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

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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

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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. Since the rate limiting step for skin absorption of most molecules is considered to be this non-viable layer, percutaneous permeation 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 better than hairy skin [19]. In these animals there is no need for hair removal (clipping or shaving) prior to the experiment, thus

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
Human	17	47	2.97

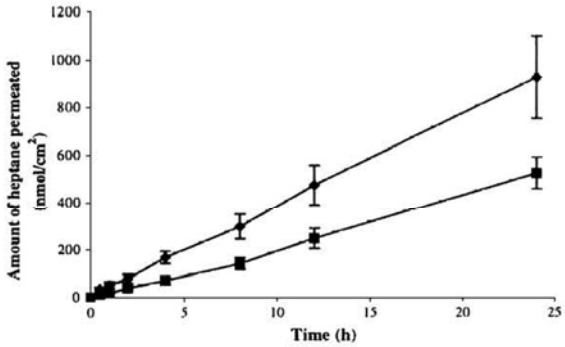

Fig. 1. *In vitro* permeation profiles of heptane across human (squares) and porcine (rhombs) skin (reproduced with permission from Ref. [23]).

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 was approximately 11-fold more permeable than human skin. These authors suggested the use of the following equation

(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 Roy et al. [33] evaluated the *in vitro* permeabilities of alkyl p-aminobenzoates through 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 with sodium laurylsulphate. In contrast all the tested enhancers performed comparably to human skin. In this study, human and

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.

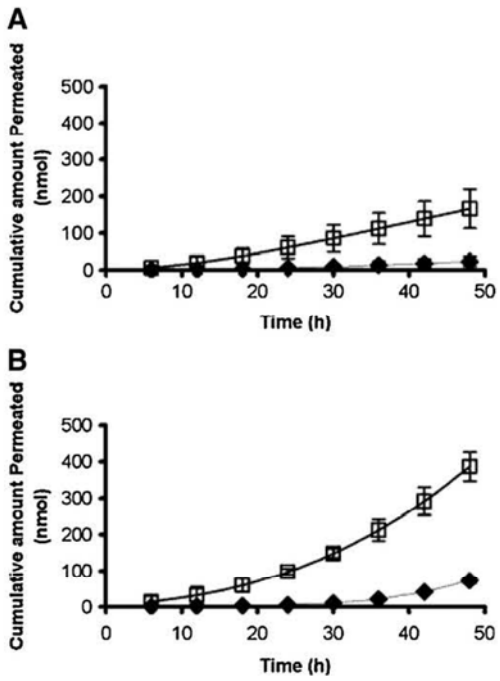

Fig. 2. Cumulative amount of naltrexone (squares, $n=7$) and 6-beta-naltrexol (rhombs, $n=8$) permeated through the human skin (A) and guinea pig skin (B) (reproduced with permission from Ref. [39]).

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 groups of data. Andersen *et al.*, used the same methodology to evaluate percutaneous absorption of ^{14}C ring-labelled

hydrocortisone, testosterone and benzoic acid *in vivo* in guinea pigs and compare the obtained results to previous human data [49]. The absorption of hydrocortisone and benzoic acid was similar to the published human absorption data, but testosterone was absorbed to a greater extent in guinea pigs than in man. Interestingly, in this work a thioglycollate based depilatory cream significantly increased the extent of transdermal permeation of testosterone. Although the above studies [43–49] used radiolabeled molecules (whose weakness is the accurate detection of the original compound), the clear advantage of these early works was their ability to compare skin absorption of a large series of molecules using the same experimental protocol.

Later reports used more advanced analytical methods for evaluation and comparison of percutaneous absorption in animals and humans. Wester et al. [50] employed inductively coupled plasma-mass spectrometry for quantitation of biological samples of boric acid, borax and disodium octaborate tetrahydrate after their application on the skin. They compared the usefulness of finite and infinite dose permeation methodologies across human skin to absorption data in humans. The results from the finite dose model were much closer to the *in vivo* absorption data, while the infinite dose methodology differed by 10-fold from the *in vivo* results. Cnubben and colleagues [51] measured the percutaneous absorption of orthophenylphenol, a fungicide, in rats, humans and a perfused pig ear model. The drug was applied in a hydroethanolic vehicle and samples from *in vivo* studies were evaluated using capillary gas chromatography with MS detector. *In vivo* results indicated that in human volunteers, approximately 27% of the applied dose was excreted with urine within 48 h versus 40% excreted in rats. Among the *in vitro* parameters tested, the fraction of applied dose most accurately predicted human *in vivo* percutaneous absorption of the drug (Fig. 3). With respect to the other parameters studied, considerable differences were observed between the various *in vitro* models.

