Pharmaceutical Research, Vol. 6, No. 8, 1989

Technical Note

A Simple Microwave Technique for the Separation of Epidermis and Dermis in Skin Uptake Studies

Saran Kumar,[1,2] Charan R. Behl,[1] Sunil B. Patel,[1] and A. Waseem Malick[1]

Received October 24, 1988; accepted March 28, 1989

KEY WORDS: skin sectioning; microwave skin sectioning; skin layer separation; skin retention; epidermal–dermal retention.

To evaluate topical/transdermal dosage forms and prodrugs, determination of the drug distribution within different layers of the skin is becoming increasingly important. In the past, almost all emphasis was placed on the permeation parameter. This communication is to introduce a new method of separating epidermis from dermis to study skin retention in these layers. In the present method, the skin is exposed to microwave energy in a conventional "household" microwave oven. A 1-cm^2 skin sample was placed in a glass petri dish and then in a microwave oven. Through several trials, it was determined that an exposure of 6 to 12 sec at medium–low setting was sufficient to allow a separation of the epidermis from the dermis of hairless guinea pig skin. The microwave oven used in this case was Sharp Carousel II, Model 5970. It was noted that the time of exposure was critical. An overexposure was found to char the skin. One actually has to calibrate the exposure time for the specific skin area, point of placement in the oven, and particular equipment being used. In our studies we have evaluated skins from hairless mouse, furry mouse, nude rat, furry rat, hairless guinea pig, furry guinea pig, and human cadaver. A general observation was that thicker skins took longer exposure times for the separation. After the exposure, the epidermis was peeled off the dermal layer with the aid of forceps. To do so, it was first necessary to lift the epidermis at one or more corners with a scalpel. If an optimal exposure time was used, the peeling process was easy. This microwave technique is being successfully and routinely used in our skin uptake studies from topical dosage forms in both *in vitro* and *in vivo* experiments. In the *in vivo* studies, the skin was excised from the animal prior to exposing it to microwave energy.

In the case of the *in vitro* studies for the evaluation of topical dosage forms utilizing finite-dose methods and vertical diffusion cells, at the conclusion of a permeation experiment, the cell was placed in a microwave oven. After an appropriate exposure time, the cell halves were unclamped and the epidermis was peeled off the dermis while the skin was still mounted on the cell. Depending upon the specific experimental needs, either or both of these layers were used to determine the drug content in the layers. It should be noted that although this method is found useful in the determination of drug content in the skin layers at the end of an experiment, it may have utility in the preparation of membranes for permeability studies. However, additional work is needed to ensure that the heat from the microwave does not cause changes in permeability characteristics. In our work, it was observed that the microwave exposure tends to shrink the skin.

The microwave technique reported here is simpler than the other techniques such as chemical (1–7), hot water immersion and hydration (8–10), enzyme digestion (11–16), adhesive tape stripping (17), and mechanical (18–20) methods. The chemical method can affect the drug distribution in the skin layers and can interfere with the drug assay. Of course, depending upon the chemical used, the skin uptake properties can also be affected. The hot water immersion method is disadvantageous because drug retained in skin layers can leach out into the surrounding hot water. The enzyme digestion method is suitable primarily to obtain the stratum corneum layer and to collect epidermal cells. In addition, the drug retained in the stratum corneum can leach out into the enzyme medium. The adhesive tape stripping method provides mainly stripped skin, as opposed to epidermis/dermis separation. Besides, this technique does not work on skins which have been used in permeation experiments in which skin undergoes hydration. The mechanical methods such as microtoming are too cumbersome to use on small animal skins and it is also not feasible to microtome precisely at the epidermal/dermal junction.

REFERENCES

1. J. P. Baumberger, V. Suntzeff, and E. V. Cowdry. *J. Natl. Cancer Inst.* 2:413–423 (1942).
2. Z. Felsher. *J. Invest. Dermatol.* 8:35–47 (1974).
3. R. D. Griesemer and E. Gould. *J. Invest. Dermatol.* 22:299–315 (1954).
4. M. Hollo and S. Zlatarov. *Arch. Geschwulstforsch.* 7:243–246 (1954).
5. Z. M. Hollo and S. Zlatarov. *J. Invest. Dermatol.* 26:383 (1956).

[1] Pharmaceutical Research and Development, Hoffmann-LaRoche, Inc., Nutley, New Jersey 07110.
[2] To whom correspondence should be addressed.

0724-8741/89/0800-0740$06.00/0

6. Z. M. Hollo and S. Zlatarov. *J. Invest. Dermatol.* 31:223–226 (1958).
7. I. Deme and M. Garazsi. *J. Invest. Dermatol.* 32:393–396 (1959).
8. S. Rothman, H. F. Krysa, and A. M. Smiljanic. *Proc. Soc. Exp. Biol. Med.* 62:208–209 (1946).
9. Chen-der Yu. Ph.D. thesis, University of Michigan, Ann Arbor (1980).
10. C. R. Behl, G. L. Flynn, W. M. Smith, T. Kurihara, K. A. Walters, O. G. Gatmaitan, W. I. Higuchi, C. L. Pierson, and N. F. H. Ho. *Abstr. 27th Ann. Meet. Am. Pharm. Assoc.*, Acad. Pharm. Sci., Kansas City, Mo., Basic Pharm. Sec. Abstr. 53.
11. G. Szabo. *J. Pathol. Bacteriol.* 70:545 (1955).
12. S. W. Becker, Jr., A. B. Lerner, and H. Montgomery. *Science* 112:223–225 (1950).
13. J. H. Cooper. *Arch. Dermatol. Syph.* 77:18–22 (1958).
14. J. Balo and I. Banga. *Biochem. J.* 46:384–387 (1950).
15. P. B. Medawar. *Lancet* 1:350–352 (1942).
16. J. Fan and M. R. Sikov. *J. Invest. Dermatol.* 30:167–171 (1958).
17. J. Wolf. *Z. Mikr. Anat. Forschung.* 47:351 (1940).
18. E. J. Van Scott. *J. Invest. Dermatol.* 17:21–25 (1951).
19. J. Tabachnick. *J. Invest. Dermatol.* 32:563–568 (1959).
20. I. J. Blank and M. H. Coolidge. *J. Invest. Dermatol.* 15:249–256 (1950).