Hairless Mouse Skin is Limited as a Model for Assessing the Effects of Penetration Enhancers in Human Skin

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The permeability coefficient of 5-fluorouracil through human abdominal and hairless mouse skins was used as an indicator of the relative effects of 12-h pretreatment of the skins with either penetration-enhancer mixtures [including laurocapram (Azone), decylmethylsulfoxide, oleic acid, and propylene glycol] or saline (control). After treatment with saline, fluxes of 5-fluorouracil through the two skin types were similar, but the mouse skin showed exaggerated responses to all the penetration-enhancer formulations. There was no consistent relationship between enhancer effects on the two skin types, and we conclude that the hairless mouse model should not be used to predict the effects of penetration enhancers in human skin. After treatment with saline, hairless mouse skin sharply increased in permeability after approximately 50 h hydration, suggesting that the stratum corneum had started to disrupt, whereas the flux through human skin remained unchanged. *J Invest Dermatol 90:810–813, 1988*

The range of drugs that can be effectively delivered via the percutaneous route is limited largely by the relative impermeability of the stratum corneum. Various methods of increasing the absorption of poorly penetrating agents have been attempted, with earlier studies concentrating often on the effects of occlusion and hydration and more recent investigations dwelling on penetration enhancers [1,2]. Such accelerants reduce the barrier properties of the stratum corneum to other permeants, thereby potentially increasing the range of drugs that can be delivered through the skin.

The development of topical formulations containing penetration enhancers often involves in vitro work with isolated skin. As human tissue is not always readily available, various animal models have been used, with hairless mouse skin currently being popular.

In this paper, we compare the effects of pretreatment with a range of penetration enhancers on the permeabilities of human abdominal and hairless mouse skins to a model permeant, 5-fluorouracil (5-FU). We conclude that hairless mouse skin is a poor mimic of human skin with respect to enhancer activity.

MATERIALS AND METHODS

We used the pseudo-steady-state permeability coefficient (K_p) of 5-FU as a test for the relative effects of 12-h pretreatments with seven potential penetration-enhancer formulations compared with normal saline (control). Previous work [3] has shown that such pretreatment optimizes penetration-enhancement effects. Effects on human abdominal and hairless mouse skins were compared to assess the suitability of the hairless mouse as a model for human skin as modified by penetration enhancers.

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Abbreviations:
5-FU: 5-fluorouracil
DCMS: decylmethylsulfoxide

Skin Sources and Preparation. Four male hairless mice (CBA/HL strain) aged 60 to 80 days were killed by spinal dislocation, and their dorsal skins were immediately excised, any underlying tissue being gently removed. Each mouse supplied 12 skin samples for use in permeation experiments.

Human midline abdominal skin from caucasian donors was obtained at autopsy and stored in evacuated polythene bags at −20°C until required [4]. Samples were sectioned with a dermatome (Davis Duplex 7) to approximately 420-μm-thick sections consisting of the epidermis and a portion of the dermis. Two pieces of human abdominal skin were used (males, 60 and 63 years), each providing 24 samples (3 from each donor for each of the 8 pretreatments).

The number of replicates allowed for occasional cell leakage with consequent rejection of data, a common problem with in vitro skin permeation work.

Pretreatment Formulations. Three potentially useful penetration enhancers of different chemical types — laurocapram (Azone, donated by Nelson Research), decylmethylsulfoxide (DCMS, donated by Procter and Gamble Co.), and oleic acid (Sigma Chemical Co., minimum assay 99%) — were tested. Oleic acid was used as a solution in propylene glycol, and laurocapram and DCMS were applied in both water and propylene glycol. Concentrations of penetration enhancers were chosen from published data, including work from this department [5]. Laurocapram 2% in propylene glycol, oleic acid 5% in propylene glycol, and DCMS 15% in propylene glycol were used by Barry and Bennett [6]. DCMS 4% in water was used by Sekura and Scala [7], and laurocapram 3% in 0.1% polysorbate 20/normal saline has also been demonstrated as effective [3,8]. As the main aim of the work was to compare the effects of a variety of enhancers on two skin types, different concentrations were deliberately chosen. A solution of 0.1% polysorbate 20 (Tween 20) in normal saline was included as a control for the emulsion of laurocapram in saline. Propylene glycol was included as a control for the enhancer solution based on this solvent and to test for enhancement effects of the solvent itself (see Table I).

