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Species difference in simultaneous transport and metabolism of ethyl nicotinate in skin

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Abstract

The objective of this research was to compare the characteristics of skin permeation and metabolism of ethyl nicotinate (EN) among humans and several animal models. In vitro simultaneous skin permeation and metabolism experiment of EN was done in side by side diffusion cells at 37°C. An EN hydrolysis experiment was carried out using skin homogenate and kinetic parameters (V_{max} and K_m) were estimated by computer data-fitting to Michaelis–Menten equation. Both EN and a metabolite, nicotinic acid (NA), were detected in all receiver solutions in permeation studies and no significant chemical hydrolysis was found, indicating that enzymatic hydrolysis of EN occurred during the skin permeation process. Difference in total (EN + NA) flux, from EN-saturated solution, was less than double among various species. The ratio of NA flux to total flux was highest for rat (0.94) followed by hairless rat, mouse, human and hairless mouse (0.76, 0.23, 0.19 and 0.13), and thus a great species difference was found in skin esterase activity. Total flux increased linearly with increase in donor concentration for all species. For hairless rat, mouse and hairless mouse, NA fluxes increased with increase in EN donor concentration and reached a plateau, suggesting that metabolic saturation occurred in skin. Species difference in NA fluxes and EN donor concentration in which the NA flux reached a plateau were also found. In rats, kinetic parameters for EN hydrolysis using skin homogenate were significantly higher than those in mice. These results suggest that species difference in permeation profiles of EN might primarily reflect the difference in esterase activity. To predict skin permeability in human using an animal model, the species difference in skin metabolism should be taken into consideration.

Keywords: Species difference; Skin permeation; Skin metabolism; Ethyl nicotinate.

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1. Introduction

The advantages and benefits of lipophilic prodrugs as a method to enhance skin permeation of drugs have been extensively reviewed and several transdermal therapeutic systems containing prodrugs have been clinically applied (Chan and Po, 1989; Kasting et al., 1992). For an even more effective system, the study on simultaneous skin permeation and metabolism of prodrug candidates must be continued. Ideally, such study should be performed in human, in which all obtained data and parameters provide the best information for clinical therapeutic application. However, limitations in obtaining fresh human skin tissue and the ethical problems involved have prompted the use of skins from laboratory animals, which is easy to handle, lower in cost and genetically uniform (Bickers, 1980; Steinstrasser and Merkle, 1995).

Although there are many reports on species difference in the skin permeability of drugs (Wester and Maibach, 1993), few data are available comparing the skin metabolism in laboratory animals to that in human. Some researchers have pointed out that the extent and rate of skin metabolism affects the skin permeability of a prodrug itself (Mukai et al., 1985; Kao, 1989; Bando et al., 1997). An incomplete skin metabolism may cause some undesirable side effects of the prodrug. Knowledge of the species difference in skin metabolism as well as permeation is important to estimate the pharmacological and side effects of prodrugs in human by extrapolation from animal data.

The present study focused on the species difference in simultaneous skin transport and metabolism. Using ethyl nicotinate (EN) as a model prodrug of nicotinic acid (NA), in vitro skin permeation characteristics and enzymatic activity in skin homogenate were compared among human and several laboratory rodents: rat; hairless rat; mouse; and hairless mouse.

2. Materials and methods

2.1. Materials

Nicotinic acid and ethyl nicotinate were obtained from Tokyo Chemical Industries (Tokyo, Japan). All other reagents and solvents were of analytical grade.

2.2. Preparation of skin membrane

Male rat (Wistar strain, 200–250 g), male hairless rat (HWY/SIC strain, 200–250 g), female mouse (BALB/c Cr Slc strain, 20–25 g) and female hairless mouse (SKH HR-1 strain, 20–30 g) were supplied from Japan SLC (Hamamatsu, Japan). All animals were 7 weeks of age. Female hairless mouse was used because of common damage in the abdominal skin of male hairless mouse, and female mouse was used to compare with hairless mouse. A round section of abdominal skin was freshly excised from various animals under pentobarbital anesthesia (50 mg/kg, i.p.) after being shaved carefully in hairy animals.

Human skin was obtained following unrelated surgical operations (Department of Secondary Surgery, Faculty of Medicine, Toyama Medical and Pharmaceutical University, Toyama, Japan); the source was the breast of 47–74-year-old female patients. The skin was preserved at -20°C prior to use in order to maintain the original activity of skin enzymes (Damen and Mier, 1982). The specimens were gradually thawed in 0.9% w/v NaCl solution at room temperature, then split-thickness skins (0.6–0.7 mm) were prepared as described by Rohatagi et al. (1997).

