ELSEVIER

International Journal of Pharmaceutics 129 (1996) 211–221

international journal of pharmaceutics

Analysis of skin penetration enhancing effect of drugs by ethanol-water mixed systems with hydrodynamic pore theory

Eiichiro Manabe[a], Kenji Sugibayashi[a,b], Yasunori Morimoto[a,b,*]

[a]*Faculty of Pharmaceutical Sciences, Josai University, 1-1 Keyakidai, Sakado, Saitama 350-02, Japan*
[b]*Life Science Research Center, Josai University, 1-1 Keyakidai, Sakado, Saitama 350-02, Japan*

Received 15 May 1995; revised 28 June 1995; accepted 21 August 1995

Abstract

Skin permeation of drugs from ethanol-water mixed systems was evaluated by the hydrodynamic pore theory. Two model drugs of similar molecule size and different polarities, isosorbide dinitrate (ISDN) and antipyrine (ANP) were used, and the relationship between drug clearance and solvent flux through excised hairless rat skin was investigated with a variety of ethanol concentrations (20–100%) in water to calculate the reflection coefficient (δ) of the drugs to the skin. The ISDN clearance was independent of the solvent flux at 20% ethanol, but was dependent on the flux at more than 40% ethanol. Linear relationships were obtained between the ANP clearance and the solvent flux at all ethanol concentrations. These results suggest high contribution of convective flow with the solvent to the total skin permeability of both drugs. Skin must be delipidized by a high concentration of ethanol in water, thus the barrier function of the stratum corneum to overall permeation of the drugs was reduced. The apparent pore radius of skin estimated from the δ value of ANP differed according to the ratio of ethanol and water in the systems: 100% ethanol was maximum (1.89 $\pm$ 1.24 nm) followed by 0% ethanol (0.98 $\pm$ 0.60 nm) and the mixed solutions had a mean value of 0.42–0.50 nm.

Keywords: Percutaneous absorption; Skin permeability; Hydrodynamic pore theory; Reflection coefficient; Ethanol-water mixed system

1. Introduction

Skin permeation of a drug from most topical vehicles is generally low and many compounds have been tested to find one with skin penetration-enhancing effect (Ghanem et al., 1987a; Akhter and Barry, 1985; Sasaki et al., 1990; Morimoto et al., 1986; Aungest et al., 1986; Barry, 1987; Ongpipattanakul et al., 1991). Ethanol has been broadly used as a disinfectant, antiseptic or preservative, and also applied to marketed transdermal delivery systems of nitroglycerin (Good et al., 1985; Campbell and Chandrasekaran, 1983), estradiol (Gale and Berggren, 1986; Gale and

* Corresponding author. Faculty of Pharmaceutical Sciences, Josai University, 1-1 Keyakidai, Sakado, Saitama 350-02, Japan. Tel.: 81 492 717685.

