Dodecyl *N,N*-Dimethylamino Acetate and Azone Enhance Drug Penetration Across Human, Snake, and Rabbit Skin

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The effectiveness of the penetration enhancers, dodecyl *N,N*-dimethylamino acetate (DDAA) and Azone, on pretreated human epidermis for the permeation of model drugs, indomethacin, 5-fluorouracil, and propranolol-HCl, was studied in *in vitro* diffusion cells. Snakeskin (*Elaphe obsoleta*) and rabbit pinna skin were compared as possible models for human skin. The drug concentrations were analyzed by HPLC. With all skins and all model drugs, DDAA increased drug permeability at least as well as Azone, and in most cases it was a more effective permeation enhancer. The relative permeation improvements in human skin, snakeskin, and rabbit skin were 10- to 20-, 5- to 50-, and 20- to 120-fold, respectively. Tritiated water served as an indicator of skin condition. Its penetration in the skin samples was independent of the drugs used, and both penetration enhancers significantly increased the flux of tritiated water through all skins. Thus, DDAA and Azone significantly increased the permeation of lipophilic and hydrophilic model compounds. Rabbit pinna skin was a poor model for human skin *in vitro*, while snakeskin was much closer to human skin in terms of transdermal permeability. In most cases drug permeability decreased in the order rabbit $\gg$ human $>$ or $<$ snake.

KEY WORDS: penetration enhancer; dodecyl *N,N*-dimethylamino acetate; Azone; percutaneous permeability; animal models.

INTRODUCTION

Human skin is poorly permeable to most foreign substances, including drugs. In addition to this low permeability, intra- and interspecimen biological variability of the skin makes systemic drug administration through the skin difficult (1). The principal barrier to drug permeation in human skin is stratum corneum. In recent years there have been many attempts to increase the flux of drugs through stratum corneum with transdermal penetration enhancers (2,3). Since most of the penetration enhancers are potential skin irritants and allergens, biodegradable penetration enhancers with reversible action are an attractive alternative. Dodecyl *N,N*-dimethylamino acetate was developed to be a possible biodegradable permeation enhancer (4,5).

Wester and Maibach (6) have compared animal skin models, but they regard human skin as the best model in permeation studies. However, it is difficult to obtain human skin. Consequently, different animal model skins are frequently used in transdermal penetration experiments. Snakeskin (4,5,7), nude mouse skin (7), and rabbit pinna skin (8) have been recently used as skin models.

The first aim of this study was to examine the effect of dodecyl *N,N*-dimethylamino acetate (DDAA) on the percutaneous absorption of model drugs, indomethacin, 5-fluorouracil, and propranolol, through human skin *in vitro*. DDAA was compared with Azone (9–11) in terms of penetration enhancement. The second aim was to compare the skins of snake (*Elaphe obsoleta*) and rabbit pinna as possible *in vitro* models for human skin. The effects of DDAA and Azone on the penetration of model drugs in the animal model skins were also studied.

MATERIALS AND METHODS

Skin Sources and Preparation

The human skin was separated from the thigh of elderly people as a 0.5-cm-thick layer during leg amputations (The University Hospital of Kuopio). The epidermis was separated by heating the skin sample in distilled water at 60°C for 2 min. These epidermis samples were stored at 4°C no longer than 1 week before experiments (drug permeability of the excised skin remained unchanged for 7 days). Snakeskin, obtained at shedding from black rat snake (*Elaphe obsoleta*) dorsal skin, was stored at −15°C (4). Rabbit pinna (ear) skin was separated from the inner side of the rabbit ear by a method described by Corbo *et al.* (8). Rabbit pinna skin was obtained from mixed breed pigmented rabbits, about 30 weeks old, both sexes, immediately after the animals were sacrificed by intravenous injection of T-61 Euthanasia solution (Hoechst, Munich, West Germany). The pinna skin was peeled away from the underlying cartilage and the pieces were stored at 4°C for at most 7 days (see above). Each skin was cut to 3 × 3-cm pieces to be used for *in vitro* diffusion experiments, six comparative determinations in each case.

