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Permeability characteristics of cultured mouse keratinocytes compared to hairless mouse skin

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Summary

The feasibility of using cultured mouse keratinocytes as an in vitro model for hairless mouse skin for permeability studies was explored in this study. Basal keratinocytes from neonatal mice were cultured onto microporous membranes using an air-liquid interface which has been reported to induce formation of stratum corneum. The permeability characteristics of the cultured keratinocytes were compared to hairless mouse skin using a series of n-alkanols. Because of the difficulty in inducing the keratinocytes to form a complete stratum corneum, the use of cultured keratinocytes as a model for studying solute transport is problematic.

Although animal skin models (e.g., hairless mouse, pig, monkey, rabbit) are extensively used to study transdermal delivery, none of these models correlates well with human skin in terms of their permeability to solutes (Montagna, 1971; Maibach, 1975; Hinz et al., 1989). A problem with other models (e.g., cadaver skin, snake skin or synthetic membranes) is that they do not contain viable cells; hence, the metabolic potential of the skin is not properly addressed. Several laboratories have attempted to develop cell culture model systems which would mimic the epidermis of the skin (Fusenig and Worst, 1975; Marcello et al., 1978; Pruniéras et al., 1983; Madison et al., 1988; Cumpstone et al., 1989; Audus et al., 1990). These keratinocyte culture systems are potentially useful to pharmaceutical scientists, since they would provide in vitro models to assess transdermal transport and metabolism of drugs (Audus et al., 1990; Wilson et al., 1990). In this article, we have explored the utility of mouse keratinocytes cultured on microporous membranes using an air-liquid interface environment as an in vitro model for hairless mouse skin for permeability studies. In a separate study our laboratory has shown the utility of cultured mouse and human keratinocytes for

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studying peptide metabolism (Bartel and Borchardt, 1988; Shah and Borchardt, 1989, 1990).

To characterize the permeability properties of hairless mouse (9–12 week old, male, HRS/J, Jackson Laboratories, Bar Harbor, ME) skin and the cultured mouse keratinocytes, *n*-alkanols were selected as solutes for diffusion studies. The [^{14}C]alkanols were purchased from American Radiolabeled Chemicals (St. Louis, MO) (methanol, ethanol, hexanol and octanol), or Pathfinder Lab. (St. Louis, MO) (propanol, butanol, pentanol and heptanol). *n*-Alkanols were selected because the permeability coefficients (P), partition coefficients (K_p) and activation energies (E_a) for these solutes in hairless mouse skin have been characterized and shown to be comparable to those in human skin (Durrheim et al., 1980; Flynn et al., 1981). Earlier permeability studies with these *n*-alkanols were done in saline or water. Neither saline nor water alone would maintain the viability of the cultured keratinocytes. Therefore, permeability studies in both hairless mouse skin and cultured mouse keratinocytes were performed in Earle's balanced salt solution, EBSS (Flow Lab., McLean, VA) which contains glucose to maintain cell viability.

The diffusion experiments across hairless mouse (male, 9–12 week old) skin were conducted at 37°C in EBSS media by essentially the same method as reported by Durrheim et al. (1980). P values of *n*-alkanols across hairless mouse skin in EBSS media and K_p (octanol/EBSS) (Table 1) were similar to the values reported by Durrheim et al. (1980). An activation energy (E_a) of 17.2 kcal/mol was obtained from the Arrhenius plot of log P of pentanol vs $1/T$ in EBSS media (Shah, 1990). Similar E_a values were obtained for alkanol permeation studies from human skin (Blank et al., 1967) and hairless mouse skin using saline (Durrheim et al., 1980).

Basal keratinocytes were obtained from neonatal mouse (0–2 days old) skin by a slight modification of the method reported by Marcelo et al. (1978), which included the addition of hydrocortisone (10 μg/ml) and insulin (10 μg/ml) to the medium (Madison et al., 1988). The cells were plated ($\sim 5 \times 10^5$ cells/insert) on Millicell-HA (Millipore Corp., Bedford, MA; surface area, 1.13 cm^2; pore size, 0.4 μm) inserts coated with a reconstituted collagen gel (Vitrogen-100®, Collagen Corp., Palo Alto, CA). After 3–4 days submerged in the medium, the Millicells were placed at the air-liquid interface as described by Madison et al. (1988, 1989) for 10–12 days. Since the inserts are lifted (and placed on dermis) to expose them to air-liquid interface, these are subsequently referred to as 'lifted cultures'.