SSDI 0378-5173(95)04328-8

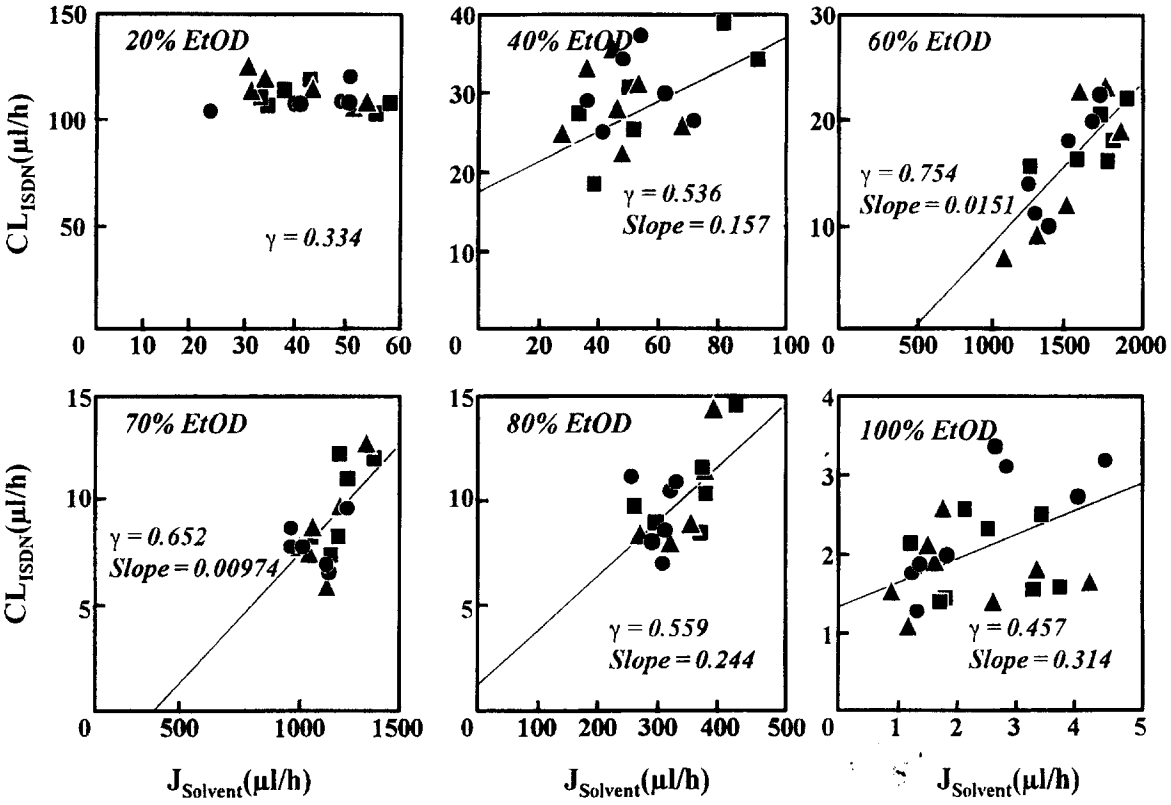

Fig. 1. Effect of solvent flow on the ISDN permeation through excised hairless rat skin from different ratios of ethanol-water mixed systems. Each line was obtained by linear regression analysis. ●, 0.272 Osmol/l; ▲, 0 Osmol/l; ■, −0.272 Osmol/l.

Berggren, 1987) and fentanyl (Gale et al., 1986) in anticipation of its skin penetration-enhancing effect. The effect of ethanol on skin penetration is dependent on its concentration in topical vehicles as well as lipophilicity of the drug used. Skin permeations of lipophilic drugs such as nitroglycerin and levonorgestrel from a high concentration of ethanol in aqueous solution were lower than those from low concentration (Friend et al., 1988; Berner et al., 1989a), while the reverse was true of hydrophilic drugs such as mannitol and tetraethylammonium bromide (Ghanem et al., 1987b). Broad studies have been made on ethanol's penetration-enhancing mechanism of action: fluidization (Chin and Goldstein, 1977; Ghanem et al., 1986; Ghanem et al., 1987a; Knutson et al., 1990) and delipidization of the stratum corneum lipid (Kurihara-Bergstrom et al., 1990), softening of the stratum corneum keratin, degradation of skin enzyme (Higuchi et al., 1987) and solvent drag (Berner et al., 1989b) were reported as possible mechanisms of this enhancing effect.

In a previous paper (Hatanaka et al., 1994), the skin permeability of four polar solutes with a variety of molecular sizes and one lipophilic drug, ISDN, from water (D_2O) was analyzed by the hydrodynamic pore theory. Skin clearance (the product of permeability coefficient across skin and effective application area) of ISDN was found to be independent of the solvent flux, whereas a linear relationship was obtained between the clearance of each hydrophilic drug of low molecular radius (though not of large molecular radius) and the solvent flux. These results suggested that the convective flow markedly contributed to the total skin permeability of a hydrophilic drug, and

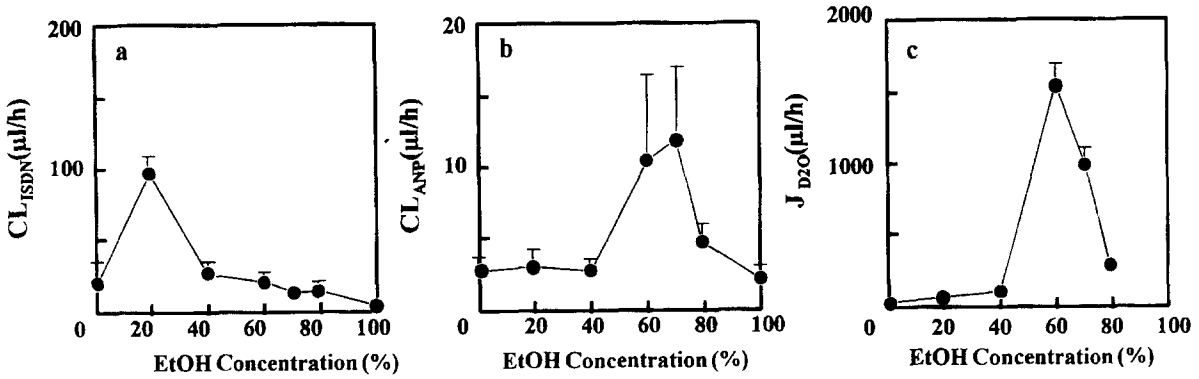

Fig. 2. Effect of ethanol concentration of ethanol-water mixed systems on the permeation of ISDN (a), ANP (b) and D_2O (c). Each point represents the mean ± S.E. of 18 experiments.

that the extent of contribution decreased with increase in molecular size of the drug.

