Enhanced Absorption through Damaged Skin: An Overview of the in vitro Human Model

S. Gattu^a H.I. Maibach^b

^aIrvine School of Medicine and ^bDepartment of Dermatology, University of California, San Francisco, Calif., USA

Key Words

Percutaneous penetration • Damaged skin • In vitro skin human model

Abstract

Background/Aim: Quantifying percutaneous absorption of topical drugs as well as those compounds relevant to occupational exposure is important for assessing delivery, efficacy and toxicology. Methods for assessing absorption are established for intact skin; however, what may be equally relevant is how much absorption occurs through damaged skin. **Methods:** The Embase database was accessed online in March 2009 in search of human in vitro studies measuring absorption through damaged or diseased skin. **Results:** Few studies have measured absorption through damaged human skin in vitro but those that have demonstrate a modest but clear enhancement in absorption with enhancement favoring hydrophilic molecules. **Conclusions:** Damaged or diseased skin may display a modest increase in absorption compared to intact skin; however, more studies with consistent methods and correlations to in vivo data are needed to fully elucidate how much absorption occurs through damaged skin.

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Introduction

The stratum corneum functions as a barrier to foreign chemicals and water loss. Absorption of industrial solvents and topical medications through intact skin is well studied and an in vitro model established [1]. However, no such model exists for diseased skin although its importance cannot be overstated in light of studies showing a high prevalence of skin disease in the workplace and skin problems being the most dominant reason for work absences [2]. Additionally, topical products are generally formulated and used for the treatment of abnormal or diseased skin. Common skin damages such as bruises, skin irritation, and dermatitis might increase dermal absorption [3–5].

The guidelines for evaluating the risks and hazards of industrial compounds through in vitro absorption studies set out by the Organization for Economic Cooperation and Development, the US Environmental Protection Agency, and the World Health Organization each use a model of normal intact skin rather than damaged skin [6–8]. The International Commission on Occupational Health committee on Occupational and Environmental Dermatoses identifies this topic as a current problem in determining skin notation for various compounds [9]. In addition, understanding the absorption of compounds through damaged skin is valuable in assessing efficacy of

Table 1. Penetration enhancement through tape-stripped/abraded skin

Method	Penetrant	MW	log K_{oct}	EF	Measured	Author
Tape stripped	caffeine	194	-0.07	19	flux	Akomeah et al. [16]
	methyl paraben	152	1.96	5.00	flux	
	angiotensin II	1,046	-1.7	25-69	flux	
	butyl paraben	194	3.6	3.00	flux	
	tritiated water	22	1.38	21.00	% dose absorbed	
Abrasion	caffeine	194	-0.07	64.00	flux	Akomeah et al. [16]
	methyl paraben	152	1.96	6.00	flux	
	angiotensin II	1,046	-1.7	57-93	flux	
	butyl paraben	194	3.57	3.00	flux	
	acyclovir	225	-1.56	36-220	flux	
	caffeine	194	0	1.60	% dose absorbed	Bronaugh and Stewart [21]
	tritiated water	22	1.38	12.50	% dose absorbed	
	benzoic acid	122	1.9	2.10	% dose absorbed	
	cortisone	360	1.6	3.90	% dose absorbed	
	nicotinic acid	123	-0.2	11.50	% dose absorbed	
	phenol	92	1.482	1.70	% dose absorbed	
	propylene glycol	76	-1.7	5.30	% dose absorbed	
	urea	60	-2.6	7.10	% dose absorbed	

Enhancement in percutaneous penetration is more pronounced in hydrophilic compounds with low log K_{oct} values compared to lipophilic compounds with high log K_{oct} values. MW = Molecular weight.

pharmaceuticals for topical use. It is therefore invaluable to know the penetrating properties of industrial compounds and drugs through compromised skin.

In the in vitro human skin model, human skin is obtained (typically from the abdomen or chest), frozen at -30°C and sectioned to split thickness, full thickness or isolated stratum corneum [1]. The Franz type diffusion cell is the most widely used device to measure both permeability and penetration which consists of donor and receptor chambers separated by skin. A jacket is in place to maintain temperature and the contents collected in the receptor chamber are analyzed periodically.

