Influence of Skin Irritants on Percutaneous Absorption

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The effects of the application of skin irritants on the *in vitro* percutaneous absorption of three model compounds of diverse physicochemical properties, caffeine, indomethacin, and hydrocortisone, were investigated. Norephedrine and imipramine, basic drugs with a known skin irritation potential, were employed to damage the skin. Treatment with norephedrine increased the permeation of caffeine and hydrocortisone by two- to fourfold, while absorption of indomethacin declined an order of magnitude. A similar result was obtained for the effect of treatment with imipramine on transport of caffeine. Pretreatment with imipramine promoted hydrocortisone absorption 10-fold but, unlike norephedrine, did not alter indomethacin permeation. While both treatments *in vivo* caused an increase (norephedrine > imipramine) in the pH on the surface of skin and after tape-stripping the skin, only norephedrine caused changes in transepidermal water loss *in vivo* in man. Since imipramine was the more severe irritant as judged by erythema, alterations by irritants of barrier function appeared rather complex.

KEY WORDS: skin irritation; percutaneous absorption; norephedrine; imipramine; hydrocortisone; caffeine; indomethacin; transepidermal water loss; skin pH.

INTRODUCTION

Skin permeation of a drug and its corresponding efficacy or toxicity may be influenced by the physiological or pathological state of the skin (1). Since various drugs are employed to control skin irritation or may be accidentally applied to irritated skin, application of drugs and other chemicals to skin compromised by chemical irritants may cause toxic symptoms due to altered absorption. The present study investigates the skin permeation of three drugs of diverse physicochemical properties through skin that has been pretreated with known chemical irritants. Such information is of clinical importance, and little quantitative information is available regarding the effect on penetration of chemically induced skin irritation.

In a previous study, the skin irritation of selected bases, in particular, norephedrine (pK_a, 9.0) and imipramine (pK_a, 9.5) was characterized, and the severity of irritation was correlated with pK_a (2). In the present probe study, concurrent effects on skin irritation, transepidermal water loss (TEWL) and surface pH were investigated in six healthy male subjects. The *in vitro* skin permeation of three model diffusants, i.e., caffeine ($\log P$ = 0.01), indomethacin ($\log P$ = 3.1), and hydrocortisone ($\log P$ = 1.61), were also examined through imipramine and norephedrine pretreated skins. Significant effects on transport and other physical parameters were observed. However, the small number of irritants and solvents employed in this study prevent generalization of these intriguing results.

MATERIALS AND METHODS

Materials

Norephedrine and imipramine were obtained from Aldrich, Milwaukee, WI. Details of the preparation of imipramine free base may be found elsewhere (2). Caffeine, hydrocortisone, and indomethacin were purchased from Sigma Chemical Co., St. Louis, MO. Radiolabeled compounds, [^{3}H]water, [^{14}C]caffeine, [4-^{14}C]hydrocortisone, and [2-^{14}C]indomethacin, were obtained from NEN Products, Boston, MA. The specific activities of the compounds were 1.0 mCi/g water, 39.9 mCi/mmol indomethacin, 51.0 mCi/mmol hydrocortisone, and 55.0 mCi/mmol caffeine. The radiochemicals had a purity greater than 95% as determined by TLC and RTLC scanner (Radiometric Instruments & Chemical Co., Tampa, FL). Skin solubilizer, Soluene-350, and scintillation counter cocktail Ultima Gold were purchased from Packard Instruments Co., Inc., Downers Grove, IL.