In the present study, different ratios of ethanol-water mixed systems were used as typical vehicles with penetration-enhancing effect to estimate the contribution of convective flow to skin permeation of an aqueous drug antipyrine (ANP) and a lipophilic drug ISDN.

2. Theoretical

(Hatanaka et al., 1994)

We have proposed a parallel permeation pathway model for describing skin penetration of drugs, based on the permeation data through excised hairless rat skin of various compounds from their aqueous solutions and suspensions (Hatanaka et al., 1990). In this model, the stratum corneum is assumed to have lipid and pore pathways, the latter being the main permeation route for hydrophilic drugs and explained by the pore theory. The net flux of a drug at the steady state, J, can be described based on this model as the sum of each flux via the two pathways:

$$J = J_L + J_P \quad (1)$$

where subscripts L and P mean lipid and pore pathways, respectively.

The net flux of lipid pathway (J_L) is mathematically expressed by Eq. (2) based on Fick's first law under assumptions that drug decrease in the donor phase is too small to be negligible and a sink condition can be maintained in the receiver phase throughout the diffusion experiment:

$$J_L = CL_L C_0 \quad (2)$$

where CL means permeation clearance of drug (μl/h), which means the product of its permeability coefficient and the application area of skin, and C_0 is the initial concentration of drug (mg/ml) in the donor phase. On the other hand, Kedem and Katchalsky (Kedem and Katchalsky, 1958) reported that the driving force of drug permeation through the pore pathway was both a concentration differential of the drug (diffusion) and an imbalance in hydrostatic and osmotic pressure (convection) across the biomembrane. We assumed the J_P by referencing their theory as follows:

$$J_P = CL_P C_0 + (1 - \delta) J_{Solvent} C_0 \quad (3)$$

where $J_{Solvent}$ is the solvent flux (μl/h). The reflection coefficient, δ, is an index of the ability of the barrier to discriminate between solute and solvent.

From Eqs. (1)–(3), the following equation can be obtained:

$$J = CL_L C_0 + CL_P C_0 + (1 - \delta) J_{Solvent} C_0 \quad (4)$$

If a parameter, CL_{Drug} (cm^3/min.), is assumed to be J/C_0, Eq. (4) becomes

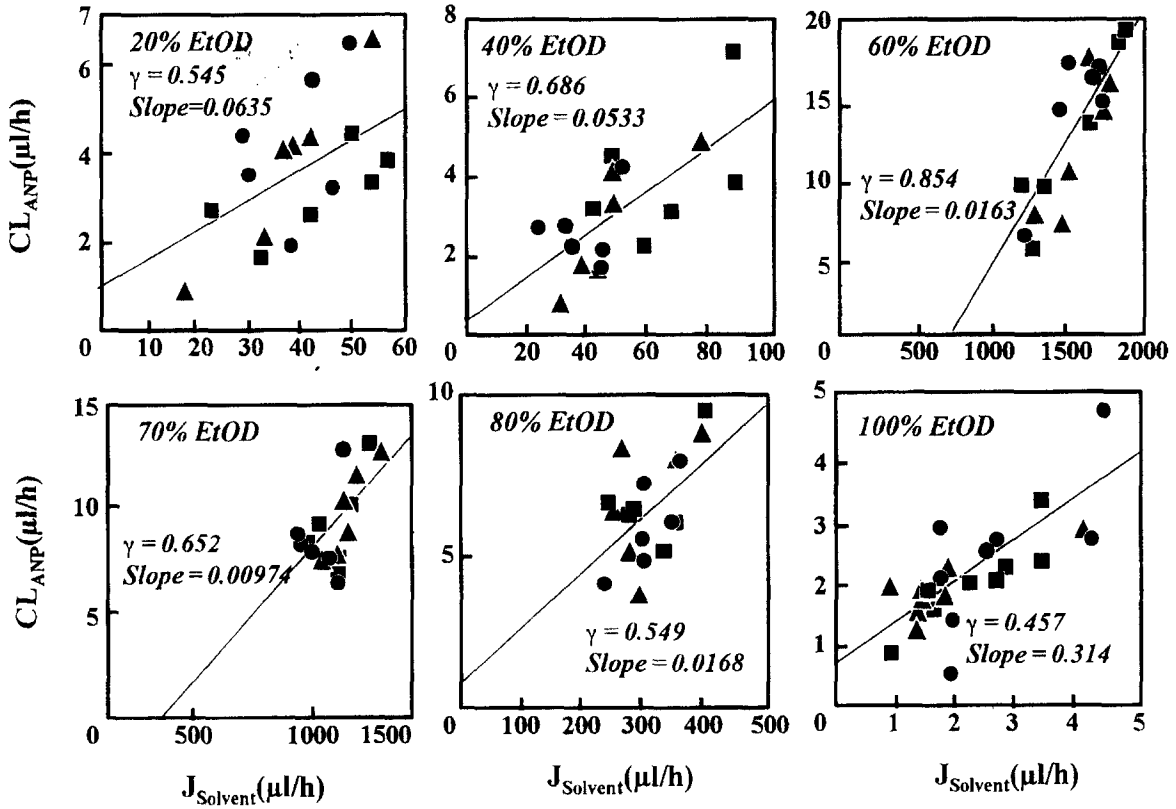

Fig. 3. Effect of solvent flow on the ANP permeation through excised hairless rat skin from different ratios of ethanol-water mixed systems. Each line was obtained by linear regression analysis. ●, 0.272 Osmol/l; ▲, 0 Osmol/l; ■, −0.272 Osmol/l.

$$CL_{Drug} = CL_L + CL_P + (1 - \delta)J_{Solvent} \quad (5)$$

Eq. (5) indicates that CL_{Drug} increases with increase in $J_{Solvent}$. δ value can be obtained by the slope of $CL_{Drug}/J_{Solvent}$ curve.

Pore size of the pore pathway in skin can be estimated by the following Levitt equation (Levitt, 1975a; Levitt, 1975b; Granger and Taylor, 1987):

$$\delta = 16/3(a/R)^2 - 20/3(a/R)^3 + 7/3(a/R)^4 - 0.345(a/R)^5 \quad (6)$$

where a and R are radius of penetrant and pore, respectively.

3. Materials and methods

3.1. Materials

ANP was purchased from Tokyo Chemical Industry Co. (Tokyo, Japan). ISDN was geneously supplied by Toko Pharmaceutical Industries Co. (Tokyo). Deuterium oxide (D_2O) and deuterium ethanol (CH_3CH_2OD, EtOD) were obtained from Merck Co. (Darmstadt, Germany) and Aldrich Chemical Co. (Milwaukee, WI, USA), respectively, and FITC-dextran (MW 71 200) was from Sigma Chemical Co. (St. Louis, MO, USA). All these drugs and chemicals were used without further purification. Other chemicals and solvents were of reagent grade and obtained commercially.

3.2. Skin permeation procedure

(Hatanaka et al., 1990)

Abdominal skin was freshly excised from male WBN/ILA-Ht hairless rats at 6 to 7 weeks (Ishikawa Laboratory Animals, Saitama, Japan), and was mounted between two half diffusion cells having 2.5 ml volume and 0.966 cm^2 effective diffusion area, with a water jacket connected to water at 37°C. The donor compartment was filled with different ratios of D_2O and EtOD (EtOD conc.: 20, 40, 60, 70, 80 and 100%) containing ISDN or ANP and FITC-dextran and the receiver compartment with distilled water. Drug concentration was adjusted to 0.5 and 1.0 mg/ml for ISDN and ANP, respectively. FITC-dextran was used as a volume marker at 50 μg/ml. The osmotic pressure differential was adjusted to 0.272, 0, or -0.272 osmol/l by addition of NaCl to donor or receiver compartment. The donor and receiver solutions were stirred with star-head bars driven in each compartment by a constant-speed synchronous motor throughout these permeation experiments. To evaluate the skin permeability of drugs and solvents at a steady state, the time dependence of the permeation was preliminarily investigated: $J_{Solvent}$ and CL_{Drug} were constant from 60 to 80 h. The permeation experiments were then made between 60 and 63 h (Those were constant from 12 to 80 h for without ethanol). One ml samples were withdrawn every half an hour from both compartments to measure skin permeation of drug and solvent.

Permeations of ISDN, ANP and EtOD from 100% EtOD through skin which was pretreated with pure water for 60 h were also measured. For simultaneous skin permeation experiment of ISDN and ANP, the donor compartment was filled with ethanol containing the two drugs. Details of these permeation experiments were the same as above.

3.3. Delipidization and deproteinization experiments

After pretreatment of skin for over 60 h in a 2-chamber diffusion cell as described in the skin permeation procedure, 1.0 ml samples were withdrawn from both compartments to determine the lipid (sterol) leached from skin.

3.4. Fourier transformation-infrared (FTIR) measurement

The abdominal skin excised from hairless rat was soaked for 60 h in various concentrations of ethanol. The resulting skin samples were dried and measured by FTIR spectrometer (JIR-MICRO 6000, Nihon Denshi Co., Tokyo) equipped with a triglycine sulfate detector.

