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Damaging effect of acetone on the permeability barrier of hairless mouse skin compared with that of human skin

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Summary

A technique widely used in dermatological research deposits drugs onto the skin from volatile solvents and often uses hairless mouse skin as a model for human tissue. We therefore compared the damaging effect of acetone on hairless mouse and human abdominal skins using an in vitro steady-state technique. The permeabilities of the two skin types to 5-fluorouracil were measured, both untreated and after acetone application. For untreated skins, the permeability coefficients of 5-fluorouracil were similar in the two skin types, suggesting a limited usefulness of the hairless mouse as a model for human skin. After acetone treatment, however, hairless mouse skin increased 16-fold in permeability ($P = 0.975$), whereas human abdominal skin was not significantly affected ($P < 0.75$). These results demonstrate that hairless mouse skin may not be confidently used as a model for human skin in techniques employing acetone. Hairless mouse skin also increased in permeability to 5-fluorouracil after longer than 36 h permeation. This was due to hydration-induced damage to the mouse stratum corneum, and represented another difference from human skin.

Introduction

For in vitro use in permeation studies, human skin is often difficult to obtain, so various animal skins have been adopted as substitutes, with hairless mouse skin currently being popular.

The deposition of drugs onto skin from volatile solvents, followed by analysis of rate and extent of penetration into either a receptor medium or the body, is a technique widely used in pharmaceutical and dermatological research (e.g. Akhter and Barry, 1984, Reifenrath et al., 1984, Bronaugh and Maibach, 1985). Implicit in such protocols is the assumption that the solvent does not alter the permeability of the skin and this assumption has not been investigated, particularly in the context of the use of hairless mouse skin as a model membrane representing human tissue. We therefore tested a commonly used volatile solvent, acetone, for its potential damaging effects on human abdominal and hairless mouse skins. The permeability coefficient of 5-fluorouracil (5-FU), a model polar drug, was used as a marker of the effects of pretreatment of both skin types with acetone.

Materials and Methods

Two male hairless mice (CBA/hl, aged 60-80 days) were sacrificed by spinal dislocation, and

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the dorsal skins excised and cleared of underlying adherent tissues. Each mouse provided 12 skin samples, 6 samples from each mouse being used as control, and 6 after acetone treatment. One sample of human abdominal skin (male, 66 years) was obtained at autopsy and stored in an evacuated polythene bag at −20 °C until required (Harrison et al., 1984). Membranes for use in diffusion studies (nominal thickness 420 μm) were obtained by dermatoming (Davis Duplex 7), and consisted of the epidermis and a portion of the dermis. 12 samples of human abdominal skin were cut, 6 being used as controls and 6 after acetone treatment.

Skin samples were mounted into stainless-steel diffusion cells (cross-sectional area 0.126 cm^2), which were maintained at 31 ± 1 °C. The cells were fitted into an automated diffusion system described previously (Akhter et al., 1984). Receptor fluid (0.002% aqueous sodium azide) flowed continuously through the receptor chambers of the cells, providing sink conditions, and was collected into glass scintillation vials.

Acetone treatment consisted of the application of 20 μl of acetone (Analar grade, BDH Chemicals Ltd., Poole) to each sample (equivalent to 160 $\mu l/cm^2$), for 2 min, after which time any remaining acetone was removed with absorbent tissue. This procedure was intended to mimic, in particular, the in vivo application procedures commonly used with human volunteer studies of drug permeation through skin. The treated skin was then exposed to a controlled atmosphere (22 °C, 60% RH) for a further 2 min, before the permeation experiment started. Control skin samples were simply exposed to the atmosphere for 4 min.

The donor preparation was a saturated solution of 5-FU in water (10.2 mg/cm^3 at 31 °C). Tritiated 5-FU (5-fluoro-6-[^{3}H]-uracil, Amersham International PLC) was diluted to 0.3 mCi/cm^3, and unlabelled 5-FU (Sigma Chemical Company, minimum assay 99%) was used to produce a saturated solution. Permeation experiments were started by the application of 160 μl of this solution to each sample, then receptor fluid was collected over 2-h intervals up to 60 h.

All samples of receptor fluid and donor solution were assayed for 5-FU content by liquid scintillation counting (Packard Tri-Carb 460C). Values for 5-FU content of receptor (cpm) were converted to cumulative weights of 5-FU penetrating the skin (mg/cm^2) and computer-plotted vs time (for examples, see Fig. 1).