TABLE 1

Octanol/EBSS partition coefficients and permeability coefficients of n-alkanols across hairless mouse skin [a,b]

Solute	Partition coefficient	Permeability ($\times 10^3$ cm/h)
Methanol	0.20	3.7 ± 0.51 [c]
Ethanol	0.49	2.7 ± 0.57
Propanol	1.62	4.3 ± 0.46
Butanol	6.73	8.7 ± 0.83
Pentanol	23.4	16.3 ± 2.3
Hexanol	104	31.7 ± 4.8
Heptanol	250	70.4 ± 8.7
Octanol	999	128.9 ± 7.1

[a] *n*-[^{14}C]Alkanols were partitioned in octanol and EBSS. Radioactivity was measured in each phase and K_p calculated. Partition coefficient value represents an average of duplicate experiments.

[b] Permeability experiments were carried out at 37°C in EBSS media.

[c] Standard deviation of at least six replicates.

Other laboratories (Cumpstone et al., 1989; Mak et al., 1989; Madison, K., personal communication) have shown that mouse keratinocytes which are grown in a submerged culture for 3–4 days followed by an air-liquid environment for 10–12 days develop a stratum corneum. The permeability properties of this stratum corneum have been partially characterized by Cumpstone et al. (1989) using a microcapillary diffusion apparatus (diffusion area = 0.02 cm^2) and studying the permeability of [^{3}H]H_2O.

In the present study we have used a more conventional diffusion apparatus with a larger surface area (1.13 cm^2) (Shah et al., 1990) and have selected butanol as the solute for permeability studies. The apparatus has the capability of stirring in both chambers, but for the experiments described here only the receiver side was stirred

since the cell layer was visibly affected by stirring on the donor side. The experiments were conducted at ambient (~ 23°C) temperature. [^{14}C] Butanol was selected for these diffusion experiments since it has an intermediate permeability in the series of *n*-alkanols used above to characterize the permeability properties of hairless mouse skin. EBSS (0.35 ml) containing [^{14}C]butanol ($< 10^{-4}$ M) and [^{3}H]sucrose ($< 10^{-4}$ M) was added in the donor side and 3.5 ml EBSS was added in the receiver side. Aliquots (200 μl) were removed every 5–10 min from the receiver side and radioactivity determined. The aliquots removed from the receiver side were replenished by adding 200 μl of fresh EBSS. The permeability of [^{14}C]butanol through the cultured mouse keratinocytes was also compared to the permeability of [^{3}H]sucrose, which is a highly polar molecule and essentially impermeable to the intact stratum corneum. For example, less than 0.3% sucrose was transported across hairless mouse skin in 5 days (data not shown). Hence, [^{3}H]sucrose was used as a marker in the diffusion experiments with the cultured keratinocytes to detect gross imperfections in the stratum corneum. Fig. 1 shows that there was little difference in the permeability of [^{14}C]butanol (Fig. 1A) or [^{3}H]sucrose (Fig. 1B) across the microporous membranes with or without the cultured cells. The permeabilities of sucrose (0.075 cm/h) and butanol (0.16 cm/h) across cultured cells were substantially higher than across hairless mouse skin. The primary reasons for the high permeability of [^{14}C]butanol and [^{3}H]sucrose through the cultured keratinocytes are: (1) the cells retract from the outer edge of the Millicell as they stratify; and (2) the stratum corneum that is formed is not continuous.

