SKIN ENZYMES DISTRIBUTION IN TRANSDERMAL DRUG DELIVERY

T. Hikima, H. I. Maibach, and K. Tojo Department of Bioscience and Bioinformatics, Kyushu Institute of Technology, 680-4 Kawazu, Iizuka 820-8502, Japan Department of Dermatology, University of California, San Francisco, CA 94143, USA

Introduction

Skin has two barriers for protective functions; a physical barrier of stratum corneum and a biochemical barrier in epidermis and dermis. Skin enzymes biotransform not only chemical toxins and tumorogens but also drugs entering from the skin surface. Pharmacokinetics in the skin, the penetration flux and distribution of drugs and its metabolites, is influenced by the distribution of metabolic enzymes in the skin. Thus, knowledge of enzymatic activities and distribution in the skin is important for developing safe and efficacious transdermal drug delivery. In this study, we have investigated the skin distribution and enzymatic activity of several enzymes (esterase, oxidase, and reductase) in human and animal skin *in vitro*. Moreover, we have developed the skin absorption model of drug with skin enzyme distribution.

Materials and Methods

Materials

Human cadaver skin samples were removed from Caucasian male (the Northern California Transplant Bank, San Rafael, CA, USA). Animal model skin samples, hairless dog (female, 7-9 years old) and hairless mouse (Hr⁻/Kud, female, 8-10 weeks old), were obtained from Nihon Nosan Kogyo Co. (Tsukuba, Ibaraki, Japan) and Kyudo Co. (Tosu, Saga, Japan), respectively.

Prednisolone 21-acetate (PNA), prednisolone (PN), β -estradiol 17-acetate (EA), estrone (E₁), and β -estradiol (E₂) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Testosterone propionate (TSP) and testosterone (TS) were obtained from MP Biomedicals Inc. (Irvine, CA) and Calbiochem (Los Angels, CA, USA), respectively. PNA, EA and TSP were chosen as substrates for esterase. E₂ and E₁ were used as substrates for oxidase and reductase. Other reagents are purchased from Sigma Chemical Co. and Wako Pure Chemical Industries (Osaka, Osaka, Japan).

Enzymatic Activity

Skin samples removed from human and animal were cut up into pieces with 20 mL of icecold Tris-Sucrose buffer (pH 7.4, 20 mM Tris, 0.25 M sucrose and 5 mM EDTA). These skin samples were homogenized by Polytrone[®] (Brinkman, Littau, Switzerland) in ice-cold bath to obtain the enzyme solution. The model parent drug was metabolized with a 1 mL enzyme solution at 36 °C.

Enzyme Distribution

After human cadaver skin removed from male was frozen in O.C.T. compound at -30 $^{\circ}$ C, the skin sample was sliced repeatedly in 10 μ m thickness with a microtome Cryostat (LEICA CM

1850, Leica Microsystems Inc., Deerfield, IL, USA). Four consecutive skin slices were placed into 5 mL 40% PEG400 solution or PBS (pH 7.4) with a known amount of the parent drug. The sliced skins were then incubated in the medium at 36 °C. At predetermined time intervals, the metabolite concentration in the medium was measured by HPLC. Animal skins were also sliced according to the above procedures and enzyme distribution in skin was investigated.

Results and Discussion

Table 1 summarized a Michaelis-Menten constant K_m and maximum rate V_{max} for hydrolytic reactions in human and hairless mouse skin.¹⁾ The values of K_m obtained from human skin was 1.8 times greater than that from

skin was 1.8 times greater than that from hairless mouse and, conversely, V_{max} from human skin was 0.81 times less than that from hairless mouse; an enzymatic affinity is a slightly difference among species. The hydrolytic activity/thickness ratio in [nmol/min/mgepidermis (4.8)± 2.3 protein/mm]) is 12 times larger than that in 0.13 [nmol/min/mgdermis (0.40)± protein/mm]) (Table 2). This result indicates that the hydro

lytic enzyme capable of metabolizing PNA mainly concentrates in the epidermis.

The concentration of metabolite catalyzed by esterase in the medium included human skin sliced layer has a peak value at 80-120 µm of the distance from the skin surface irrespective of age and parent drug (Figure 1). Therefore, the esterase activity may be mainly distributed at the

Table 1. Kinetic parameters of hydrolytic enzymes for PNA in the enzyme solution

	K _m [µM]	V _{max} [nmol/min/mg]
Human skin	25.1	0.46
Hairless mouse	14.2	0.57

 $K_{\rm m}$ is the Michaelis-Menten constant and $V_{\rm max}$ is the maximum rate.

