

Objective

Evaluate potential *in vitro* percutaneous absorption and penetration differences of human dermatomed skin and dermis-only skin following topical application of two model compounds under conditions with different flow rates and receptor phase compositions used with the Braough Flow-Through Diffusion Cell system. Clotrimazole and hydrocortisone, both at 1% in a cream emulsion formulation, were selected as model compounds based upon their clinical topical use as antifungal and steroidal anti-inflammatory agents, differences in partition coefficient ($\log K_{ow}$ 6.26 and 1.62), but comparable molecular weights (384.5 and 362.5 g/mol), respectively. Skin penetration of the model compounds should inversely correlate with relative saturation of compound in the receptor phases after exceeding sink conditions. Relative saturation (dependent upon skin permeability, model compound solubility in receptor phase, and receptor phase flow rate) will be characterized to assess whether sink conditions are met under the various experimental test situations.

Methods

Tissue Source and Preparation

Human abdominal skin was obtained and prepared within 24 hours following elective surgery from three donors. The epidermis along with approximately the upper quarter to a third of the dermis was collected by dermising the skin at a setting of 0.813mm thickness (0.022") on a Padgett Model 6 dermatome. The remaining dermis-only tissue was also collected for study as a model for wounded skin. Tissues were then stored frozen at -20 °C in vacuum-sealed packages prior to use. Tissue thickness was measured using a snap gauge micrometer.

Tissue Thickness (mm), Mean \pm SD	
Dermatomed Skin (Donor 1)	0.836 \pm 0.112
Dermatomed Skin (Donor 2)	0.828 \pm 0.103
Dermatomed Skin (Donor 3)	0.676 \pm 0.108
Dermis-Only (Donor 1)	1.488 \pm 0.304
Dermis-Only (Donor 2)	1.124 \pm 0.194
Dermis-Only (Donor 3)	1.545 \pm 0.202

Experimental Details

Clotrimazole and hydrocortisone emulsion creams¹ (10% Stearyl alcohol, 5% Glycerin, 2% Isopropyl Myristate, 1% API, c.s.) emulsion creams were manufactured with 1%Clotrimazole or ³H-clotrimazole or ³H-hydrocortisone. ³H-Clotrimazole or ³H-hydrocortisone were added to the oil phase along with sufficient unlabeled API to achieve 1% API in the two emulsion creams.

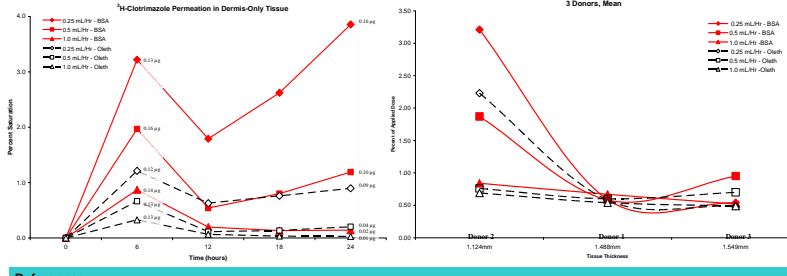
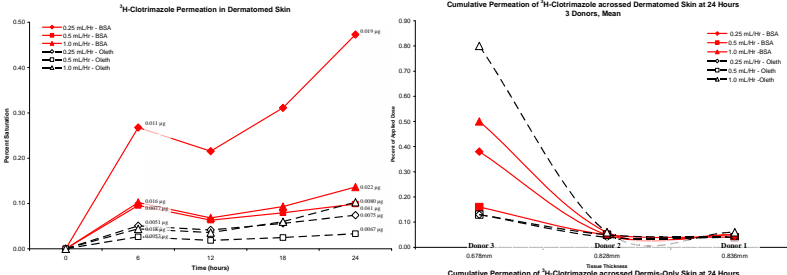
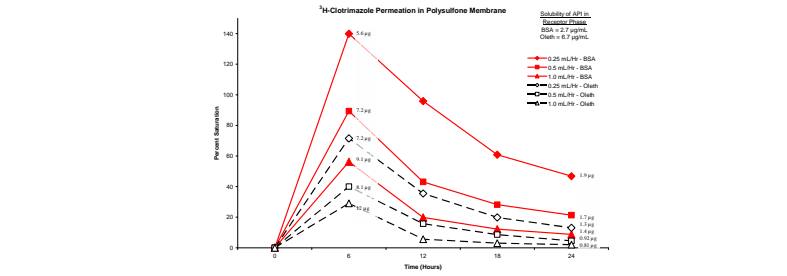
All tissues were mounted in Braough Flow-Through Diffusion Cells at 32°C and dosed with a clinically relevant amount (5mg/cm²) of either 1% ³H-clotrimazole or 1% ³H-hydrocortisone emulsion cream. The receptor phase (Phosphate Buffered Saline with 0.1% Sodium Azide and 1.5% Oletn-20 (Oletn), pH 7.4, or Phosphate Buffered Saline with 0.1% Sodium Azide and 4% Bovine Serum Albumin (BSA), pH 7.4) was pumped across the underside of the tissue at three flow rates (0.25, 0.5 and 1.0 mL/hr).

