea pig skin. Rigg and Barry [40] investigated the skin permeability of two species of snake (*Elaphe obsoleta, Python molurus*) compared to *in vitro* experimental results for human skin and for hairless mouse. The effect of typical enhancers on the permeabilities of the membranes to a model penetrant 5-fluorouracil (5-FU) was evaluated. The studied enhancers were 3% Azone in Tween 20/ saline, propylene glycol (PG), 2% Azone in PG, and 5% oleic acid in PG. The data from snake membranes showed minor effects of the enhancers, while for hairless mouse skin, the enhancer effects were significant. None of the membranes was a completely reliable model for human percutaneous absorption in assessing the effect of skin permeation enhancers. The authors concluded that human skin should be used in skin permeation studies and not hairless mouse or snake skin; otherwise, misleading results may be obtained.

Kanikkannan and colleagues [41] evaluated the effect of species variation (rat, rabbit, mouse, guinea pig and human) on the transdermal iontophoretic permeation of timolol maleate.

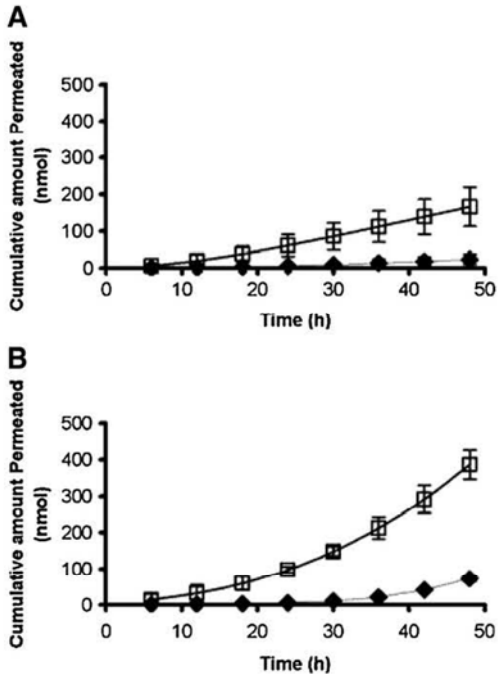

Fig. 2. Cumulative amount of naltrexone (squares, $n=7$) and 6-beta-naltrexol

Interestingly, the amount of timolol transported during iontophoresis (2 h) was significantly different among the various skin species, but the final quantity of timolol crossing the skin during 24 h (2 h iontophoresis and 22 h post-iontophoretic passive diffusion) was comparable in the different species. According to this data, iontophoresis may diminish interspecies variations in *in vitro* skin permeation studies. Microinvasive techniques (microneedles, RF skin ablation, etc.) represent another means of skin permeation enhancement. Recently Wang et al. [42] imaged infusion of dye molecules, insulin, polymer microparticles, and cells into the skin by brightfield and fluorescence microscopy following the insertion of hollow glass microneedles into hairless rat skin *in vivo* and human cadaver skin *in vitro*. Studying the flow mechanism the authors reported that using both models, partial retraction of the needle by withdrawing 100–300 μ or vibrating the microneedle array dramatically increased infusion flow rate.

7. Animal models for evaluation of skin absorption in humans: molecules

In studies conducted in the 1970s and 1980s, transdermal absorption of various radio-labeled molecules in human volunteers and animals was assessed [43–45]. In these studies, the same concentration of substance (4 μg/cm^2) was applied on the forearm of subjects in an attempt to standardize the application conditions, and percutaneous absorption was quantified by following the excretion of the tracer for 5 days. Bartek et al. [45] undertook a comparative study of percutaneous absorption of haloprogin, acetylcystein, cortisone, caffeine and testosterone *in vivo* in various animal species (rats, rabbits, miniature swine) and humans. The highest extent of percutaneous absorption was observed with haloprogin, with complete absorption in rats and rabbits but not in humans and pigs. In rats and rabbits the absorbed fraction of applied dose followed the order: acetylcystein<cortisone<caffeine=testosterone<haloprogin. *In vivo* data from man and pigs indicated that the order of absorption was: acetylcystein<cortisone<haloprogin<testosterone<caffeine. The authors concluded that the transdermal absorption in rats and rabbits was not predictive for human data, while results obtained in porcine model and humans were comparable.

Using the same technique, Wester and Maibach [46,47] compared the percutaneous absorption of various molecules between rhesus monkey and humans. They found that the *in vivo* percutaneous absorption of hydrocortisone, testosterone and benzoic acid was similar for rhesus monkey and man. For example, when hydrocortisone, testosterone and benzoic acid were applied at a dose of 4 μg/cm2, the absorbed dose was 2.9, 18.4 and 59.2% vs. 1.9, 13.2 and 42.6% in monkey vs. humans, respectively. Bronaugh and Maibach [48] measured the percutaneous absorption extent of five nitroaromatic compounds (p-nitroaniline, 4-amino-2-nitrophenol, 2,4-dinitrochlorobenzene, 2-nitro-p-phenylenediamine, nitrobenzene) in humans and monkeys using both *in vitro* and *in vivo* techniques. It was found that except for the highly volatile nitrobenzene, no significant differences were observed in the four

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