In Vitro Permeation Studies

The model drugs used were indomethacin (Sigma, St. Louis, MO), 5-fluorouracil (Aldrich-Chemie, Steinheim, West Germany), and propranolol hydrochloride (Amsa, Milano, Italy). The solubilities of indomethacin, 5-fluorouracil, and propranolol-HCl in the phosphate buffers (pH 7.2 and 7.4) were 1.03, 10.57, and 123.6 mg/ml, respectively. The octanol/phosphate buffer distribution coefficients (DC) of the drugs were determined and calculated by a method described by Schoenwald and Huang (12) at 32°C. Indomethacin (log DC 1.20) and propranolol (log DC 1.16) are lipophilic and 5-fluorouracil (log DC −0.56) is hydrophilic.

Saturated solutions of the model drugs were used as donor solution to achieve maximum chemical potential across the skin in diffusion cell. The drugs were dissolved in pH 7.2 (indomethacin and 5-fluorouracil) or pH 7.4 (propranolol-HCl) phosphate buffer at 32°C. The ionic strength of the buffer solutions was adjusted to 0.15 *M* with NaCl. Blank buffer was placed in the receiver compartments of the diffusion cells, and because the permeabilities of the three

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skins were low, sink conditions were maintained during the experiments.

Tritiated water (sp act, 18 mCi/g; Du Pont, New England Nuclear Research Products, Boston, MA) was used as an indicator of the condition of the skin samples during the diffusion experiments. ^{3}H-Water was added to the donor chamber along with the saturated drug solution and the flux of ^{3}H-water through skin samples was followed. All *in vitro* permeation studies were performed in DC-100-B horizontal diffusion cells (Crown Glass Company Inc., Somerville, NJ). The volume of each cell was 3.0 ml, the area of the skin exposed was 0.64 cm^2, and the temperature was 32 ± 1°C. Samples of 300 μl were withdrawn from the receiver compartment at fixed times. Part of each sample (250 μl) was used for HPLC analysis and the rest (50 μl) for determination of ^3H_2O with liquid scintillation counting. The withdrawn sample volumes were immediately replaced with blank buffer solution.