Skin permeation studies using inadequate protocols will generate inaccurate data. Currently used sunfilters are lipophilic substances with relatively low molecular weight, thus possessing a good potential to be systemically absorbed across the skin. In fact, for a long period of time scientists have been aware of the issues of potential toxicity caused by the percutaneous absorption of chemical sunscreens. Recently these concerns have been confirmed in numerous reports [52–54]. However, the experimental conditions, such as a hydrophilic receiver fluid that is used in many *in vitro* skin permeation experiments with sunscreens, generally do not permit a good clearance of these molecules from the skin. For example, one study compared the skin penetration of benzophenone-3 (BPH), ethylhexyl methoxycinnamate, butyl methoxydibenzoyl methane, ethylhexyl salicylate and homosalate, from two vehicles, an oil-in-water (O/W) emulsion gel and petrolatum jelly, both *in vitro* and *in vivo*. The receptor fluid used in *in vitro* experiments was saline containing 1.5% BSA and at these conditions none of the filter agents permeated through the skin and negligible amounts were detected in various skin layers after 6 h of product application. Also, the effect of the vehicle was minimal in the *in vitro* permeation experimental setup. On the other hand, in humans

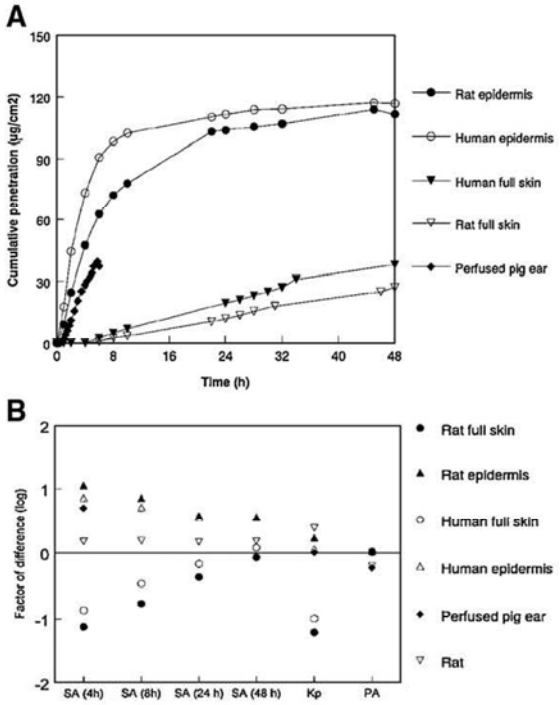

Fig. 3. Transdermal absorption of [^{14}C]ortho-phenylphenol, in vitro-in vivo correlation: (A) Cumulative amount of [^{14}C]ortho-phenylphenol ($n=6$) permeated *in vitro* through human viable skin, rat viable skin, human epidermal membranes, rat epidermal membranes and perfused pig ears; (B) Factor of difference (FOD) between in vitro and in vivo skin absorption of [^{14}C]ortho-phenylphenol based on the systemically available (SA) amount at 4, 8, 24, and 48 h after a 4-h exposure period of 120 µg/cm^2, the permeability coefficient (Kp), and the potentially absorbed dose (PA) in humans and rats (reproduced with permission from Ref. [51]).

the amount of sun filtering agents accumulated in the SC was significantly higher (around 3 times) with the O/W emulsion gel than with the petroleum jelly, which was reflected also in SPF measured *in vivo* 30 min after application of the products [55]. Yet, when an appropriate receiver fluid was used, a large amount [9% from the applied dose] of octylmethoxycinnamate (OMC) permeated the skin [56]. In this study, the OMC skin permeation flux was 27 µg/cm^{2}h. It is important to keep in mind that sunscreen formulations are applied to a large skin area (>1.5 m^2) and for a long period, producing a constant and high input of the chemical into the viable skin strata and to the systemic circulation. These *in vitro* results are supported by data from a number of human and animal *in vivo* studies. Hayden et al. [57] reported that BPH has been detected in human urine and in breast milk, with up to 1–2% of the applied amount estimated to be absorbed into the body. Treffel and Gabard [58] have shown significant amounts of BPH, OMC, and octylsalicylate recovered from tape-stripped stratum corneum demonstrating that these UV filters penetrate into the epidermis.