Skin Permeation

Human cadaver skin excised from the thigh of a 23-year-old Caucasian male was dermatomed set at 250-μm thickness and stored at −20°C until used within 7 days (3). Two hours prior to the experiment, frozen skin samples were thawed at room temperature. The integrity of each skin membrane was assessed by a tritiated water procedure similar to that of Bronaugh *et al.* (4). The skin transport studies were conducted with flow-through diffusion cells (Model LG-1084-SPC, Laboratory Glass Apparatus Inc., Berkeley, CA). The skin area was 1 cm^2 and the receptor fluid, 0.01% (w/v) gentamicin sulfate in physiological saline, was perfused through the chamber with a multichannel peristaltic pump at a rate of 1.5 mL/h. The donor compartment of the cell was charged with a 200-μl dose of tritiated water (1 μCi) and occluded with Parafilm (American National Can, Greenwich, CT). The receptor fluid was collected hourly for 2 hr in 25-mL scintillation vials, and after adding 10 mL of cocktail, the radioactivity was measured using a liquid scintillation counter (Packard LSC, Tri-Carb. 1500). All measurements were made in triplicate. Skin samples with permeability coefficients (K_p) within the range of 0.5 to 1.0 × 10^{-3} cm/hr were considered to be intact and were used in permeation studies.

Initial studies have demonstrated that imipramine base and norephedrine at a concentration of 5% (w/v) induce moderate to intense erythema in man. Therefore, a 5% aqueous solution of norephedrine and a 5% (w/v) ethanolic solution of imipramine base were used to assess the damage to skin by treatment with irritants. After gently absorbing the

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tritiated water with a cotton-tipped applicator, 0.2 mL of imipramine or norephedrine solutions was applied to the donor side for 4 or 24 hr and the cell was occluded as before. To observe the effect of solvent on drug permeation, skin samples were treated with 0.2 mL of water or ethanol as control pretreatments. After 4 or 24 hr of pretreatment with norephedrine, the excess solution was removed and rinsed four times with 200 μL of distilled water. For imipramine, an alternate treatment with 200 μL of ethanol and water was used. After the washing procedure, the application site was blotted with filter paper and loaded with 200 μL of saturated solutions of caffeine (3850.0 μg/cm^2), indomethacin (35.5 μg/cm^2), or hydrocortisone (55.8 μg/cm^2) and occluded. The drug samples were collected hourly in the initial 4 hr and every 2 hr subsequently up to 24 h on an automated fraction collector (Retriever IV, Isco Inc., Lincoln, NE).

After the experiment, mass balance was obtained (5). This was performed by absorbing the drug solution with a filter paper, followed by an alternating treatment with soap–water solution (1:15; Liquid Ivory, Procter & Gamble, Cincinnati, OH), water, soap–water solution, water, and, again, water. To quantitate the drug content in different layers of the skin, the epidermis was separated from the dermis with forceps, and the layers were immediately dissolved in 1 mL of Soluene 350 (Packard Instrument Co.). Finally, the upper surfaces of the cell were washed with an acetone–methanol solution (1:2). In all experiments more than 95% of the applied dose was recovered. The radioactivity in the samples and in the dissolved skins was determined by liquid scintillation counting (Tri-Carb 4640, Packard Instrument Co.). All experiments were performed in triplicate.

Skin Irritation Study

Six healthy male volunteers aged 25–35 years, who had given their informed consent, participated in this study. Occlusive plastic chambers (Hill Top Laboratories, Cincinnati, OH) were loaded with 200 μL of 5% (w/v) aqueous solution of norephedrine and 5% (w/v) ethanolic solution of imipramine base, applied to the subjects' volar forearms, and covered with adhesive transparent dressings (Tegaderm, 3M, St. Paul, MN). Water and ethanol served as vehicle controls. After 4 and 24 hr of application, the patches were removed and the sites were marked with a marking pen. Transepidermal water loss (TEWL) and skin pH were then measured on intact and irritated skin.

TEWL Measurements

Thirty minutes after removing the patches, TEWL (g/m^2/hr) was measured with an Evaporimeter (Servomed, EP1, Stockholm, Sweden) under neutral-environmental conditions (room temperature, 19–23°C; relative humidity, 48–57%) (6). Skin temperature was monitored with a digital thermistor (Tele-Thermometer, Yellow Springs Instruments, Yellow Springs, OH). TEWL values were corrected for a skin temperature of 30°C (7).

pH of the Skin Surface

The pH measurements of the skin surface were performed with a flat-surface electrode (Model 91-35, Orion Research Inc., Boston, MA) immediately after removing the patches, after washing the application site repeatedly, and after stripping the sites with tape (Scotch 600, 3M, Co., St. Paul, MN) repeatedly 5 and 10 times.