3.5. Analytical methods

ISDN and ANP were determined by HPLC as described previously (Hatanaka et al., 1994). Ethanol was assayed by GC-FID (GC-14A, Shimadzu, Kyoto, Japan). D_2O and EtOD concentrations were measured by the obtained ethanol concentration and absorbance of O-D stretching vibration at 2512 cm^{-1} by IR spectroscopic spectra (JIR-3510, Nihon Denshi Co.). Fluorescence intensity of FITC-dextran was measured by a spectrofluorometer (RF-5000, Shimadzu) at an excitation wavelength of 495 nm and an emission wavelength of 515 nm. The amounts of sterol and protein leached were determined by the method of Zak-Henly and BCA protein assay (Pierce, IL, USA) using bovine serum albumin as a standard. Each assay method was sensitive enough for the present experiments.

4. Results and discussion

4.1. Effect of ethanol-water mixed systems on the skin permeation of ISDN

$J_{Solvent}$ and CL_{ISDN} were evaluated using various ethanol concentrations in water. Fig. 1 shows the effect of $J_{Solvent}$ on the CL_{ISDN} among different ethanol concentrations (20, 40, 60, 70, 80 and 100% EtOD). CL_{ISDN} was almost constant (100–120 μl/h) independent of $J_{Solvent}$ at 20% ethanol. CL_{ISDN} was also independent of $J_{Solvent}$ when pure water was used (15–35 μl/h, Hatanaka et al., 1994). We earlier reported that ISDN permeated

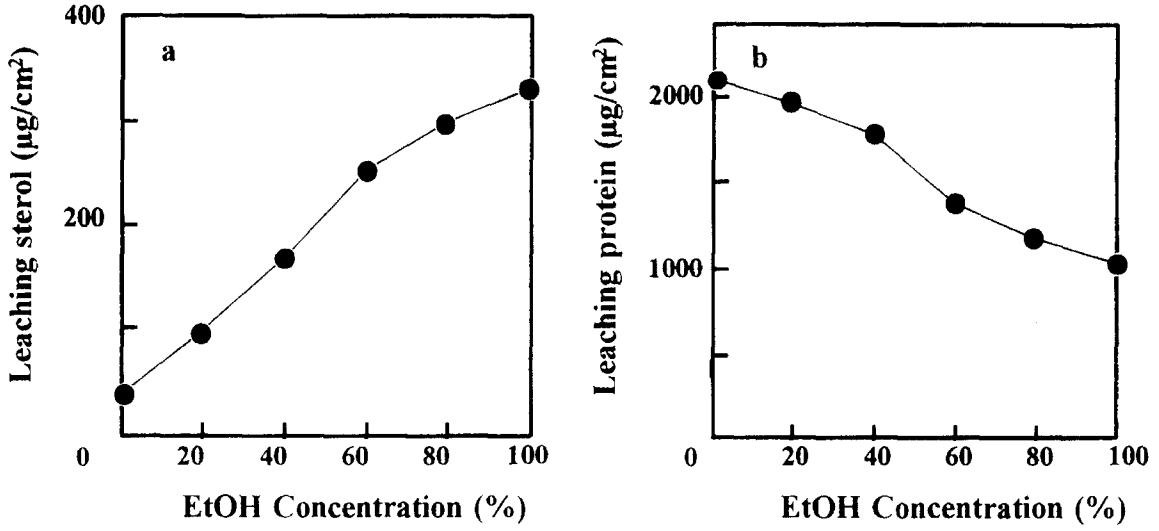

Fig. 4. Effect of ethanol concentration of ethanol-water mixed systems on the sterol (a) and protein (b) leaching from hairless rat skin. Each point represents the mean ± S.E. of six experiments.

primarily via the lipid pathway of skin (Hatanaka et al., 1994). The ratio of ISDN permeated through the pathway was so large that no contribution could be recognized of the convective flow of ISDN with these solvents. In contrast, a linear relationship was found between $J_{Solvent}$ and CL_{ISDN} from 40–100% ethanol in water.

Fig. 2a shows the relation between CL_{ISDN} and ethanol concentration. Increase in the ethanol concentration from 20 to 100% decreased CL_{ISDN} (1–3.5 μl/h for 100% EtOD, for example), although the mean CL_{ISDN} from 20% ethanol was about four times higher than that from 0% ethanol. These results can be explained as follows: ratio of lipid pathway was lowered and that of pore pathway (solvent filled domain) was increased by delipidization with a higher concentration of ethanol. The pore pathway can be assumed as a domain filled with D_2O-EtOD mixed solvent. If this assumption is right, the solubility of ISDN in skin is dependent on that is this mixed solvent. The drug solubility in skin would thus be changed by the concentration of ethanol used as a donor solvent (Silvieri and DeAngelis, 1975).