Automatic Diffusion Apparatus. Skin samples were mounted into stainless-steel diffusion cells (cross-sectional area 0.126 cm²) maintained at 31 ± 1°C on hollow copper arms through which thermostated water was pumped. Receptor fluid (0.002% aqueous

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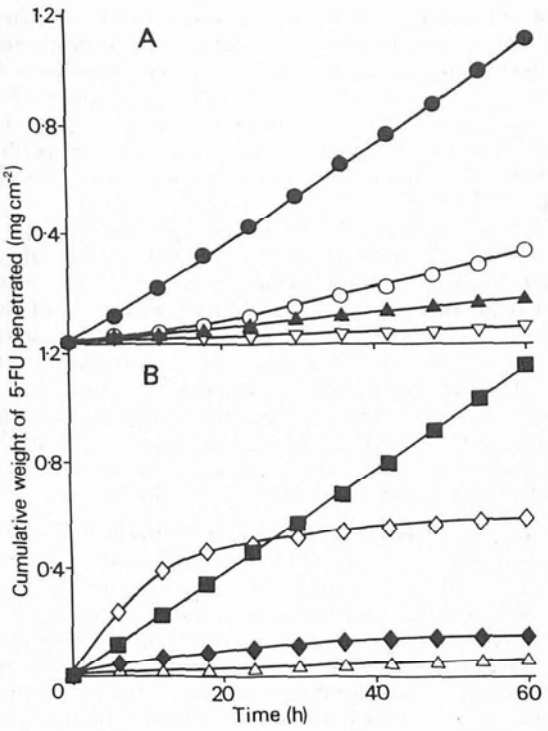


Figure 1. Sample penetration plots for 5-FU through human abdominal skin after pretreatment of the skin with one of the test mixtures. *A*. Polysorbate 20 in saline *(inverted open triangles)*, propylene glycol *(closed triangles)*, laurocapram in polysorbate 20/saline *(open circles)* and laurocapram in propylene glycol *(closed circles)*. *B*. Normal saline *(open triangles)*, aqueous decylmethylsulfoxide *(open diamonds)*, decylmethylsulfoxide in propylene glycol *(closed diamonds)* and oleic acid in propylene glycol *(closed squares)*.

sodium azide) flowed continuously through the receptor chamber and was collected in glass scintillation vials. Flow rate was 2 cm^3 h^{-1}, corresponding to 40 changes of receptor volume per hour, ensuring sink conditions. The vials were changed automatically at 2-h intervals; a detailed description of the diffusion system has been published by Akhter et al [9].

Pretreatment of Skin Samples and Permeation Studies. Each treatment mixture was applied to six samples of both skin types, consisting of 150 μL of water-based mixtures (≡ 1200 μL cm^{-2}) and 10 μL of propylene glycol-based mixtures (≡ 80 μL cm^{-2}). Liquids remained on the skin for 12 h; then they were gently removed with absorbent tissue and permeation studies commenced immediately.

The donor solutions consisted of 160 μL of a radiolabeled saturated (10.2 mg cm^{-3}) solution of 5-FU in distilled water [5-fluoro-6-[^{3}H]uracil (Amersham International PLC) was diluted to 0.3 mCi cm^{-3}]. Receptor samples were collected over 2 h intervals, up to 60 h, and assayed for 5-FU content by liquid scintillation counting (Packard Tri-Carb 460C) after the addition of 10 cm^3 of Scintran Cocktail T (BDH Chemicals Ltd.).