The slopes of the linear regions of these plots were calculated by regression analysis (typically $r = 0.998$) to give the steady state flux (J, $mg \cdot cm^{-2} \cdot h^{-1}$). Permeability coefficients for 5-FU (K_p, cm/h) were calculated from steady state flux (J) and donor concentration (C, mg/cm^3) using the relationship

$$K_p = \frac{J}{C}$$

Of the 24 samples of hairless mouse skin used, the flux of 5-FU for 13 rose after 36 h or longer of the permeation experiments (see Fig. 1B). In these instances, fluxes were calculated from the linear region of the penetration plot after the lag phase and before the stage where permeability started rising.

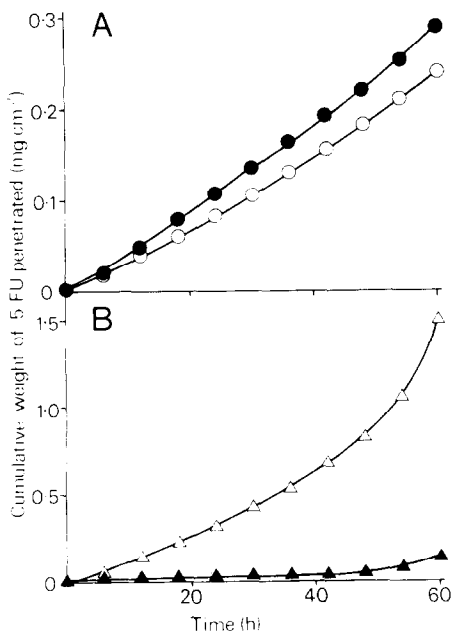

Fig. 1. Cumulative penetration plots for 5-FU through control (closed symbols) and acetone-pretreated (open symbols) human abdominal (A) and hairless mouse (B) skins.

Results and Discussion

Fig. 1 shows sample plots of 5-FU penetration through control and acetone-pretreated hairless mouse and human abdominal skins, and Fig. 2 presents the mean permeability coefficients (K_p) for 5-FU through each skin type

For the two control skins, K_p values of 5-FU are of similar magnitudes, the mean permeability through the animal skin being 2.2 times that through human skin. This suggests that hairless mouse skin may act as a reasonable substitute for human skin in permeation experiments, at least for simple, aqueous solutions at short contact times. However, after acetone treatment, the permeability of human skin is not significantly altered ($P = 0.75$, 2-sample t-test), whereas that of hairless mouse skin increases 16-fold ($P = 0.975$). We therefore suggest that data obtained using acetone-exposed hairless mouse skin (and possibly other solvents?) may not relate to similar data for human skin, as the hairless mouse skin barrier is apparently weakened more easily than is human stratum corneum. This feature has obvious implications for its use as a substitute for human skin in investigational work.

The rise in permeability of hairless mouse skin, both control and acetone-pretreated samples, after prolonged use may be explained by previous work (Bond and Barry, 1985). Extended hydration destroys the stratum corneum structure of hairless mouse skin, but not that of human abdominal skin. The rise in mouse skin permeability therefore signals the commencement of this breakdown, as the protocol involves the presence of water on the stratum corneum. Once again, this behaviour militates against the confident use of the hairless mouse model as a true representation of human skin.

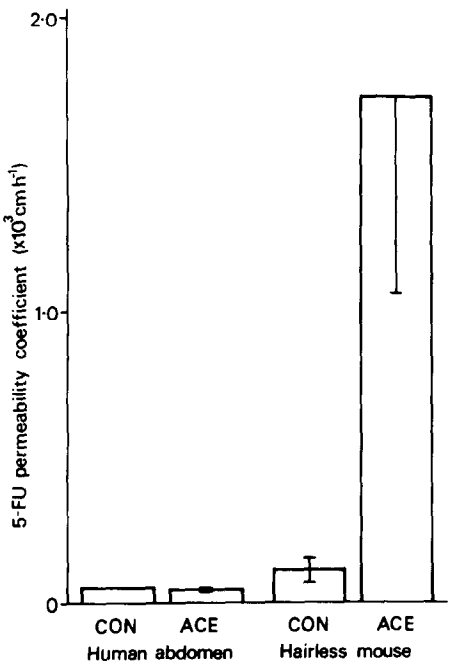

Fig. 2. Mean ($\pm$S.E.M. $\times 10^3$ cm/h) permeability coefficients of 5-FU through control (CON) and acetone-pretreated (ACE) human abdominal and hairless mouse skins.

Acknowledgements

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