2.3. In vitro permeation studies

The skin samples excised from various animals and human were mounted between two diffusion half-cells with a waterjacket connected to a water bath at 37 ± 0.5°C, each having 3.0 ml volume and 0.95 cm^2 effective diffusion area (Okamura et al., 1989). The receiver and donor compartments were filled with 0.1 M phosphate buffered saline, pH 7.4 (PBS) and stirred with a Teflon magnetic stirrer at 1200 rpm. One hour of equilibration was allowed and then the receiver solution was replaced with fresh PBS and the donor solution with various concentrations of EN (6–244 mM) in PBS solution. Samples of 0.3 ml were with-

drawn from the receiver solution every hour until 8 h and replaced with the same volume of drug-free PBS solution. The samples were stored at 4°C until analyzed.

2.4. Hydrolysis of ethyl nicotinate

The kinetics of enzymatic hydrolysis of EN was assessed in skin homogenates. Skin homogenates (25% w/w) were made with full-thickness skin freshly excised from various animals and PBS solution using a tissue homogenizer. The homogenates were centrifuged for 10 min at 9000g and 4°C. The supernatant and various concentrations of EN in PBS solution were preincubated for 15 min, mixed to make 5% w/w homogenate and maintained at 37 ± 0.5°C. Chemical degradation of EN was also assessed in enzyme-free PBS at 37 ± 0.5°C. Samples were taken at an appropriate time point and kept at 4°C until analyzed. A non-linear least squares regression program based on the logarithm of Gauss–Newton and Berman et al. (1962) which was run on a personal computer (PC-9801 RA, NEC, Tokyo), was used to estimate the kinetic parameters (V_{max} and K_m). Protein content in skin homogenate was determined by Lowry's method (Lowry et al., 1951) and used to obtain V_{max} values in unit of nmol/min per mg protein. The V_{max} was recalculated in unit of μmol/cm^2 per h using the values of protein content of 5% w/w skin homogenate and weight per area of skin.

2.5. Analytical methods

The HPLC system for analyzing EN and NA concentrations consisted of a pump (LC10AD, Shimadzu, Kyoto, Japan), a 4.6-mm × 150-mm stainless steel column packed with Nucleosil 100-5 C18 (Macherey Nagel, Germany), an auto-injector (SIL-10Axl, Shimadzu), a variable UV detector (SPD-10A, Shimadzu) and an integrator (C-R6A Shimadzu). Mobile phase of 0.1% v/v of phosphoric acid and methanol (35:65), and 0.1% v/v of phosphoric acid and methanol (20:80) containing 5 mM of sodium 1-heptane sulfonate were used for EN and NA. The column temperature was 33 and 40°C, and internal standards were methyl paraben and *p*-hydroxy benzoic acid for EN and NA. The flow rate of mobile phase was 1.0 ml/min and the detector wavelength was set at 260 nm.

3. Results

3.1. Permeation study

In vitro simultaneous skin permeation and metabolism experiments of EN in PBS solution at various concentrations were carried out using excised skin from human, rat, hairless rat, mouse and hairless mouse. For example, the permeation profile through human skin from EN saturated solution (244 mM) is shown in Fig. 1. Both EN and NA were detected in receiver solution, indicating that hydrolysis of EN occurred during the skin permeation process. The cumulative amount of EN and NA and thus the total increased linearly with time after a short lag time. This linear increase in the three amounts after a short lag time was also found in other profiles although the slope of the linear portion differed depending on initial donor concentration and species.

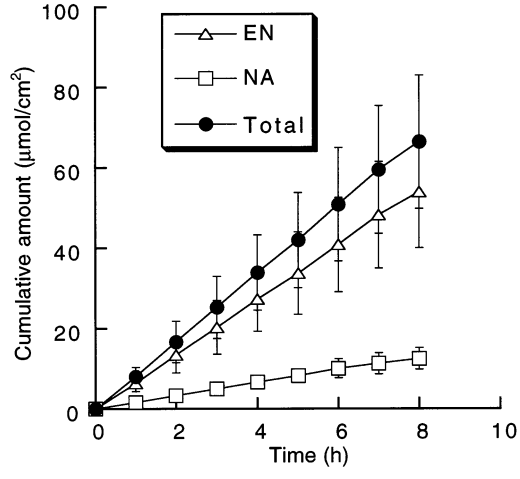

Fig. 1. Permeation profiles through human skin from EN-saturated solution (244 mM). Each point represents the mean ± S.D. of three experiments.