8. Animal models for evaluation of skin absorption in humans: delivery systems

Enhanced delivery of drugs by means of specially designed vesicular carriers is currently one of the more exciting ways to

enhance drug delivery into and across the skin. An ethosomal antibiotic system was evaluated in a mouse model for deep skin infection [59,60]. The efficiency of ethosomal erythromycin applied to the skin-infected site was compared with parenteral or topical drug administration in a hydroethanolic solution. It was found that ethosomal erythromycin was as effective as the systemically administered erythromycin. A very efficient healing of *S. aureus*-induced deep dermal infections and zero bacterial skin counts were measured when the mice were treated topically with ethosomal erythromycin [59,60]. These results obtained in an animal infection model with antibiotic ethosomes correlate well with data obtained *in vitro* with fluorescent probes. In a clinical trial on another anti-infective agent, acyclovir (ACV), ethosomal ACV cream was compared to a commercial product (Zovirax®, ZC) in 40 subjects (61 herpetic episodes) [61]. Ethosomal acyclovir significantly improved all evaluated clinical parameters (time to crust formation, the healing period and the percentage of abortive lesions) in both parallel and cross-over arms. As an example, in the crossover arm, both the time to crust formation and the healing period were significantly reduced with ethosomal acyclovir vs. ZC (1.8 vs. 3.5 days and 4.2 vs. 5.9 days, respectively). These data could be explained by the efficient delivery of the drug to its target tissue in the deep epidermis as demonstrated in the animal infection model.

A study by Chien and co-workers [62] examined several transdermal patch formulations of levonorgestrel and 17 beta-estradiol. Based on the results of *in vitro* permeation experiments through human cadaver skin and *in vivo* data obtained in rabbits, the authors selected the most adequately performing system to be tested in humans. A Phase I clinical bioavailability/dose proportionality study, was carried out in 12 healthy female volunteers. Results of the pharmacokinetic and pharmacodynamic analyses demonstrated that the hormone patch was capable of maintaining a dose dependent steady-state serum level of levonorgestrel throughout the 3-week treatment period by weekly applications of one or two patches (10 or 20 cm^2). A good correlation was therefore found between the rates of transdermal delivery measured in *in vitro* studies using human cadaver skin, the *in vivo* measurements in rabbits, and the results obtained in humans.

9. Mathematical models of skin absorption

Skin permeation of molecules is a multifactorial phenomenon depending on diverse types of physical, chemical and biological interactions. A large portion of these interactions is nonlinear, making mathematical modeling of percutaneous absorption difficult. Reviews by Yamashita and Hashida [63] and Moss et al. [64] summarize the main approaches for mathematical modeling of skin permeation. Below we give examples of some frequently reported mathematical correlations for prediction of transdermal transport of a permeant, quantitative structure–permeability relationship (QSPR) and estimation of the *in vitro-in vivo* correlation (IVIVC) in skin transport for a drug and dosage form.

Since the main obstacle of skin permeation of solutes lies in a nonviable SC layer, Fick's diffusion law has generally been accepted for the description of skin transport of permeant. According to this law, diffusion is assumed to be a course of mass transfer of individual solutes, driven by random molecular movement and the rate of transport is expressed in Eq. (2).

$$dC/dt = K \times D \times C_0/h \quad (2)$$

where C_0 is the donor concentration; K is the partition coefficient; D is the diffusion coefficient; and h is the thickness of the barrier. However, when a broad variety of molecules (especially highly hydrophilic ones) is taken into account, the mathematic relationships are more complicated due to the heterogeneity of skin structure having at least two parallel diffusion patterns (polar and nonpolar). These models consider skin permeation of drugs as a function of the transport across two layers, lipophilic SC and hydrophilic viable tissue, and polar and nonpolar pathways exist in the former layer [62,64–66]. For example, Anissimov and Roberts [65] developed a diffusion model for the percutaneous absorption of a molecule present at a constant donor concentration. This model takes into account a viable epidermal and a donor-stratum corneum interfacial resistance and a receptor removal rate.