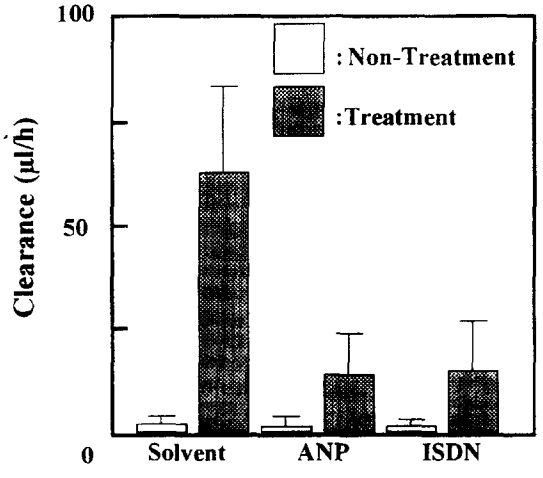

Fig. 5. Effect of pretreatment by water on the solvent and drug clearances through excised hairless rat skin. Each column represents the mean ± S.E. of 18 experiments.

4.2. Effect of ethanol-water mixed systems on the skin permeation of ANP

Fig. 3 shows a linear relation between the $J_{Solvent}$ and CL_{ANP} from 20–100% ethanol in water. All relations were significant (at least $P < 0.05$). Almost no enhancing effect was found at 20–40% ethanol in water compared to pure water (about 3 μl/h, Hatanaka et al., 1994). Fig. 2b and c show

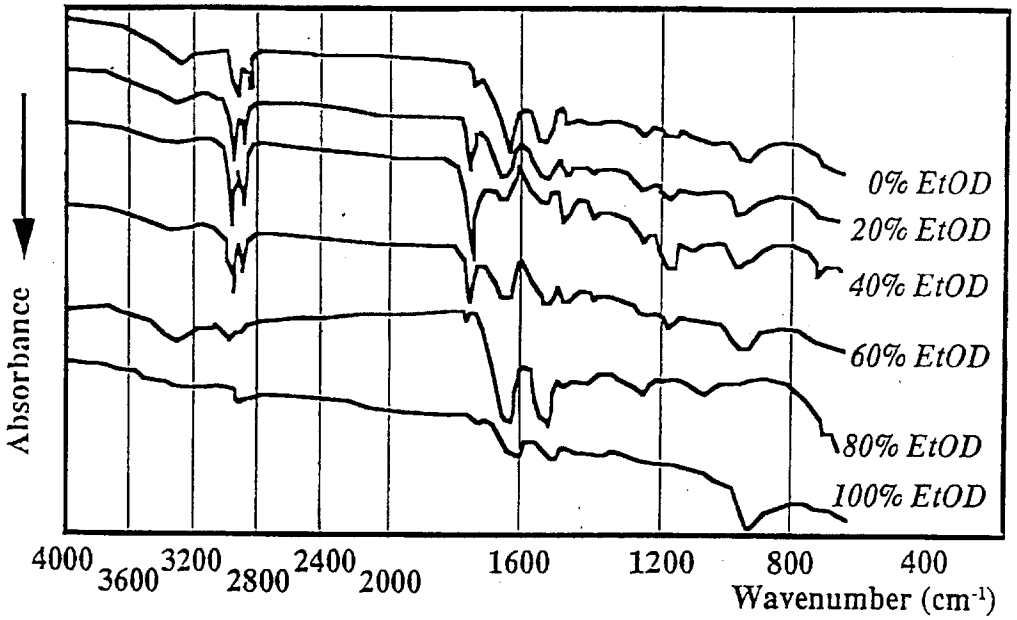

Fig. 6. FTIR spectra of skin surface pretreatment with different ratios of ethanol-water mixed systems.

relations between CL_{ANP} or J_{D2O} and ethanol concentration, respectively. The relation for ANP (Fig. 2b) was similar to that for D_2O (Fig. 2c): the permeation clearances of both were maximum at 60% and increased at higher ethanol concentration (see following discussion for the effect of ethanol concentration on the water permeation). The great enhancement effect by higher ethanol is probably due to new pore formation.