Table 1
Apparent fluxes of EN and NA through skin of five species from EN-saturated solution[a]

Species	Flux (μmol/cm^2 per h)			
	EN	NA	Total	NA/total
Human	6.85 ± 1.73	1.62 ± 0.35	8.47 ± 1.08	0.191 ± 0.038
Rat	0.392 ± 0.077	5.97 ± 0.42	6.36 ± 0.62	0.939 ± 0.124
Hairless rat	2.84 ± 0.26	9.03 ± 0.16	11.9 ± 0.3	0.759 ± 0.214
Mouse	5.26 ± 0.71	1.60 ± 0.17	6.86 ± 0.80	0.233 ± 0.005
Hairless mouse	10.4 ± 0.1	1.57 ± 0.23	12.0 ± 0.7	0.131 ± 0.006

[a] Each value represents the mean ± S.D. of three experiments.

Apparent steady state fluxes of EN, NA and total were calculated from the linear portion in each permeation profile and the values from EN-saturated solution are listed in Table 1. EN flux was highest in hairless mouse followed by human, mouse, hairless rat and rat, whereas the highest NA flux was noticed in hairless rat followed by rat, human, mouse and hairless mouse. Difference in total flux among all species was less than double. Total flux of hairless rodents was about two times higher than hairy rodents. The ratio of NA flux to total flux for rats was higher than that for mice or human.

Fig. 2 shows the effect of EN donor concentration on the fluxes of EN, NA and total for: rat (a); hairless rat (b); mouse(c); and hairless mouse (d). The total flux increased linearly with increase of donor concentration for all species. In rat, a large contribution of NA flux was observed throughout the donor concentration range (50–244 mM). For hairless rat, EN flux was very low and gradually increased in the lower donor concentration range (50–150 mM) then sharply increased in the higher donor concentration range (150–244 mM). In contrast, NA flux sharply increased in the lower donor concentration range and reached a plateau in the higher range. The same pattern of EN and NA flux was found in mouse and hairless mouse. The plateau values of NA flux for mouse, hairless mouse and hairless rat were 1.45, 1.60 and 9.05 μmol/cm^2 per h, while the donor concentrations in which the NA flux reached a plateau were around 100, 50 and 200 mM, respectively.

3.2. EN hydrolysis with skin homogenate

Hydrolysis experiment of EN was done with and without skin homogenate from rat, hairless rat, mouse and hairless mouse at 37 ± 0.5°C and EN degradation profiles from various initial concentrations in 5% w/w skin homogenate are shown in Fig. 3. Although no significant chemical hydrolysis of EN to NA in the PBS solution was found for 25 h (data not shown), hydrolysis was found after skin homogenate was added to EN solution, indicating that hydrolysis of EN was caused by skin enzymes. The semilogarithmic plots of EN concentration versus time were linear at low concentration but EN seemed to decline at a constant rate at higher concentrations, suggesting that degradation of EN follows Michaelis–Menten kinetics. Kinetic parameters (V_{max} and K_m) for EN hydrolysis which were estimated by computer data-fitting to Michaelis–Menten equation are listed in Table 2 and the fitting curves are shown in Fig. 3. Both parameters and intrinsic metabolic clearance (V_{max}/K_m) of rats were higher than those of mice, whereas the difference was small between hairy and hairless rodents.

4. Discussion

Species difference in the skin permeation profile of EN was found in this study. Both EN and NA were detected in all receiver solutions for all species in the skin permeation evaluation. No significant chemical hydrolysis of EN to NA was found

in PBS at 37°C for 25 h. In the other preliminary study, NA could not be detected after incubation of EN with receiver fluid, which had been in contact with the dermal site of excised skin at 37 ± 0.5°C for 3 h, indicating that enzymes were not leached from the skin into the receiver solution. These results indicated that hydrolysis of EN to NA was due to skin enzymes during the permeation process. In permeation studies, the cumulative amount of NA increased linearly with time for all species, suggesting that esterase activity was not changed through 8 h. EN penetrated intact through stratum corneum and was partly metabolized only to NA in viable skin by esterase, and both EN and NA continuously diffused into the receiver solution. The back diffusion of NA can be ignored because the permeability coefficient of NA through intact skin of hairless mouse was about 470 times lower than that through stripped skin (data not shown). Thus, flux of EN through stratum corneum equals the sum of EN and NA fluxes, namely, the total flux through intact skin. For hairless mouse, total flux through the intact skin was about six times lower than that through stripped skin (data not shown). This suggested that the main skin permeation barrier against EN is in the stratum corneum and thus the total flux through intact skin can be regarded as the EN intrinsic flux over all skin.