Human dermatomed and dermis-only skin from 3 donors and polysulfone filter (0.45µm pore size) controls were evaluated over a 24-hour duration following topical application of cream.

Discussion

Flow rates used in *in vitro* studies have been documented to vary from 1.5 to 5 mL/hr⁴; this study was designed to investigate if drug penetration is affected by flow rates ranging from 0.25 to 1.0 mL/hr and if sink conditions could be maintained based on API solubility in each receptor phase. Comparable mass of API was observed in the receptor phase at flow rates of 0.5 mL/hr and 1.0 mL/hr. Whereas, with 0.25 mL/hr, a slight decrease in drug permeability was observed, most likely due to API increased saturation in the receptor phase.

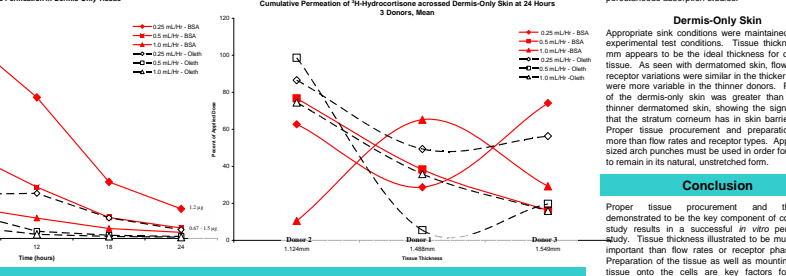
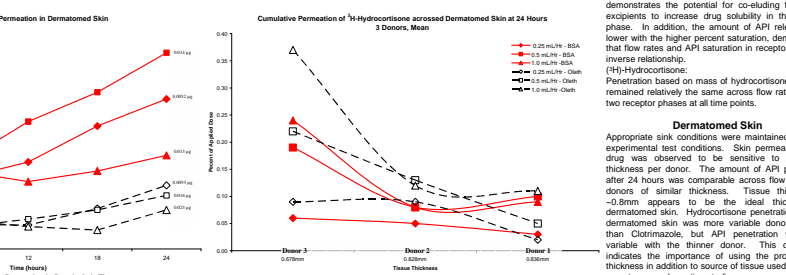
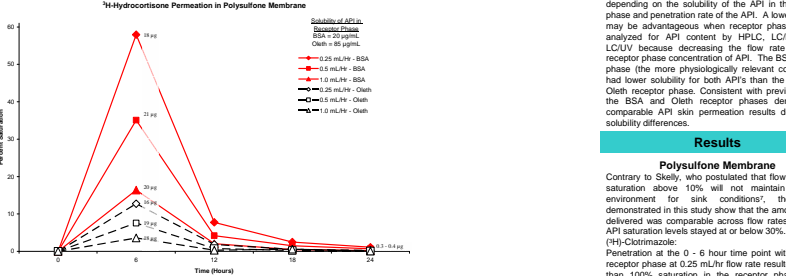
³H-Clotrimazole - 24 Hour Exposure