4.3. Effect of ethanol-water mixed systems on the pore radius in skin and the skin permeation of water

Table 1 summarizes skin pore size calculated from results in Fig. 3 by the Levitt equation (Eq. (3)) and J_{D2O} as an index of porous regions (porosity). The pore size of skin treated by pure water was calculated to be about 0.98 ± 0.60 nm (mean ± S.E., the variation was due to the range of reflection coefficient and the minimum and maximum radii of solute). The pore size obtained by 20% ethanol was 0.50 ± 0.29 nm, and this value was about half that for pure water. The value was not significantly different from 40–80% ethanol. Pore size of the skin barrier with pure ethanol, however, was markedly higher (1.89 ± 1.24 nm). In contrast, apparent water influx, J_{D2O}, with pure water was 6.99 ± 1.3 μl/h, and that with 20% EtOD was about five times greater. This result shows that ethanol fluidizes lipid domain of skin and forms new pores in skin. The maximum J_{D2O} was 1568.8 ± 223.8 μl/h from 60% ethanol in water, which was 200 times larger than pure water. J_{D2O} from 70–80% ethanol in water was smaller than from 60%.

4.4. Effect of ethanol-water mixed systems on the skin barrier

Ethanol-water mixed systems have an effect on the skin permeation of drugs, probably due to increase in lipid fluidization by low concentration of ethanol or new pore formation by high concentration. No clear reason was found for decrease in skin permeability by high ethanol concentration. Several experiments evaluated the mechanism of ethanol-water mixed system on the stratum corneum, the main barrier membrane filled with lipid among keratinized cells (White et al., 1988). Ethanol was thought to have an effect on the lipid or protein in the stratum corneum, and the following experiments were done.

Table 1
Effect of EtOD concentration on the morphological parameter of hairless rat skin

EtOD concentration	δ_{Drug}	Pore radius (nm)	J_{D2O} (μl/h)
0%	0.606 ± 0.221	0.98 ± 0.60	6.99 ± 1.3
20%	0.925 ± 0.076	0.50 ± 0.29	36.3 ± 9.5
40%	0.947 ± 0.032	0.50 ± 0.21	49.4 ± 17.9
60%	0.948 ± 0.007	0.45 ± 0.16	1568.8 ± 223.1
70%	0.975 ± 0.013	0.47 ± 0.17	1116.3 ± 124.5
80%	0.893 ± 0.015	0.42 ± 0.21	318.1 ± 46.4
100%	0.303 ± 0.184	1.89 ± 1.24	—

Mean ± S.E. of at least 18 experiments.

First, sterol and protein leaching from skin was measured for 60h using diffusion cells. Fig. 4a and b show the effect of ethanol concentration in water on the amount of sterol and protein leached, respectively, for 60 h. Although pore formation would be maximum at 60% ethanol, the amount of sterol and protein leached evenly increased and decreased, respectively, with increase in ethanol concentration. Thus both leachings may be complicatedly related to new pore formation.

To determine whether the low permeability of solute by high concentration of ethanol could be due to alteration of the protein structure, skin permeation was tested using pure ethanol after deproteinization with pure water. Fig. 5 shows clearances of ethanol, ANP and ISDN. All values were significantly high compared to non-treatment. These data suggest the low permeation by pure ethanol is related to the amount and structure of protein in skin.

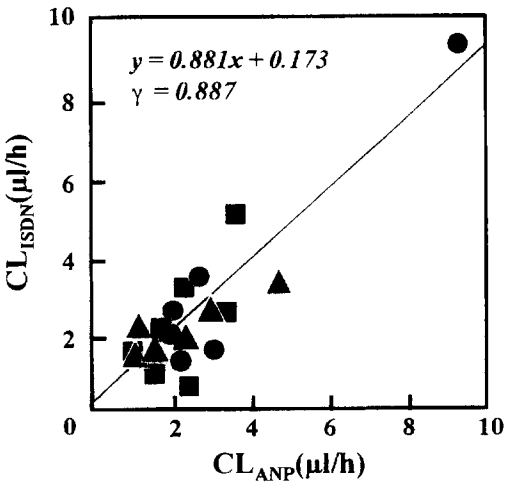

Fig. 7. Relationship between permeation clearances of ANP (CL_{ANP}) and ISDN (CL_{ISDN}) through excised hairless rat skin pretreatment with pure water. The line was obtained by linear regression analysis. ●, 0.272 Osmol/l; ▲, 0 Osmol/l; ■, −0.272 Osmol/l.