Calculation of Permeability Coefficients. Raw data from scintillation counting were converted to cumulative amounts per unit area (mg cm^{-2}) and computer-plotted versus time; for examples, see Fig 1. Steady-state penetration fluxes, J (mg cm^{-2} h^{-1}), were calculated by regression analysis from the linear regions of the plots (r typically equaled 0.998). Pretreatment with aqueous DCMS, however, consistently produced an atypical penetration plot, with a rapid initial absorption followed by a fall in rate; fluxes were calculated from the initial slope after this pretreatment (r typically 0.98). Permeability coefficients, K_p (cm h^{-1}), were calculated from the steady-state flux and donor concentration, C (mg cm^{-3}), using the relationship

$$K_p = J/C$$

RESULTS

Table 1 shows the mean permeability coefficients (K_p) calculated for 5-FU, for both skin types, after each treatment. From these values, we calculated enhancement ratios for each enhancer treatment, and both skin types, from the formula

$$\text{enhancement ratio} = \frac{K_p \text{ of 5-FU after enhancer treatment}}{K_p \text{ of 5-FU after saline treatment}}$$

The ratios calculated for each treatment and skin type are compared in Fig 2.

The cumulative 5-FU penetration plots for saline-pretreated hairless mouse skin differed markedly from those obtained with human abdominal skin (Fig 3). Fluxes through hairless mouse skin increased dramatically after 35 to 40 h permeation, corresponding to 47 to 52 h hydration.

Table I. Formulas and Volumes of the Eight Pretreatments Applied to the Skin Samples and Resultant Permeability Coefficients (K_p) of 5-Fluorouracil Through Human Abdominal and Hairless Mouse Skins

Pretreatment Formula	Code[a]	Human Abdomen			Hairless Mouse		
		Mean K_p[b]	SEM[c]	n[d]	Mean K_p	SEM	n
Normal saline (0.9% sodium chloride)	S	0.951	0.451	5	1.07	0.457	6
0.1% Polysorbate 20 in normal saline	TS	1.03	0.466	5	3.44	0.610	5
3% w/v Laurocapram in 0.1% Polysorbate/saline	LTS	6.48	1.14	6	11.4	1.04	6
4% w/v Decylmethylsulfoxide in water	DCAQ	71.3	23.9	6	107	8.18	6
Propylene glycol	PG	2.53	0.785	6	4.88	1.21	5
2% w/v Laurocapram in propylene glycol	LPG	17.7	5.12	6	142	36.2	6
15% w/v Decylmethylsulfoxide in propylene glycol	DCPG	2.15	0.688	4	6.59	0.938	6
5% w/v Oleic acid in propylene glycol	OAPG	19.3	6.20	4	159	15.5	6

[a] Codes used in Fig 2 to denote treatment type.

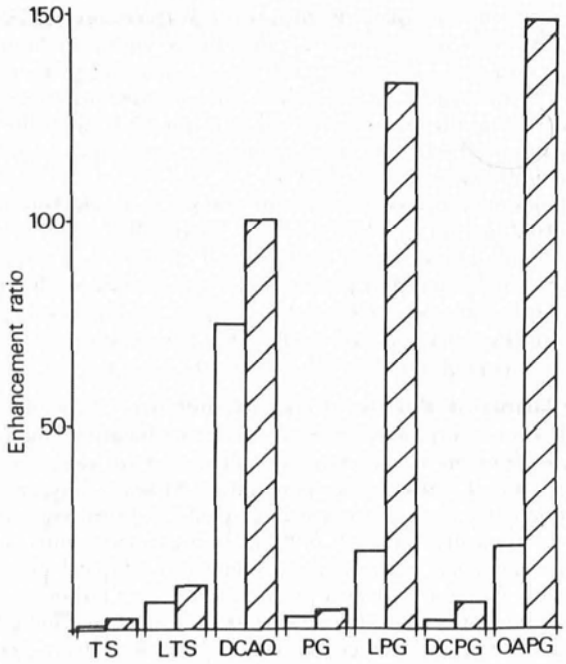


Figure 2. Enhancement ratios for 5-FU through human abdominal skin *(open bars)* and hairless mouse skin *(hatched bars)* after 12-h pretreatment with the enhancer mixtures. Enhancement ratios are calculated by the equation.