Visual Irritation Scoring

Each site was examined and scored for erythema by the same investigator. The following visual scoring system was adopted: (0) no erythema; (1) very slight erythema (barely perceptible); (2) well-defined, uniform erythema; (3) moderate to marked erythema; (4) marked erythema (beet redness) to slight eschar formation; and (5) fiery erythema with severe edema.

RESULTS

Table I shows the mean TEWL values and erythema scores observed after treatment with norephedrine and imipramine and their respective aqueous and ethanolic controls. All subjects showed an erythematous response with both basic irritants. Application of norephedrine resulted in uniform to moderate erythema after 4 hr of application; the mean erythema was not statistically different after 24-hr treatments. Compared to the control, norephedrine increased the TEWL value approximately threefold. Treatment with imipramine for 24 hr resulted in an intense erythema greater than that produced by norephedrine. After this 24-hr pretreatment, the erythemal response was statistically different from that with all other treatments ($P \leq 0.05$), but the response after a 4-hr pretreatment with imipramine was not statistically different from the control. These results concur with those observed by Berner *et al.* (1990) (2). In spite of the severity of the erythema, little difference in the TEWL was observed after treatment with imipramine, and the values were similar to that of the ethanolic control.

The effects of treatment with these basic irritants on the apparent pH of the skin, in particular that of the deeper

Table I. TEWL and Erythema Score Observed with Basic Irritants

	TEWL (g/m^2/hr; mean ± SD)		Erythema score[a]	
Treatment	4 hr	24 hr	4 hr	24 hr
Water	4.2 ± 0.9	4.7 ± 0.6	0.0 ± 0.0	0.1 ± 0.2
Ethanol	4.0 ± 1.3	4.4 ± 2.1	0.0 ± 0.0	0.3 ± 0.4
Norephedrine	11.1 ± 3.6*	16.7 ± 2.8*	1.2 ± 0.2*	0.9 ± 0.2*
Imipramine	3.8 ± 0.8	4.8 ± 1.3	0.2 ± 0.3	2.4 ± 0.8*

[a] Five-point scale from 0 to 5; see text for details.
* $P < 0.05$ (t test for unpaired data).

layers, are shown in Table II. After 4 and 24 hr of application, norephedrine increased the surface pH of the skin from 4.6 ± 0.2 to 9.7 ± 0.3 and 9.2 ± 0.2 ($P < 0.05$), respectively. The changes in pH observed with imipramine were comparatively smaller. In both treatments, 10 strippings resulted in glistening suggesting that the stratum corneum had been removed. In this tape-stripped skin, the norephedrine and imipramine treatments for 24 hr resulted in significant increases ($P \leq 0.05$, unpaired t test) in pH from 4.9 ± 0.1 to 8.9 ± 0.1 and 7.3 ± 0.2, respectively.

The cumulative skin permeations at 24 hr of caffeine, indomethacin, and hydrocortisone after treatment with vehicle controls, norephedrine, or imipramine for 4 or 24 hr are shown in Table III. Treatment with either norephedrine or imipramine for 24 hr resulted in increased skin transport of caffeine ($P < 0.05$). Note that transport of caffeine from ethanol was twice that from water.

While barrier function might be expected to deteriorate with treatment with irritants, the reverse appeared to be true of indomethacin transport following treatment with norephedrine (Table III). In contrast to treatment with imipramine, which did not affect permeation of indomethacin, the skin transport of indomethacin was reduced by nearly an order of magnitude by pretreatment with norephedrine ($P < 0.05$).

Alteration of hydrocortisone transport (Table III) appeared to be the most sensitive measure of barrier damage. Norephedrine pretreatment for 4 or 24 hr induced two- to threefold increases in the penetration of hydrocortisone ($P < 0.05$). While treatment with imipramine for 4 hr had no significant effect on delivery of hydrocortisone, treatment for 24 hr produced an order of magnitude increase in the delivery of hydrocortisone ($P < 0.05$).