Difference in total flux among all species was less than double, and less than the species difference in skin permeation of hydrophilic permeants reported by other investigators (Scott et al., 1991; Surber et al., 1991; Cornwell and Barry, 1995). Some reports showed that the variation in permeability of lipophilic drugs in human and hairless rat skin was less than that in hydrophilic drugs (Morimoto et al., 1991; Roy et al., 1994). EN is a neutral and lipophilic prodrug ($\log P = 0.92$) and

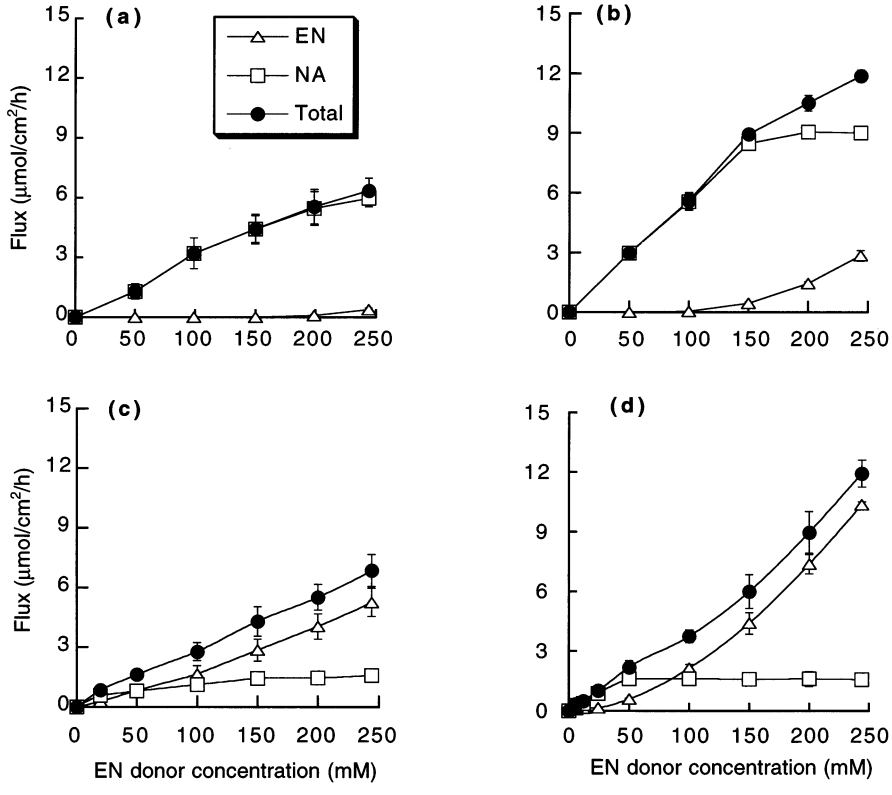

Fig. 2. Effect of EN donor concentration on fluxes of EN, NA and total for rat (a), hairless rat (b), mouse (c) and hairless mouse (d) skin. Each point represents the mean ± S.D. of three to four experiments.