QSPR methods attempt to relate statistically the experimentally determined kinetic data of percutaneous absorption of a range of exogenous chemicals to known physicochemical parameters [64]. The main criticism associated with these models is related to the uncertainty in their application to percutaneous absorption, predominantly due to (a) the limitations of the models developed in terms of statistical fit; (b) their obvious failure under severe non-linear conditions; and (c) the inability to extrapolate their conclusions to other systems, particularly when carrier effects have to be taken into account [64,67]. There are also other issues and concerns regarding the validity of such approaches to understanding mechanisms of skin permeation.

Many of QSPR models are based on Flynn's dataset [68] of 97 permeability coefficients for 94 compounds obtained *in vitro* through human skin (with the exception of *in vivo* studies for toluene, ethylbenzene and styrene). Flynn's heterogeneous human skin permeation database until now remains the largest one. Since it brings together kinetic results from 15 various literature sources, it contains a high degree of experimental error resulting from inter-laboratory variability. Mathematical QSPR relationships based on this dataset [63–72] and other QSPR models were exhaustively reviewed by Moss et al. [64]. It is important to note that, most existing QSPR models are based on experimental results obtained with saturated aqueous or ethanolic solutions of a permeant, therefore these aspects should be taken in account when considering issues of dermal toxicology and risk assessment.

In vitro skin permeation studies are frequently performed for screening of molecules and drug carrier systems aimed at optimising dermal or transdermal delivery. Therefore, one of the main objectives of *in vitro* permeation studies is prediction of *in vivo* absorption. A number of reports present attempts to mathematically correlate or predict from *in vitro* permeation data to *in vivo* drug levels based on a diffusion model [37,73–76] or a convolution technique [77]. In their study, describing for the first

time transdermal delivery of cannabinoids, Touitou and colleagues [37] assessed the permeability of tetrahydrocannabinoid (THC) from an enhancing carrier *in vitro* through human and rat skin and *in vivo* in rats. By substituting the permeability coefficient (Kp), calculated from the *in vitro* experiments across human skin, into Eq. (3) the authors predicted THC steady-state blood levels in humans following application of the transdermal formulation.

$$\mathrm{Css} = A \times C \times \mathrm{Kp}/\mathrm{Vd} \times \mathrm{Ke} \quad (3)$$

where A is the area of application to the skin; C is the initial drug concentration in the donor; Vd is the volume of distribution; and Ke is the elimination constant. Using the same equation, Ainbinder and Touitou [75] estimated that the required application area for obtaining physiological testosterone human plasma levels for a nonpatch system is 40 cm^2, which is approximately 10 fold less than for the currently marketed products. Yamashita et al. [74] applied a deconvolution method to obtain *in vivo* absorption profiles of mannitol (a hydrophilic compound) and butylparaben (a lipophilic compound) and to correlate between them and corresponding *in vitro* permeation profiles. The mathematical analysis was based on the diffusion model and indicated (a) that the diffusion length of a viable layer was shorter *in vivo*, probably due to the washout by microcirculation, and (b) that the effective area of the polar pathway was larger *in vitro*, probably due to the hydration effect.

Empirical approaches for prediction of skin transport of solutes, based on their molecular structures, have been proposed. These models take into account various molecular or structural descriptors without mechanistic consideration, thus resulting in permeability–descriptor relationship. Artificial neural network (ANN) is a powerful and promising technique for non-linear modeling of complex causal–effect relationships. Lim and colleagues [78] utilized a combination of molecular orbital (MO) calculations and ANN to predict the human skin permeability constant (log K_p) of molecules listed in Flynn's dataset from 3D molecular structure (Fig. 4). The molecular descriptors assessed from MO-calculations included dipole moment, polarizability, sum of charges of nitrogen and oxygen atoms, and sum of charges of hydrogen atoms bonding to nitrogen or oxygen atoms. The resulting ANN model was much superior to the conventional multiple linear regression model in terms of root mean square errors (0.528 vs. 0.930, respectively). ANN analysis was used in a study by Degim et al. [79] for estimation of skin permeability of 40 compounds. In this study, ANN produced log K_p values that correlated well with the experimental data (r^2=0.997). Further, the authors experimentally tested Kp for some new molecules across human skin, demonstrating that it was possible to predict the experimental data from the proposed ANN model. In practice, the lack of correlation between *in vivo* parameters and *in vitro* drug permeation data is attributed a number of reasons related to variables in study design, complexity of physicochemical and physiological conditions of transdermal absorption and numerous other biological, physical and chemical factors [63,64]. Comparative *in vitro–in vivo* studies, reviewed in this article indicate that *in vitro* and *in vivo* transport is certainly correlated but highly variable in extent. To summarize,