References

- Marketed product use level from the USP DI, Drug Information for the Health Care Professional, Volume I, Micromedex, 2007 27th Edition pp. 326, 862 and 936. <http://www.asppharmaceut.com/view.asp?an=pt070104>
- USP DI, approved Drug Products and Legal Requirements, Volume III, Micromedex, 1999 19th Edition pp. IV/138, and IV/249
- Brain KR, Walters KA & Watkinson AC (1998a) Investigation of skin permeation *in vivo*. In: Roberts MS & Walters KA eds. Dermal absorption and toxicity assessment. New York, Marcel Dekker, pp. 161-187 (Drugs and the Pharmaceutical Sciences, Vol. 91).
- Brough RL & Maibach HI (1985) Percutaneous absorption of nitroaromatic compounds: *in vivo* and *in vitro* studies in the human and monkey. J Invest Dermatol. 84(3): 180-183.
- Bucks D, Lund T, Winkles C, Richardson C, Crawford B. An *In Vitro* Model Using Newborn Bovine Eyes to Assess Cornea & Sclera Permeation. The AAPS Journal 2006; Available from: <http://www.aapspharmaceut.com>
- Skelly JP, Shan VP, Guj RH, Wester RC, Flynn G & Yacobi A (1987) FDA and AAPS report of the workshop of principles and practices of *in vitro* percutaneous penetration studies; relevance to bioavailability and bioequivalence. Pharm Res. 4(3): 265-267.

³H-Hydrocortisone - 24 Hour Exposure



Discussion, cont.

This demonstrates that lower flow rates can be used depending on the solubility of the API in the receptor phase and penetration rate of the API. A lower flow rate may be advantageous when receptor phase is to be analyzed for API content by HPLC, LC/MSMS or LC/LUV because decreasing the flow rate increases receptor phase concentration of API. The BSA receptor phase (the more physiologically relevant composition) had lower solubility for both APIs than the respective Oletn receptor phase. Consistent with previous work⁶, the BSA and Oletn receptor phases demonstrated comparable skin permeation results despite API solubility differences.

Results

Polysulfone Membrane

Contrary to Skelly, who postulated that flow rates with saturation above 10% will not maintain favorable environment for sink conditions⁴, the results demonstrated in this study show that the amount of API delivered was comparable across flow rates when the API saturation levels stayed at or below 30%.

(H)-Clotrimazole

Penetration at the 0 - 6 hour time point with the BSA receptor phase at 0.25 mL/hr flow rate resulted in more than 100% saturation in the receptor phase. This demonstrates the potential for co-formulating excipients to increase drug solubility in the receptor phase. In addition, the amount of API released was lower with the higher percent saturation, demonstrating that flow rates and API saturation in receptors have an inverse relationship.

(H)-Hydrocortisone

Penetration based on mass of hydrocortisone delivered remained relatively the same across flow rates and the two receptor phases at all time points.

Dermatomed Skin

Appropriate sink conditions were maintained under all experimental test conditions. Skin permeation of the drug was observed to be sensitive to the tissue thickness per donor. The amount of API penetration after 24 hours was comparable across flow rates with donors of similar thickness. Tissue thickness of <0.8mm appears to be the ideal thickness for dermatomed skin. Hydrocortisone penetration through dermatomed skin was more variable donor to donor than Clotrimazole, but API penetration was more variable with the thinner donor. This observation indicates the importance of using the proper tissue thickness in addition to source of tissue used for *in vitro* percutaneous absorption studies.

Dermis-Only Skin

Appropriate sink conditions were maintained under all experimental test conditions. Tissue thickness of 1.5 mm appears to be the ideal thickness for dermis-only tissue. As seen with dermatomed skin, flow rates and receptor variables were similar in the thicker tissue and were more variable in the thinner donors. Penetration of the model compound was greater than the much thinner dermatomed skin, showing the significant role that the stratum corneum has in skin barrier function. Proper tissue procurement and preparations matter more than flow rates and receptor types. Appropriately-sized arch punches must be used in order for the tissue to remain in its natural, unstretched form.

Conclusion

Proper tissue procurement and thickness demonstrated to be the key component of consistent study results in a successful *in vitro* permeation study. Tissue thickness illustrated to be much more important than flow rates or receptor phase type. Preparation of the tissue as well as mounting of the tissue onto the cells are key factors for tissue structure to remain intact.