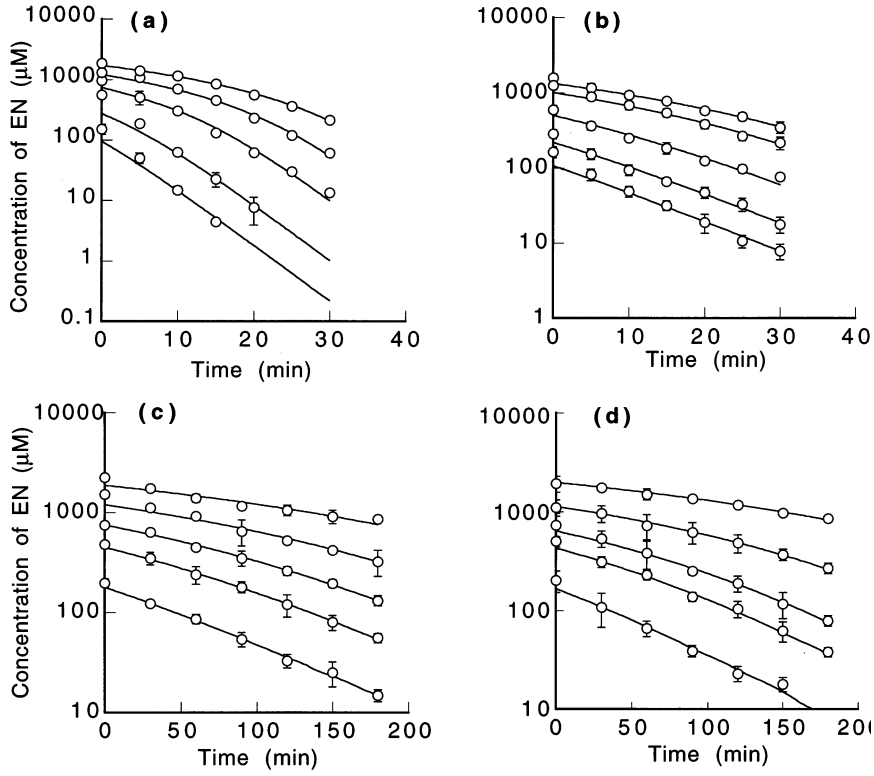

Fig. 3. Hydrolysis profiles and fitting curves of EN at various initial concentrations in skin homogenate for rat (a), hairless rat (b), mouse (c) and hairless mouse (d). Each point represents the mean ± S.D. of three experiments. Solid curve shows the calculated data best fitting to Michaelis–Menten equation.

thus a small variation among different species can be expected. Interestingly, skin permeability of EN in hairless rodents was higher than that in hairy rodents; these results corresponded to the data on nitroglycerin (Santus et al., 1987), water and paraquat (Walker et al., 1983). Difference in skin permeability between hairless and hairy rodents may be explained in terms of lipid content, water uptake of the stratum corneum, epidermal and dermal thickness, density of skin appendages and so on (Morimoto et al., 1991; Sato et al., 1991; Scott et al., 1991; Dick and Scott, 1992).

Compared with species difference in skin permeability, that in skin metabolism was very large. NA/total flux ratio was about 7.5-fold different between the maximum of rat and the minimum of hairless mouse (Table 1). The donor concentration dependency of NA/total flux also differed among animal species (Fig. 2). Such a difference in skin metabolism was reported for nitroglycerin, where nitroglycerin and two dinitrate metabolises recovered in the receiver solution following nitroglycerin delivery to skin varied among BALB/c mouse, hairless mouse and human (Santus et al., 1987). In hairless rat, mouse and hairless mouse, NA fluxes increased with donor concentration and then reached a plateau level, suggesting metabolic saturation. The values of NA fluxes and donor concentrations when metabolic saturation occurred differed among animal species. The maximum hydrolysis rate (V_{max}) and intrinsic metabolic clearance (V_{max}/K_m) of rats were much higher than those of mice while the difference was small between hairy and hairless rodents (Table 2). The difference in skin metabolism observed in this study may partially be due to gender difference, which is known in the activity of esterase and alcohol dehydrogenase in skin of hairless

guinea pig (Boehnlein et al., 1994). But this remains unclear because male mice could not be used in the skin permeation experiment due to the skin damage commonly found.