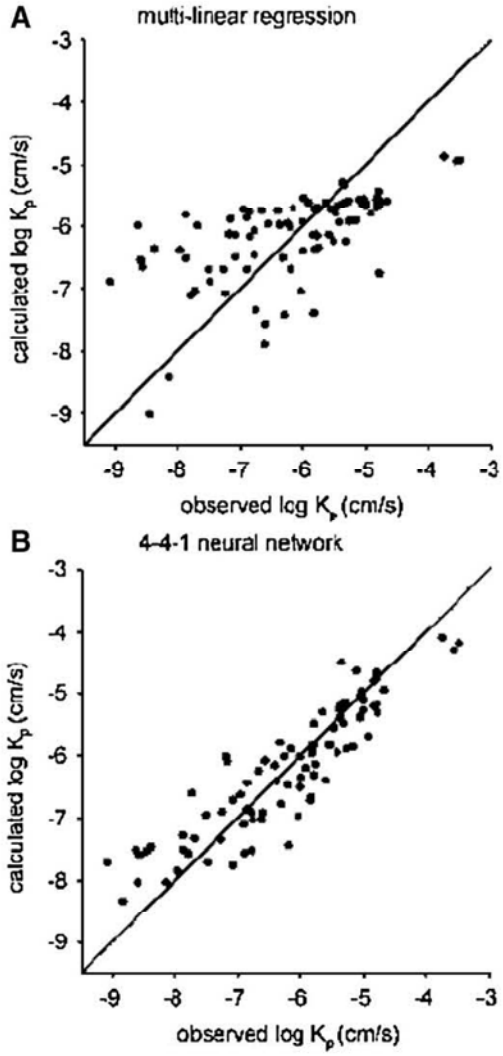

Fig. 4. Relationship between experimental and calculated skin permeability coefficients: (A) analysis using multiple linear regression and (B) analysis using 4–4–1 neural network (reproduced with permission from Ref. [78]).

mechanistic models (e.g. QSPR) can provide us with some information on understanding transdermal transport of molecules. Alternatively, empirical models (e.g. ANN), can offer better predictions since they overcome some of the issues related to uncertainties present in mechanistic models.

10. Conclusions

Dermal/transdermal absorption is a multi-factorial multi-step process, which is affected by a number of factors including the animal source and type of skin, physicochemical properties of the tested compound and delivery systems, as well as possible skin pretreatment and environmental factors. For a number of tested systems there is a correlation between *in vitro/in vivo* data acquired in animals and in humans. Yet, there are also many examples indicating poor correlation. To date, a limited number of validated correlations have been reported. It is noteworthy that in some cases, the pharmacodynamic effect and absorption profiles are higher than could be presumed from *in vitro*

permeation data. The main reason for this behaviour may lie in the efficient clearance of the penetrant by skin microcirculation.

To draw conclusions from the existing information on the suitability of the various animal models for transdermal absorption in man, it can be summarized that: (1) in *in vitro* experiments, porcine skin has been shown to perform comparably with human skin. (2) tissue culture human skin and epidermis equivalents generally possess lower barrier characteristics than human skin, making them questionable for permeation studies. On the other hand, these models could be of significant assistance in the evaluation of skin irritation and metabolism; (3) selected data obtained using *in vivo* and *in vitro* models correlate well with studies in humans.

In conclusion, *in vitro* permeation experiments and animal models, with all their limitations, provide important tools for screening drug delivery systems, skin permeation enhancers and drug delivery carriers. Also, these tools make it possible to estimate the rank order of percutaneous absorption of a series of molecules.

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