Table 2. Specific activity of PNA hydrolysis in the whole skin, dermis, and epidermis.

Tissue	Thickness	Specific activity
	[mm]	[nmol/min/mg]
Whole skin	0.94 ± 0.06	0.65±0.17
Dermis	0.81±0.01	0.33±0.11
Epidermis	0.05 ± 0.004	0.26±0.12*

Data represent mean±S.D.

*Significantly different from whole skin at p<0.1, which are calculated using t-test.



Figure 1. Time variation of metabolite concentration (a: PN, b: E_2 , and c: TS) in its parent drug solution after several incubation time. The age of human skin samples are 29 (a), 71 (b), and 21 (c) years olds. The columns indicate mean \pm S.D of triplicated data.

basement layer of epidermis. On the other hand, the distribution of reductase and oxidase shows the almost same mountainous pattern (a peak at 40-120 μ m) throughout the skin irrespective of age (Figure 2).



Figure 2. Distribution of metabolite concentration in medium by reductive 17 β -HSD (a) and oxidative 17 β -HSD (b) after 24 hrs incubation. The columns indicate mean ± S.D. of triplicated data.

In human cadaver skin, skin enzymes distribution has a peak value around 80-120 μ m. The distance from skin surface is corresponded to the layer just before capillary vessel.²⁾ This result shows that skin enzymes effectively metabolize drugs before entering the systemic circulation.

Figures 3 shows the distribution of EA metabolite (E_2) in hairless dog skin. The thickness of epidermis of hairless dog was about 50 μ m³⁾ and the amount of metabolite in the medium was approximately plateau from 0 to 180 μ m. Therefore, the hydrolytic activity in the hairless dog skin was equally distributed through epidermis layer and decreased with increasing the skin thickness. On the other hand, hairless mouse has the highest metabolic activity at 180-240 μ m from the skin surface (Figure 4) although the thickness of epidermis was 50 μ m.³⁾ Hairless mouse



Metabolite (E₂) concentration $[\mu g/mL]$ 0.8 6hrs 0.6 12hrs 0.4 0.2 0.0 0~00 60~120 20~180 180~240 240~300 300~360 360~420 Distance from skin surface $[\mu m]$

Figure 3. Distribution and time variation of metabolite (E_2) concentration in the EA solution after 3 hrs and 12 hrs incubation. Skin samples are obtained from dorsal skin of hairless dog. The columns indicate mean \pm S.D (n=3).

Figure 4. Distribution and time variation of metabolite (E_2) concentration in the EA solution after 6 hrs and 12 hrs incubation. Skin samples are removed from abdomen of hairless mouse. The columns indicate mean \pm S.D (n=3).

had the highest activity hydrolyzed EA at the area of the basement layer of dermis which are surrounding area of the cutaneous plexus not as in human and hairless dog skin. Therefore, hairless mouse takes topically applied drugs into the circulatory system from the papillary plexus before metabolizing and detoxifying them.

In conclusion, the biological barrier, an enzymatic activity and enzyme distribution in the skin, differs among species. Skin distribution reveals that an efficacy of enzymatic reactions in hairless mouse skin may be lower than that in human and hairless dog skin. The physical barrier, a low diffusivity through stratum corneum, also has a different behavior among species.⁴⁾ Therefore, we should take account of the difference for developing the transdermal drug delivery system.

References

- 1. T. Hikima, Y. Ohno, and H. I. Maibach (2001), "Metabolism of prednisolone 21-acetate in hairless mouse skin," *Skin Pharmacol. Appl. Skin Physiol.*, 14: 203-209.
- 2. P. Petzelbauer, J. R. Bender, J. Wilson, and J. S. Pober (1993), "Heterogeneity of dermal micro-vascular endothelial cell antigen expression and cytokine responsiveness in situ and in cell culture," *J. Immuno.*, 151: 5062-5072.
- 3. T. Hikima, K. Yamada, T. Kimura, H. I. Maibach, and K. Tojo (2002), "Comparison of skin distribution of hydrolytic activity for bioconversion of ß-estradiol 17-acetate between man and several animals *in vitro*," *Eur. J. Pharm. Biopharm.*, 54: 155-160.
- 4. R. Panchagnula, K. Stemmer, and W. A. Ritschel (1997), "Animal models for transdermal drug delivery," *Methods Find Exp. Clin. Pharmacol.*, 19: 335-41.