When EN concentration C, in skin is significantly lower than K_m, the metabolic clearance $[V_{max}/(K_m + C)]$ is almost equal to the intrinsic metabolic clearance (V_{max}/K_m). The stratum corneum permeation of EN might thus be the rate limiting step in the NA permeation process in the lower donor concentration range. As donor concentration was increased and concentration in skin also increased, metabolic clearance was reduced, so that the rate limiting step might change from permeation to metabolism of EN in the higher donor concentration range. Under the metabolic saturated condition, NA flux must be equal V_{max}. Metabolic saturation was confirmed in hairless rat, mouse and hairless mouse. In rats, contribution of NA to total flux was decreased in saturated EN solution, suggesting that metabolism of EN to NA in skin was close to saturation. To compare NA flux in the skin permeation experiment with V_{max} in the skin homogenate experiment, V_{max} was recalculated in unit of μmol/cm^2 per h. The recalculated V_{max} of rat and hairless rat (6.33 and 5.80 μmol/cm^2 per h) was close to the permeation rate of NA at the plateau region, whereas that of mouse and hairless mouse (0.231 and 0.300 μmol/cm^2 per h) was much lower. It has been reported that the enzymes related to prednisolone farnesylate metabolism are enriched in the lower layer of viable skin of hairless mouse (Tojo et al., 1994). On the other hand, good data-fitting to the homogeneous enzyme distribution model in viable skin was obtained for rats (Bando et al., 1996) and hairless rats (Sugibayashi et al., 1996). Taking our results together with previous reports, it appears that species difference in skin enzyme distribution can be expected. In hairless mouse, esterase may enrich in specific skin layers and thus the dilution of esterase during skin homogenate preparation might produce the difference between V_{max} in hydrolysis experiment and NA flux reached a plateau in permeation study. On the other hand, esterase may homogeneously distribute in rat skin then no dilution effect on the activity of esterase might be observed. The effects of enzyme distribution on skin permeation require further study.

The result of permeation studies and K_m values from the skin homogenate experiment cannot be directly compared without determination of the EN concentration in skin. However, the EN concentration, in which the NA flux reached a plateau (about 250, 200, 100 and 50 mM for rat, hairless rat, mouse and hairless mouse, respectively) and K_m values (Table 2) had the same order in rats or mice. The similar skin structure, thickness and enzyme distribution might produce a similar relationship between EN concentration in skin and that in donor solution among these rodents. Although K_m value of hairless rat from the skin homogenate experiment was about doubled that of rat, the EN donor concentration in which NA reached a plateau was lower for hairless rat. Because total permeability coefficient for hairless rat skin was about doubled that for rat skin, EN concentration may be higher in the former than that in the latter.

The present study confirmed species differences in simultaneous skin transport and metabolism of EN among human, rat, hairless rat, mouse and hairless mouse. The difference in permeation

Table 2
Kinetic parameters for the hydrolysis from EN to NA in the skin homogenate[a]

Species	V_{max} (nmol/min per mg protein)	K_m (μM)	V_{max}/K_m (ml/min per mg protein)
Rat	27.3 ± 11.7	352.0 ± 72.0	0.0776
Hairless rat	25.8 ± 8.8	665.0 ± 110.0	0.0388
Mouse	6.46 ± 1.14	595.0 ± 157.0	0.0108
Hairless mouse	4.84 ± 1.52	473.0 ± 101.0	0.0102

[a] Each value represents the mean ± computer-calculated S.D.

profiles of EN among all species tested was primarily due to the difference in esterase activity. To predict skin permeability in human by extrapolating from an animal model, therefore the species difference in skin metabolism should be considered. We cannot decide on the best animal model for human skin because kinetic parameters from the hydrolysis experiment using human skin homogenate could not be obtained. For human, the value of NA/total flux ratio was small (Table 1), suggesting low esterase activity. Compared to other tested species, esterase activity in human skin may be more similar to mice than rats. To identify the best animal skin model for human skin, more data using human skin, other species and other permeants should be obtained both in vitro and in vivo.

References

Bando, H., Sahashi, M., Takagi, T., Yamashita, F., Takakura, Y., Hashida, M., 1996. Analysis of in vitro skin permeation of acyclovir prodrugs based on a diffusion model with a metabolic process. Int. J. Pharm. 135, 91–102.

Bando, H., Sahashi, M., Yamashita, F., Takakura, Y., Hashida, M., 1997. Effects of skin metabolism on percutaneous penetration of lipophilic drugs. J. Pharm. Sci. 86, 759–761.

Berman, E., Shahn, E., Weiss, M.F., 1962. The routine fitting of kinetic data to models: a mathematical formalism for digital computer. Biophys. J. 2, 275–287.

Bickers, D.R., 1980. The skin as a site of drug and chemical metabolism. In: Drill, V.A., Lazar, P. (Eds.), Current Concepts in Cutaneous Toxicity. Academic Press, New York, pp. 95–126.

Boehnlein, J., Sakr, A., Lichtin, J.L., Bronaugh, R.L., 1994. Characterization of esterase and alcohol dehydrogenase in skin. Metabolism of retinyl palmitate to retinol (vitamin A) during percutaneous absorption. Pharm. Res. 11, 1155–1159.

Chan, S.Y., Po, L.W., 1989. Prodrug for dermal delivery. Int. J. Pharm. 55, 1–16.