The lifted cultures were retracted from the outer edges of the Millicells as they stratified, presumably due to the degradation of the underlying collagen (Woodley et al., 1986; Souren et al., 1989), leaving extensive regions uncovered in the inserts. Several cross-sections of the lifted culture also showed that the stratum corneum was not continuous and top layers of the cells were poorly anchored. Other laboratories have reported similar discontinuous stratum corneum formation with keratinocytes cultured at the air-liquid interface

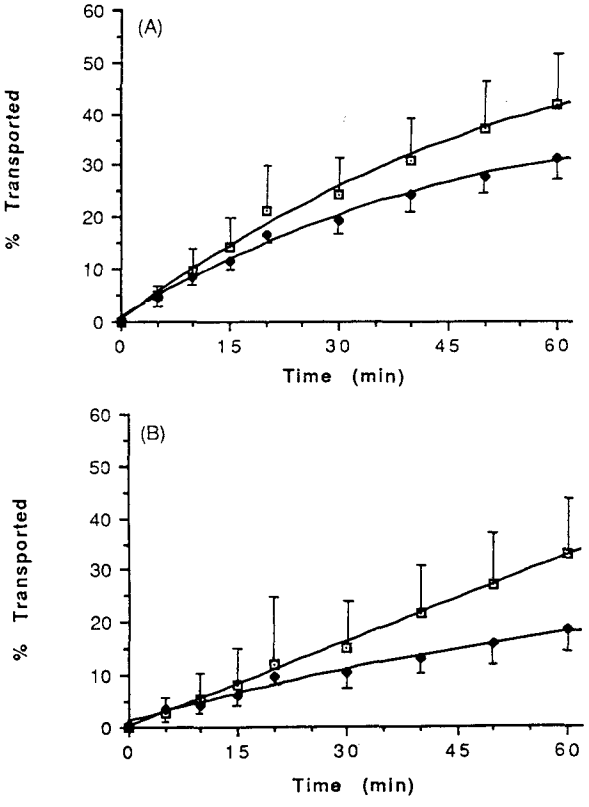

Fig. 1. Transport of (A) [^{14}C]butanol and (B) [^{3}H]sucrose across Millicells with or without cultured mouse keratinocytes. The diffusion experiments were carried out using the device previously reported (Shah et al., 1990). The error bars are standard deviations of five replicates. The lines are drawn to visualize the trends. (◆———◆) Millicells with cells and (⊡———⊡) Millicells without cells.

(Mak et al., 1989; Madison, K., personal communication).

In order to use the cultured mouse keratinocyte systems that have been developed to date as in vitro models for permeability studies, one would have to use the micro-diffusion apparatus (diffusion area = 0.02 cm^2) described by Cumpstone et al. (1989). This apparatus requires one to select regions of the culture that appear to have an intact stratum corneum. This subjective selection may lead to high variability in permeability studies. The inconvenience of using this apparatus may limit its utility and thus the utility of cultured keratinocytes for permeability studies.

Although the existing cultured mouse keratinocytes model is a poor model for transport studies, we have shown it to be an excellent in vitro model for metabolism studies (Shah and Borchardt, 1990). Peptidase activity (K_m, V_{max} values) and Leu-enkephalin metabolism (rate constants and distribution of metabolites profile) in cultured mouse keratinocytes and hairless mouse were shown to be similar. Thus, the existing technology for culturing keratinocytes may be useful for conducting drug metabolism studies but not transport experiments.

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References

Audus, K.L., Bartel, R.L., Hidalgo, I.J. and Borchardt, R.T., The use of cultured epithelial and endothelial cells for drug transport and metabolism studies. *Pharm. Res.*, 7 (1990) 435–451.

Bartel, R.L. and Borchardt R.T., Characterization of epidermal keratinocyte cultures as a potential model for skin transport and metabolism. *Pharm. Res.*, 5 (1988) S-79 (Abstract no. PD828).

Blank, I.H., Scheuplein R.J. and Macfarlane, D.J., Mechanism of percutaneous absorption. III. The effect of temperature on the transport of non-electrolytes across the skin. *J. Invest. Dermatol.*, 49 (1967) 582–589.