Table III. Skin Permeation After Treatment with Irritants

Permeant	Treatment	Duration of treatment (hr)	Amount permeated at 24 hr ($\mu g/cm^2$)
Caffeine	Water	4	187 ± 102
Caffeine	Norephedrine	4	318 ± 42
Caffeine	Water	24	159 ± 10
Caffeine	Norephedrine	24	322 ± 62*
Caffeine	Ethanol	4	399 ± 136
Caffeine	Imipramine	4	530 ± 130
Caffeine	Ethanol	24	399 ± 193
Caffeine	Imipramine	24	1141 ± 408*
Indomethacin	Water	4	6.2 ± 2.1
Indomethacin	Norephedrine	4	0.75 ± 0.25*
Indomethacin	Water	24	7.6 ± 3.0
Indomethacin	Norephedrine	24	1.4 ± 0.4*
Indomethacin	Ethanol	4	8.6 ± 1.5
Indomethacin	Imipramine	4	8.6 ± 2.1
Indomethacin	Ethanol	24	6.2 ± 1.1
Indomethacin	Imipramine	24	8.0 ± 1.9
Hydrocortisone	Water	4	1.82 ± 0.49
Hydrocortisone	Norephedrine	4	4.57 ± 1.50*
Hydrocortisone	Water	24	1.43 ± 0.45
Hydrocortisone	Norephedrine	24	5.38 ± 0.37*
Hydrocortisone	Ethanol	4	2.57 ± 0.61
Hydrocortisone	Imipramine	4	3.03 ± 0.43
Hydrocortisone	Ethanol	24	0.89 ± 0.35
Hydrocortisone	Imipramine	24	10.4 ± 4.1*

* $P < 0.05$.

DISCUSSION

Skin irritation of drugs has been correlated with the pK_a of the irritant (2), and consequently, for a series of basic irritants, correlations of erythema with the local epidermal pH and perhaps with decreased barrier function might be expected. While treatment with both imipramine and norephedrine for 4 and 24 hr caused increases in the surface pH of the skin, after removal of the stratum corneum by tape stripping, imipramine, the more severe irritant (Table I), produced smaller increases in pH than norephedrine.

The effects of treatment with skin irritants on TEWL and on the steady-state transport of model drugs with dis-

Table II. pH of the Human Skin Following Treatment with Basic Irritants[a]

Procedure adopted & time	Normal skin	Water	Ethanol	Imipramine	Norephedrine
A					
4 hr	4.6 ± 0.2	5.2 ± 0.1	4.6 ± 0.1	8.2 ± 0.6	9.7 ± 0.3
24 hr	4.6 ± 0.1	5.4 ± 0.1	4.8 ± 0.1	6.8 ± 0.1	9.2 ± 0.2
B					
4 hr	4.6 ± 0.1	5.2 ± 0.3	4.8 ± 0.2	7.4 ± 0.5	9.3 ± 0.2
24 hr	4.5 ± 0.1	4.9 ± 0.1	4.8 ± 0.1	6.4 ± 0.1	8.9 ± 0.1
C					
4 hr	4.8 ± 0.1	5.1 ± 0.1	4.9 ± 0.1	6.5 ± 0.3	9.1 ± 0.1
24 hr	5.1 ± 0.1	5.0 ± 0.1	4.8 ± 0.1	7.1 ± 0.1	9.1 ± 0.2
D					
4 hr	4.8 ± 0.1	5.4 ± 0.3	4.9 ± 0.1	6.7 ± 0.7	8.3 ± 0.4
24 hr	4.9 ± 0.1	5.5 ± 0.1	4.9 ± 0.1	7.3 ± 0.2	8.9 ± 0.1

[a] A, measurements made after application of drugs; B, measurements made after surface removal of drugs with four repeated washings; C, measurements made after stripping the stratum corneum 5 times; D, measurements made after stripping the stratum corneum 10 times. All measurements are expressed as mean ± SD.

similar physicochemical characteristics are summarized in Fig. 1. While norephedrine increased the fluxes of water, caffeine, and hydrocortisone, treatment with imipramine, the more severe irritant (2), improved permeation of only caffeine and hydrocortisone. Surprisingly, norephedrine decreased indomethacin transport by an order of magnitude.