Cornwell, P.A., Barry, B.W., 1995. Effects of penetration enhancer treatment on the statistical distribution of human skin permeabilities. Int. J. Pharm. 117, 101–112.

Damen, F.J., Mier, P.D., 1982. Cytochrome P-450-dependent O-dealkylase activity in mammalian skin. Br. J. Pharmacol. 75, 123–127.

Dick, I.P., Scott, R.C., 1992. The influence of different strains and age on in vitro rat skin permeability to water and mannitol. Pharm. Res. 9, 884–887.

Kao, J., 1989. The influence of metabolism on percutaneous absorption. In: Bronaugh, R.L., Maibach, H.I. (Eds.), Percutaneous Absorption. Marcel Dekker, New York, pp. 259–282.

Kasting, G.B., Smith, R.L., Anderson, B.D., 1992. Prodrug for dermal delivery: solubility, molecular size and functional group effects. In: Sloan, K.B. (Ed.), Prodrug: Topical and Ocular Drug Delivery. Marcel Dekker, New York, pp. 117–161.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193, 265–275.

Morimoto, Y., Hatanaka, T., Sugibayashi, K., Omiya, H., 1991. Prediction of skin permeability of drug: comparison of human and hairless rat skin. J. Pharm. Pharmacol. 44, 634–639.

Mukai, E., Arase, K., Hashida, M., Sezaki, H., 1985. Enhanced delivery of mitomycin C prodrugs through the skin. Int. J. Pharm. 25, 95–103.

Okamura, M., Sugibayashi, K., Ogawa, K., Morimoto, Y., 1989. Skin permeability of water-soluble drugs. Chem. Pharm. Bull. 37, 1404–1406.

Rohatagi, S., Barrett, J.S., Mcdonald, L.J., Morris, E.M., Darnow, J., DiSanto, A.R., 1997. Seligenine percutaneous absorption in various species and metabolism by human skin. Pharm. Res. 14, 50–55.

Roy, S.D., Hou, S-Y.E., Witham, S.L., Flynn, G.L., 1994. Transdermal delivery of narcotic analgesics: comparative metabolism and permeability of human cadaver skin and hairless mouse skin. J. Pharm. Sci. 83, 1723–1728.

Santus, G.C., Nobutoshi Watari, N., Hinz, R.S., Benet, L.Z., Guy, R.H., 1987. Cutaneous metabolism of transdermally delivered nitroglycerin in vitro. In: Shroot, B., Schaefer, H. (Eds.), Skin Pharamcokinetics, Vol. 1, Pharmacology and the Skin. Karger, Basel, pp. 240–244.

Sato, K., Sugibayashi, K., Morimoto, Y., 1991. Species differences in percutaneous absorption of nicorandil. J. Pharm. Sci. 80, 104–107.

Scott, R.C., Corrigan, M., Smith, A.F., Mason, H., 1991. The influence of skin structure on permeability: an intersite and interspecies comparison with hydrophilic penetrant. J. Invest. Dermatol. 96, 921–925.

Steinstrasser, I., Merkle, H.P., 1995. Dermal metabolism of topically applied drugs: pathways and models reconsidered. Pharm. Acta. Hel. 70, 3–24.

Sugibayashi, K., Hayashi, T., Hatanaka, T., Ogihara, M., Morimoto, Y., 1996. Analysis of simultaneous transport and metabolism of ethyl nicotinate in hairless rat skin. Pharm. Res. 13, 855–860.

Surber, C., Wilhelm, K., Maibach, H.I., 1991. In-vitro skin pharmacokinetics of acitretin percutaneous absorption studies in intact and modified skin from three different species using different receptor solutions. J. Pharm. Pharmacol. 43, 836–840.

Tojo, K., Yamada, K., Hikima, T., 1994. Diffusion and metabolism of prednisolone farnesylate in viable skin of the hairless mouse. Pharm. Res. 11, 393–397.

Walker, M., Dugard, P.H., Scott, R.C., 1983. In vitro percutaneous absorption: a comparison of human and laboratory species. Hum. Toxicol. 2, 561–562.

Wester, R.C., Maibach, H.I., 1993. Animal models for percutaneous absorption. In: Shah, V.P., Maibach, H.I. (Eds.), Topical Drug Bioavailability, Bioequivalence and Penetration. Plenum Press, New York, pp. 333–349.