Cumpstone, M.B., Kennedy, A.H., Harmon, C.S. and Potts, R.O., Water permeability of primary mouse keratinocyte cultures grown at air-liquid interface. *J. Invest. Dermatol.*, 92 (1989) 598–600.

Durrheim, H., Flynn, G.L., Higuchi, W.I. and Behl, C.R., Permeation of hairless mouse skin. I: Experimental methods and comparison with human epidermal permeation by alkanols. *J. Pharm. Sci.*, 69 (1980) 781–786.

Flynn, G.L., Durrheim, H. and Higuchi, W.I., Permeation of hairless mouse skin. II: Membrane sectioning techniques and influence on alkanol permeabilities. *J. Pharm. Sci.*, 70 (1981) 52–56.

Fusenig, N.E. and Worst, P.K.M., Mouse epidermal cell cultures. II. Isolation, characterization and cultivation of epidermal cells from perinatal mouse skin. *Exp. Cell Res.*, 93 (1975) 443–457.

Hinz, R.S., Hodson, C.D., Lorence, C.R. and Guy, R.H., In vitro percutaneous penetration: evaluation of the utility of hairless mouse skin. *J. Invest. Dermatol.*, 93 (1989) 87–91.

Madison, K.C., Swartzendruber, D.C., Wertz, P.W. and Downing, D.T., Lamellar granule extrusion and stratum corneum intercellular lamellae in murine keratinocyte cultures. *J. Invest. Dermatol.*, 90 (1988) 110–116.

Madison, K.C., Swartzendruber, D.C., Wertz, P.W. and Downing, D.T., Murine keratinocytes cultures grown at the air/medium interface synthesize stratum corneum lipids and recycle linoleate during differentiation. *J. Invest. Dermatol.*, 93 (1989) 10–17.

Maibach, H., *Animal Models in Dermatology*, Churchill Livingstone, New York, 1975.

Mak, V.H.W., Cumpstone, M.B., Kennedy, A.H., Harmon, C.S., Guy, R.H. and Potts, R.O., Barrier function of mouse and human keratinocyte cultures grown at the air-liquid interface. *J. Invest. Dermatol.*, 92 (1989) 475.

Marcelo, C.L., Kim, C.L., Kaine J.L. and Voorhees, J.J., Stratification, specialization and proliferation of primary keratinocyte cultures. *J. Cell Biol.*, 79 (1978) 356–370.

Montagna, W., Cutaneous comparative biology. *Arch. Dermatol.*, 104 (1971) 577–591.

Pruniéras, M., Régnier, M. and Woodley, D., Methods for cultivation of keratinocytes with an air-liquid interface. *J. Invest. Dermatol.*, 81 (1983) 28s–33s.

Shah, P.K., Ph. D. Dissertation. Cultured keratinocytes: An in vitro model to study transport and metabolism in skin, University of Kansas, 1990.

Shah, P.K. and Borchardt, R.T., Cultured human keratinocytes as an in vitro model to study peptide metabolism in human skin. *Pharm. Res.*, 6 (1989) S-148, Abstract No. PD 1085.

Shah, P.K. and Borchardt, R.T., A comparison of peptidase activities and peptide metabolism in cultured mouse keratinocytes and neonatal mouse epidermis. *Pharm. Res.*, 8 (1991) 70–75.

Shah, P.K., Hidalgo, I.J. and Borchardt, R.T., A simple device to study transport across Millicells. *Int. J. Pharm.*, 63 (1990) 281–283.

Souren, J.E.M., Ponec, M. and Wijk, R.V., Contraction of collagen by human fibroblasts and keratinocytes. *In Vitro Cell. Dev. Biol.*, 25 (1989) 1039–1045.

Wilson, G., Davis, S.S. and Illum, L., *Pharmaceutical Applications of Cell and Tissue Culture*, Plenum, New York, in press.

Woodley, D.T., Kalebee, T., Banes, A.J., Link, W., Pruniéras, M. and Liotta, L., Adult human keratinocytes migrating over nonviable dermal collagen produce collagenolytic enzymes that degrade type I and type IV collagen. *J. Invest. Dermatol.*, 86 (1986) 418–423.