Only the steady-state flux of hydrocortisone (Fig. 1) exhibited increases that ranked in the same order as erythema. Perhaps permeation of hydrocortisone, as the most poorly absorbed model compound through skin, is the most sensitive test of barrier function. The 10-fold increase in transport of hydrocortisone with treatment with imipramine for 24 hr was the largest change observed in this study. Similar results with poorly transported compounds may be found in the literature concerning diseased, tape-stripped, or abraded skins (4).

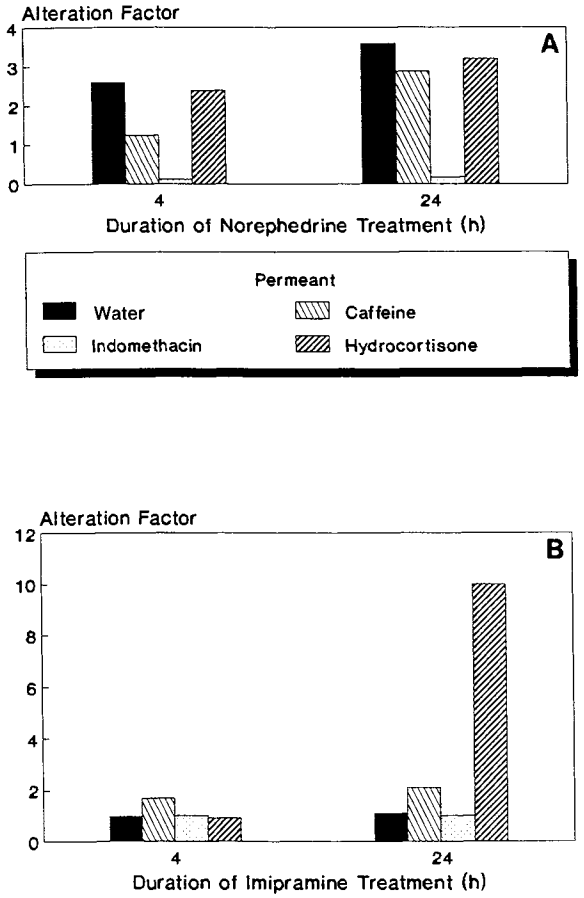

Fig. 1. Alteration factor induced by treatment with (A) norephedrine and (B) imipramine on the permeation of water, caffeine, hydrocortisone, and indomethacin. "Alteration factor" is the ratio of the steady-state flux for the permeant after pretreatment with the selected irritant to that for pretreatment with the respective vehicle as a control.

While it is clear that the pH of the skin and its barrier function may be altered by irritants, the small number of irritants and vehicles examined precludes generalization of these data. The most perplexing result was the decrease in the steady-state flux of indomethacin subsequent to pretreatment with norephedrine. Since the pK_a of indomethacin is 4.5 and the pH in the stratum corneum following treatment with norephedrine was 8.3–9.7 (Table II), the decreased transport of indomethacin could reflect a loss of driving force as the drug was ionized (8). If this were true, the data for transport of indomethacin over the 24-hr experiment would be expected to exhibit convexity as norephedrine desorbed from the skin, but the data were highly linear.

In conclusion, this study demonstrates that treatment with basic irritants can influence skin permeation and that this alteration in penetration varies depending on the permeant, the solvent, and the irritant. Although the consensus suggests increased absorption of drugs through irritated skin, a large decrease in steady-state transport is reported in the present study. The relevance of this *in vitro* study to the real clinical situation may be debated, because exposure to a skin irritant results in a cascade of reactions that do not occur in human cadaver skin. Moreover, long-term changes are initiated in skin irritation (9) that are not reflected in measurements at 4 or 24 hr. Future *in vitro* and *in vivo* studies with a series of diverse irritants and penetrants should ascertain the broader applicability of the present work.

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