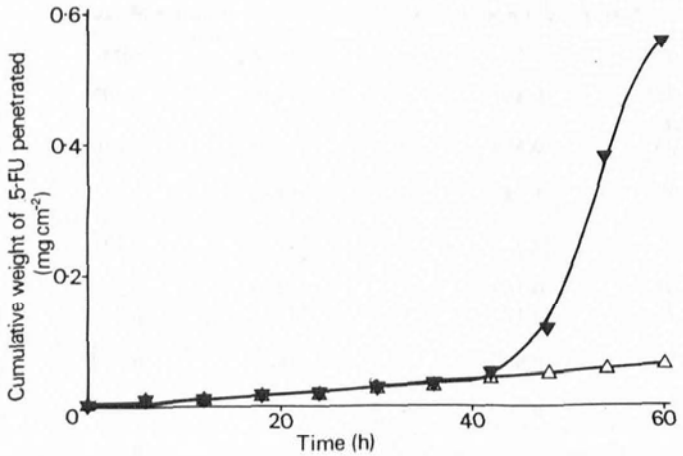
$$\text{enhancement ratio} = \frac{K_p \text{ of 5-FU after enhancer treatment}}{K_p \text{ of 5-FU after saline treatment}}$$

Codes are defined in Table I.

DISCUSSION

Effects of Penetration Enhancers on Human Skin. Statistical analysis was performed using the Wilcoxon–Mann–Whitney rank sum test [10], taking a level of significance (α) of 0.05. In testing for effects of the penetration enhancers (compared with saline control) a one-tailed test was used, but in comparing human abdominal and hairless mouse skins we used a two-tailed test.

All the effects of penetration enhancers shown by human abdominal skin agree with previous studies. Laurocapram was effective when used as an emulsion (e.g., [3,8]), but other workers found that its action was heightened by propylene glycol [11]. We discovered a near 7-fold rise in skin permeability after treatment with the emulsion of laurocapram ($\alpha < 0.005$), increasing to 18-fold when a solution in propylene glycol was used ($\alpha < 0.0005$). Propylene glycol alone had a moderate enhancing effect, increasing permeability to 5-FU some 2.6 times ($\alpha < 0.025$). The polysorbate 20 used to emulsify laurocapram in water insignificantly changed human skin permeability to 5-FU ($\alpha > 0.05$), in agreement with previous work that showed that nonionics are the least damaging class of surfactants (e.g., [12,13]).



DCMS in aqueous solution initially produced a high flux of 5-FU, the effect being reversible as the DCMS was washed out of the skin [14]. DCMS in propylene glycol, in contrast, exerted very little effect on skin permeability, slightly less than that of propylene glycol alone. The effect of DCMS may have been reduced here because propylene glycol was a good solvent for the enhancer and inhibited its partitioning into the stratum corneum.

Oleic acid is an effective penetration enhancer for lipophilic compounds, when used as a solution in propylene glycol [15]. We have found it to be as effective as laurocapram in promoting permeation of 5-FU (a polar drug) when applied in this way.

Comparison of Hairless Mouse and Human Skins. The permeability coefficients for 5-FU through human abdominal and hairless mouse skins pretreated with saline were similar, suggesting that the mouse model may have some validity in simple, ideal situations; however, after penetration-enhancer pretreatment, the hairless mouse model was misleading. Application of aqueous polysorbate 20, which had no significant effect on human abdominal skin ($\alpha > 0.05$), increased the permeability of hairless mouse skin 3-fold ($\alpha < 0.01$).

Figure 1 demonstrates that all pretreatments modified hairless mouse skin more than they did human skin. The relative effect of each enhancer formulation on the two skins was not consistent. Thus, laurocapram in propylene glycol was 7 times more active in promoting 5-FU penetration through hairless mouse skin than through human abdominal skin, whereas the corresponding ratio for the aqueous emulsion of laurocapram was only 1.6. As there was no consistent relationship between penetration-enhancement effects on the two skin types, we conclude that hairless mouse skin cannot be used as a reliable model for human percutaneous absorption as modified by accelerant treatment. The enhancement ratios found for the accelerants used here were calculated with respect to 5-FU. It is likely that enhancement effects will change according to the chemical nature of the permeant used [6,16], and this would add additional variability and therefore potential inaccuracy to use of the hairless mouse model.

Previous work explains the rise in permeability after 50 h hydration of hairless mouse skin pretreated with saline [17]. Prolonged hydration completely disrupts hairless mouse skin and the rise in permeability seen in the present work probably coincided with the start of stratum corneum breakdown, which would allow rapid permeation of 5-FU through weakened regions of the horny layer.

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