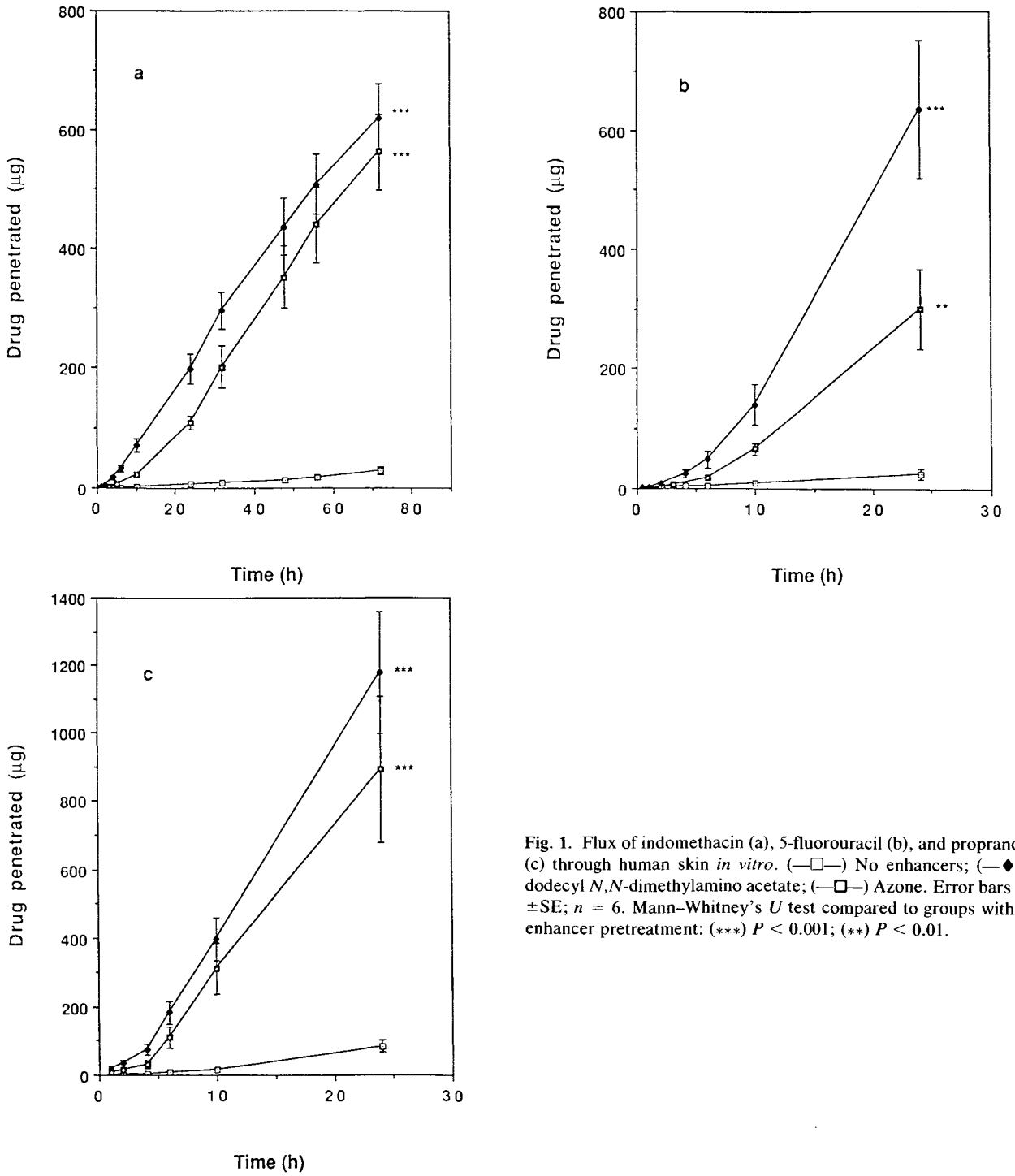

Fig. 1. Flux of indomethacin (a), 5-fluorouracil (b), and propranolol (c) through human skin *in vitro*. (—□—) No enhancers; (—♦—) dodecyl *N,N*-dimethylamino acetate; (—□—) Azone. Error bars are ±SE; $n = 6$. Mann–Whitney's U test compared to groups with no enhancer pretreatment: (***) $P < 0.001$; (**) $P < 0.01$.

Analysis

The drugs were analyzed by HPLC (Beckman System Gold, Beckman Instruments Inc., San Ramon, CA). Indomethacin was analyzed with a Supelco LC-18-DB column (5 μm, 150 × 4.6 mm; Supelco Inc., Rohm and Haab Company, Bellefonte, PA). The mobile phase was 35% (v/v) acetonitrile and 65% (v/v) phosphate buffer solution pH 7.2 (USP) and the detection wavelength was 260 nm. 5-Fluorouracil was analyzed with the same column, the mobile phase was pure phosphate buffer solution pH 7.2 (USP), and the detection wavelength was 266 nm. The concentration of propranolol was determined with a Hypersil RP-18 column (3 μm, 50 × 4.6 mm; Shandon Southern Instruments, Sewicley, PA). The mobile phase was 30% (v/v) acetonitrile and 70% (v/v) acetic acid at pH 4.0. The detection wavelength was 254 nm. The amount of penetrated ^{3}H-water was determined with a Rackbeta 1216 liquid scintillation counter (Wallac OY, Turku, Finland). The samples of 50 μl were diluted with 450 μl distilled water, and 4.5 ml aqueous counting scintillant (ACS, Amersham Co., Arlington Heights, IL) was added before determination of radioactivity. Counting time was 480 sec and counting limit 12,000.

Application of Penetration Enhancers

The penetration enhancers studied, dodecyl *N,N*-dimethylamino acetate (DDAA) and Azone (Whitby Research Inc., Irvine, CA), both contain a C-12 alkyl chain. The synthesis of DDAA is described by Wong *et al.* (4). The effectiveness of the enhancers was studied by pretreating the skin samples with pure liquid enhancer (8 μl/skin sample) for 3.5 hr before the drug permeation experiment. With this procedure the maximum effect was achieved. Before their placement in diffusion chambers the untreated skin samples were shortly hydrated for 10 min with distilled water to remove possible wrinkles from dry skin samples. Six parallel experiments were conducted with each enhancer treatment and with each model drug.

Data Treatment

Steady-state fluxes, J_{ss}, for the drugs (μg/hr cm^2) and tritiated water (%/hr cm^2) were calculated using linear regression analysis of the straightline portion of the cumulative drug penetrated vs time plots. The permeabilities (cm/sec) of the three model drugs were calculated by dividing the J_{ss} by the concentration of the saturated drug solutions, respectively. The significance of the differences between the groups was tested using Kruskall–Wallis one-way analysis of variance, and thereafter the comparison of each two groups with Mann–Whitney's *U* test.