While data from animal skin are abundant, some studies suggest that animal skin may be more permeable than human skin [10, 11]. To date, there has been no consensus on under what circumstances animal and human skin share similar permeability. Human models are currently considered a 'gold standard' for studying human dermal risk assessment. Human in vitro studies, compared to in vivo studies, may be advantageous in that multiple replicate experiments are more easily performed and toxic compounds may be studied.

Few studies have examined absorption through damaged human skin in vitro. Moon and Maibach in 1991 [5] previously examined this issue; readers will find their

overview an introduction to this subject. This paper surveys the literature and estimates the degree to which absorption is affected in damaged skin and how the type of damage and properties of the diffusants affect both permeation (diffusion through skin) and penetration (diffusion through various layers of the skin).

Methods

The Embase database was accessed in March 2009 and was searched utilizing the key words 'absorption, permeability, penetration, damaged skin, compromised skin, and barrier perturbation'. In vitro studies measuring differences in absorption through damaged human skin compared to intact skin were selected. Studies were limited to human, in vitro, those written in English, and permeability/penetration enhancement data extracted. Studies were excluded if damage occurred simultaneously with the application of the diffusant rather than sequentially.

Results

Eight studies meeting the criteria are summarized in tables 1-3 and were categorized by method of damage: mechanical, chemical, and biocidal. Because absorption

Table 2. Penetration enhancement through surfactant/solvent/delipidization/irritant induced skin

Method	Penetrant	MW	log K _{oct}	EF	Measured	Author
SLS 0.25%	tritiated H ₂ O	22	1.38	1.80	total pp	Frankild et al. [28]
	¹⁴ C-hydrocortisone	364	1.4	n.s.		
	nickel	134	0.05	0.00		
SLS 2%	tritiated H ₂ O	22	1.38	3.30		
	¹⁴ C-hydrocortisone	364	1.4	n.s.		
	nickel	134	0.05	0.00		
SLS 10%	tritiated H ₂ O	22	1.38	4.20		
	¹⁴ C-hydrocortisone	364	1.4	4.30		
	nickel	134	0.05	increased		
SLS 0.1%	dimethoate	229	0.7	1.85	total pp	Nielsen [30]
	pirimicarb	238	1.7	1.80	K _p	Nielsen et al. [29]
	paclobutrazol	293	3.2	2.00		
	methiocarb	225	3.34	n.s.		
	glyphosate	170	-1.7	24.00		
	caffeine	194	0.16	5.00		
	benzoic acid	122	1.83	n.s.		
	malathion	330	2.75	2.00		
SLS 0.3%	pirimicarb	238	1.7	1.70	total pp	Nielsen [30]
	paclobutrazol	293	3.2	2.00		
	methiocarb	225	3.34	n.s.		
	dimethoate	229	0.7	2.75		
Ethanol	methyl paraben	152	1.96	4.08	flux	Akomeah et al. [16]
	butyl paraben	194	3.57	3.75		
	caffeine	194	-0.07	15.46		
Delipidization	methyl paraben	152	1.96	3.85		
	butyl paraben	194	3.57	4.16		
	caffeine (infinite/finite)	194	-0.07	10.93		
	angiotensin II	1,046	-1.7	44-75		

Enhancement in percutaneous penetration is overall modest but more pronounced in hydrophilic compounds with low log K_{oct} values compared to lipophilic compounds with high log K_{oct} values. MW = Molecular weight; total pp = total percutaneous penetration; n.s. = not significant.

Table 3. Modest penetration enhancement through biocide-damaged skin

Method	Penetrant	MW	log K _{oct}	EF	Measured	Author
ADBAC	¹⁴ C-proxopur	209	1.5	increased	K _p	Buist et al. [33]
	tritiated H ₂ O	22	1.38	increased		
Deltamethrin	¹⁴ C-proxopur	209	1.5	increased		
	tritiated H ₂ O	22	1.38	n.s.		
DDAC	¹⁴ C-proxopur	209	1.5	increased		
	tritiated H ₂ O	22	1.38	increased		
Permethrin	¹⁴ C-proxopur	209	1.5	increased		
	tritiated H ₂ O	22	1.38	increased		

Enhancement in percutaneous penetration is overall modest. MW = Molecular weight; ADBAC = alkyl dimethyl benzyl ammonium chloride; DDAC = didecyl dimethyl ammonium chloride; n.s. = not significant.

enhancement may rely on the lipophilic nature of the compound [12, 13], the log of the octanol:water coefficient ($\log K_{\text{oct}}$) of compounds was recorded. The $\log K_{\text{oct}}$ values reported here were taken from publications cited in the tables, or from values obtained from an online search engine accessing chemical data from suppliers (www.emolecules.com), and The PubChem Project (<http://pubchem.ncbi.nlm.nih.gov/>).