RESULTS AND DISCUSSION

Both dodecyl *N,N*-dimethylamino acetate and Azone significantly ($P < 0.01$) increased drug permeability of all three model drugs in human epidermis (Figs. 1a–c). Permeabilities of indomethacin, 5-fluorouracil, and propranolol through human skin are presented in Table I. The relative permeation enhancements are listed in Table II. It appears that dodecyl *N,N*-dimethylamino acetate was at least as potent a penetration enhancer in human skin as Azone. In all cases it increased the flux of model drugs as well as or better than Azone. In human skin, DDAA increased the penetration of lipophilic indomethacin and hydrophilic 5-fluorouracil over 20-fold (Table II). The permeation increasing effect of DDAA and Azone on the permeability of propranolol, a lipophilic base which is ionized and very water soluble at physiologic pH, was about 13- and 10-fold, respectively. The DDAA and Azone appeared to increase the permeability of lipophilic and hydrophilic model drugs equally well in human skin.

Fleeker *et al.* (5) have shown the permeability improv-

Table I. Permeability of Indomethacin (IND), 5-Fluorouracil (5FU), and Propranolol (PRO) Through Human Skin, Snakeskin and Rabbit Pinna Skin After Pretreatment of 3.5 hr with Enhancers: Mean ± SE; $n = 6$

	Permeability (cm/sec) ×10 E-8					
	Human skin			Snakeskin		
	Control[a]	DDAA[b]	Azone	Control	DDAA	Azone
IND	10.06 ± 2.97	380.21 ± 29.72**	351.10 ± 48.63**	3.24 ± 0.81	161.75 ± 19.65**	130.64 ± 29.67**
5FU	5.05 ± 1.89	110.63 ± 18.91**	50.68 ± 13.71*	9.97 ± 1.37	71.84 ± 7.62**	51.35 ± 8.07*
PRO	1.32 ± 0.07	10.89 ± 2.67**	9.06 ± 3.59**	1.37 ± 0.28	8.90 ± 3.22**	6.64 ± 1.23**

	Rabbit pinna skin		
	Control	DDAA	Azone
IND	29.69 ± 7.28	2106.14 ± 81.66**	991.06 ± 71.63**
5FU	21.30 ± 3.71	893.35 ± 41.37**	392.13 ± 80.42**
PRO	8.83 ± 0.52	1033.47 ± 157.89**	306.36 ± 41.35**

[a] No enhancer pretreatment.

[b] Dodecyl *N,N*-dimethylamino acetate.

* $P < 0.01$. Mann–Whitney's *U* test compared to groups with no enhancer pretreatment.

** $P < 0.001$. Mann–Whitney's *U* test compared to groups with no enhancer pretreatment.

Table II. Penetration Enhancement of Model Drugs in Human Skin, Snakeskin, and Rabbit Pinna Skin After Pretreatment of 3.5 hr with the Enhancers

Drug + enhancer	Enhancement factor[a]		
	Human skin	Snakeskin	Rabbit pinna skin
Indomethacin + DDAA[b]	21.0	51.9	62.3
Indomethacin + Azone	19.6	44.4	29.1
5-Fluorouracil + DDAA	22.3	6.4	44.6
5-Fluorouracil + Azone	11.0	4.8	19.8
Propranolol + DDAA	13.1	10.1	117.8
Propranolol + Azone	9.7	4.8	34.5

[a] Permeability with enhancer divided by drug permeability without enhancer.
[b] Dodecyl *N,N*-dimethylamino acetate.

ing effects of dodecyl *N,N*-dimethylamino acetate on snakeskin. In our study both DDAA and Azone were potent enhancers in increasing the flux of the drug probes in snakeskin and also in rabbit pinna skin (Table I). In snakeskin DDAA and Azone increased drug permeability approximately equally (Tables I and II). The permeation of indomethacin was increased most in snakeskin. In rabbit pinna skin both enhancers increased the flux of the model drugs more than in human skin or snakeskin (Table II). In contrast to human skin and snakeskin, the strongest effect of DDAA in rabbit skin was observed in the case of propranolol (118-fold increase). The stronger penetration enhancement by DDAA compared to Azone was most apparent in rabbit pinna skin. DDAA increased the flux of indomethacin, 5-fluorouracil and propranolol through rabbit pinna skin more effectively than Azone ($P < 0.01$). This finding may be due to differences in stratum corneum structure in rabbit pinna skin (e.g., number of hair follicles) compared with human skin or snakeskin, or the enhancers may have different mechanisms of action in rabbit pinna skin, e.g., the composition of skin lipids can be different in rabbit pinna skin. Wertz and Downing (13) have shown that there are differences in the composition of skin lipids in human, pig, mouse, and rat skin.

Rabbit pinna skin allowed permeation of all model drugs more readily than human skin or snakeskin (Table I). The structure of snakeskin is similar to that of human stratum corneum (4), and in many cases the permeation of 5-fluorouracil or propranolol in snakeskin did not differ significantly from that in human skin. In most cases the permeability decreased in the order rabbit pinna skin $\gg$ snakeskin $>$ or $<$ human skin. The penetration enhancers significantly increased the flux of all three model drugs through each skin model. According to our studies, snakeskin is a better model than rabbit pinna skin. Recently, Rigg and Barry (7) have shown that snakeskin is more similar to human skin in terms of drug permeability than is hairless mouse skin. Corbo and co-workers (8) used rabbit pinna skin as a model membrane in transdermal drug delivery system studies. They found a good correlation between human cadaver skin and rabbit pinna skin in the case of propranolol permeation. This may be explained by the fact that in that study the delivery system, not the skin, was the rate-determining step in propranolol penetration across the skins. In our study, indomethacin, 5-fluorouracil, and propranolol penetrated through rabbit pinna skin significantly faster (1.5- to 12-fold) than through human skin or snakeskin.

Tritiated water proved to be a useful indicator of skin condition because even little damage to the skin was indicated by a faster tritium permeation rate. The flux of water increased in the order snakeskin $<$ human skin $<$ rabbit pinna skin. Penetration enhancers dodecyl *N,N*-dimethylamino acetate and Azone increased clearly the flux of ^{3}H-water through the model skins (Table III). This effect was again strongest in rabbit pinna skin. The model drugs had no

Table III. Penetration of ^{3}H-Water Through Skin Models with Saturated Drug Solutions, After 3.5 hr of Pretreatment with the Enhancers: Mean ± SE, $n = 6$

	^{3}H-Water flux (%/hr cm^2)					
	Control[a]			DDAA[b]		
	Indomethacin	5-Fluorouracil	Propranolol	Indomethacin	5-Fluorouracil	Propranolol
Human skin	0.385 ± 0.024	0.343 ± 0.040	0.449 ± 0.081	1.190 ± 0.091	1.515 ± 0.204	1.295 ± 0.230
Snakeskin	0.221 ± 0.119	0.161 ± 0.014	0.197 ± 0.028	0.487 ± 0.064	0.818 ± 0.067	0.467 ± 0.172
Rabbit skin	0.791 ± 0.261	0.653 ± 0.111	0.923 ± 0.170	6.257 ± 0.129	6.619 ± 0.403	10.617 ± 0.305*

	Azone		
	Indomethacin	5-Fluorouracil	Propranolol
Human skin	1.405 ± 0.142	1.097 ± 0.114	1.980 ± 0.229
Snakeskin	0.423 ± 0.067	0.337 ± 0.041	0.645 ± 0.037
Rabbit skin	6.383 ± 0.190	5.854 ± 0.979	9.276 ± 0.302*

[a] No enhancer pretreatment.
[b] Dodecyl *N,N*-dimethylamino acetate.
* $P < 0.05$. Mann–Whitney's U test compared to the other model drugs with similar pretreatment.

effect on water penetration in the skins (Table III), except in rabbit pinna skin pretreated with enhancer, where propranolol increased ^{3}H-water flux by an unknown mechanism, in comparison with indomethacin or 5-fluorouracil ($P < 0.05$). Under these conditions the permeability of propranolol increased more than 100-fold (Table II).

In conclusion, dodecyl *N,N*-dimethylamino acetate is an effective penetration enhancer in human skin for both lipophilic and hydrophilic compounds. The mechanism of action and biodegradability of this enhancer remain to be elucidated. Snakeskin is a better animal model than rabbit pinna skin for *in vitro* drug permeation studies.

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