Mechanical Damage

Mechanical damage provides one model for a diseased state of the skin [14, 15]. Mechanical damage and stratum corneum removal through tape stripping can increase transepidermal water loss (TEWL) to a degree possibly seen in various dermatoses and is the most widely used method of barrier perturbation mainly due to its accessibility and simplicity. Abrasion by means of a brush bristle or a needle drawn across the skin's surface has been shown, by transmission electron microscopy, to loosen the top layers of the skin [16]. Abrasion may also enhance and control the delivery of vitamin C, 5-aminolevulinic acid, vaccines and biopharmaceuticals [17–20]. However, mechanical damage has infrequently been employed *in vitro* in human skin as a means to study absorption enhancement.

Akomeah et al. [16] and Bronaugh and Stewart [21] examined the effects of tape stripping and abrasion on human abdominal epidermis and found that caffeine, tritiated water, acyclovir, urea and nicotinic acid (K_{ow} –2.12 to 1) permeation was greater than that observed for the more lipophilic substances, i.e. benzoic acid, cortisone, phenol, methyl paraben and butyl paraben (K_{ow} 1.5–3.6) (table 1).

However, the degree of permeation enhancement differed between them. While Akomeah et al. [16] reported a 64-fold enhancement of caffeine using abrasion, Bronaugh and Stewart [21] only observed a 1.6-fold increase. In addition, Akomeah et al. [16] found that abrasion produced a higher enhancement factor (EF) than tape stripping, while Bronaugh and Stewart [21] observed the opposite. This discrepancy can partly be explained by the differing abrasion methods employed by each author. Akomeah et al. [16] utilized a bristle type brush made of hog skin applied to the skin for 25 s while Bronaugh and Stewart [21] had drawn the point of a needle across the surface. The latter method damages only 6.4% of the surface area that is subsequently exposed to the diffusant. The variability in damage methodology highlights the methodological inconsistency across many human *in vitro* permeability studies.

Chemical Damage and Delipidization

Organic solvents are found in degreasers, cleaning agents, solvents for plastics, paints and rubber. Like many industrial chemicals, organic solvents may damage the skin through delipidization by extraction of fatty materials resulting in karyopyknoses on histology [22]. Clinically, this may present as hand dermatitis in industrial workers [23]. Similarly, surfactants such as sodium lauryl sulfate (SLS) are commonly found in cosmetics and drug vehicles and may be irritating. Surfactants may change the composition of the stratum corneum by extracting lipids and converting α -keratin to β -keratin which in turn produces swelling and hydration of the stratum corneum [24–27].

Four studies measuring the penetration of chemically damaged skin were examined (table 2). The duration of pretreatment with the chemical was between 2 and 6 h simulating common situations in an occupational setting.

Frankild et al. [28] showed that increasing the concentration of SLS subsequently increased the amount of cumulated nickel in the receptor. The accumulation of nickel was thought to be secondary to the SLS effect on nickel's binding capacity to the epidermis. The relationship between SLS and nickel, however, was not seen with hydrocortisone until the concentration of SLS was increased to 10% indicating the possible unresponsiveness of damaged skin to increasing lipophilicity.

Neilsen et al. [29] studied the effect of a 0.3% SLS concentration on penetration. Penetration was measured as K_p taking flux into account. After analyzing the epidermis and dermis separately, Neilsen et al. [29] noted that the more hydrophilic compounds had a preference for the epidermis while more lipophilic compounds had a preference for the dermis and damage attracted both compounds towards the dermis. The observation that hydrophilic compounds preferentially deposit in the epidermis may explain why these compounds are more sensitive to damage to the epidermis compared to lipophilic compounds. Also, a decreased lag time was seen with damaged skin for hydrophilic compounds more than lipophilic compounds. In an earlier study by Neilsen [30] in 2005, similar conclusions were drawn through the penetration of pesticides of various lipophilicities with penetration measured as flux and total penetration expressed as amount/surface area $\times$ hour, and amount/surface area, respectively. Increasing the concentration of SLS generally did not increase penetration of lipophilic pesticides. In fact, prochloraz (K_{ow} 4.4; not shown) did not penetrate intact or damaged skin, most likely secondary to its extreme lipophilic nature.

The only study involving damage induced by organic solvents used ethanol and another unidentified form of delipidization and measured penetration of compounds with various lipophilicities. Solvent damage produced enhancing effects more dramatically for hydrophilic molecules like caffeine than for lipophilic molecules such as methyl paraben and butyl paraben [16].

Biocide

Biocidal products are used by professional operators as disinfectants for veterinary hygiene (e.g. slaughterhouses), drinking water, private and public health areas, and as insect repellents. The European Union has labeled many of these as skin irritants or corrosives [31].

Quaternary ammonium may cause cellular swelling and separation of the epidermis and dermis in *in vitro* human skin cultures [32]. Repeated exposure may chronically damage the skin but further effect on permeability is minimal. Only alkyldimethylbenzylammonium chloride and didecyldimethylammonium chloride showed a clear increase in enhancement with single and repeated exposure of the damaging factor while permethrin permeability increased with single exposure only [33]. However, single and repeated exposure to all other biocides including boric acid, formaldehyde, piperonyl butoxide, tebuconazole, and sodium bromide did not increase the skin's permeability to tritiated water or the pesticide proxopur suggesting that workers exposed to these biocides may not be at increased risk of skin permeability to similar compounds. Studies examining a wider array of compounds with varying lipophilicity need to be performed to examine their permeability rates in biocidal damaged skin.

Clinical Disease

Two *in vitro* human studies were found measuring permeability in diseased skin. The lack of data may be explained by the difficulty in obtaining specimens of diseased skin, difficulty in reproducibility, and variability of skin damage.

Atopic dermatitis (AD) primarily has a barrier defect attributed to abnormalities in barrier ceramides [34] resulting in increased TEWL [35]. Correlations between TEWL values and skin permeability of compounds have been suggested [36]. Yoshike et al. [37] reported that theophylline flux through the stratum corneum in lesional AD skin and non-lesional AD skin was roughly twice that observed in non-AD skin. This underscores the importance of considering risks of occupational exposure in patients with a history of atopy even when the patient does not manifest active disease.

Psoriasis has a compromised barrier function by means of partially or completely damaged stratum corneum [38–40] which may result in an increased TEWL. Yet studies on the permeability of exogenous compounds in psoriatic skin *in vitro* are scarce. Wang et al. [41] noted that involved psoriatic skin was much more permeable to anthralin than uninvolved psoriatic skin and that the rate-limiting step to the permeation of anthralin products was their release rates rather than their permeability.

Conclusion

All methods of damage often result in some enhancement of the absorption of molecules, most pronounced with hydrophilic molecules and less so with lipophilics. An additional overall trend was noted consisting of smaller molecules having a higher EF and larger molecules having a lower EF. In general, enhancement was modest on the order of 1 magnitude of 10 implying that despite mechanical, chemical, biocidal damage and clinical disease, not all barrier properties are removed.

While *in vitro* studies produce precise reproducible models, studies are variable in their designs (e.g. skin thickness used, pretreatment time, and how permeability was measured). Many studies measured flux ($\mu\text{g cm}^{-1} \text{s}^{-1}$) or percentage of total dose absorbed; flux differs from percentage of total dose absorbed in that it provides a rate of absorption that may differ temporally (e.g. rate of absorption is faster immediately after contact than 28 h after contact). However, K_p (cm s^{-1}), also a rate, offers a more reliable means of comparison between studies because its measure is not concentration dependent. More *in vitro* tests of skin with clinical disease are needed before considering a modification to the skin notation system.

Just as there are mathematical models correlating *in vitro* permeability to *in vivo* permeability [42, 43] and *in vivo* permeability to the physiochemical properties of the compound [44] with more data and with consistent studies, a mathematical model may be developed to predict absorption enhancement as an economic alternative to *in vitro* and a safe alternative to *in vivo*.

Once the *in vitro* model utilizing compromised skin has elucidated a clear and consistent understanding of absorption of compounds with widely ranging physical and chemical properties, broad comparisons and even correlations to the *in vivo* studies can be drawn.

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