Third, structure change in skin pretreated with different concentrations of ethanol aqueous systems was evaluated by FTIR. Fig. 6 illustrates the obtained spectra, and Table 2 summarizes change in stretching bands coming from skin lipid and protein. The stratum corneum has absorption spectra of about 2920 cm^{-1} for C–H asymmetric stretching, about 2850 cm^{-1} for C–H symmetric stretching and about 1740 cm^{-1} for C=O stretching from lipid domain, and about 1645 cm^{-1} for C=O stretching from amide (Park and Baddiel, 1972). C–H and C=O frequencies for the stratum corneum lipid pretreated with 20–60% EtOD increased more than 4 cm^{-1} of resolution. This effect may be due to lipid fluidization, and is similar to the reported data for the stratum corneum lipid treated with oleic acid (Bommannan et al., 1991) or high temperature (Knutson et al., 1985). C=O frequency from the stratum corneum protein was increased with 40% ethanol, and was decreased with 80 and 100% ethanol. This showed that the protein bond was loosened by 40% ethanol, perhaps due to hydration effect as suggested by Oertel (1977). Frequency increase was proportional to ethanol concentration, and may be related to protein denaturation. This potential mechanism is consistent with the maximum J_{D2O} flux at 60% ethanol.

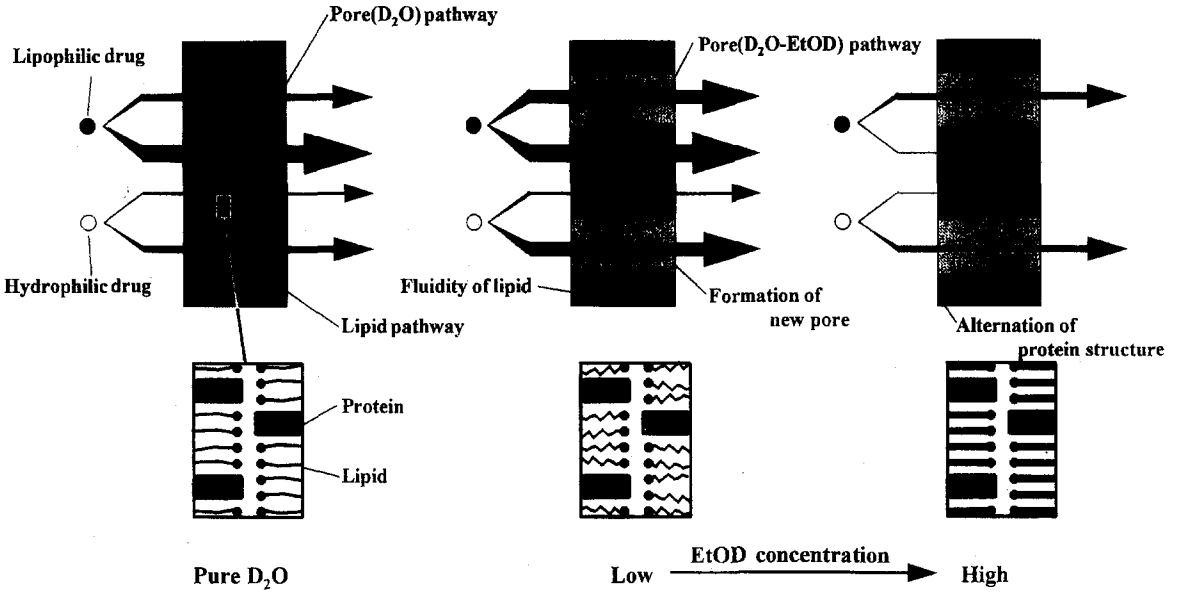

Fig. 8. Permeation model of drugs through skin by ethanol-water mixed systems.

4.5. Simultaneous permeation of ISDN and ANP through skin

Simultaneous permeation of ISDN and ANP through skin treated by pure ethanol was measured. Fig. 7 shows the relationship between obtained CL_{ISDN} and CL_{ANP}. A linear relationship existed between the CL_{ISDN} and CL_{ANP} (γ = 0.887, $P < 0.05$). The slope and intercept were 0.881 and 0.173, respectively, which were not significantly different from 1.00 and 0.00, respectively. ISDN and ANP, having similar molecule size, show similar reflection coefficient independent of the lipophilicity, which suggests that both drugs permeated through the same skin pathway, and skin lost the ability for selective permeation. In other words, skin is not able to distinguish drug polarity.

5. Conclusion

In the present study, we evaluated ethanol-water (20–100%) mixed systems as vehicles that enhance skin permeation of drugs. The enhancing effect was evaluated based on the hydrodynamic pore theory. Fig. 8 illustrates a schematic model of skin. In the neat water system (without ethanol), lipophilic and hydrophilic drugs permeate mainly via lipid and pore pathway, respectively (Hatanaka et al., 1990). A low concentration of ethanol (20–60%) extracts lipids from skin and promotes the permeation of a drug through the pore pathway independent of the drug polarity. Contribution of convective flow with solvents on the skin permeation of lipophilic drugs may be also increased. FTIR experiment showed increase in lipid fluidity, which may enhance the permeation of lipophilic drugs, especially through the lipid pathway. A high concentration of ethanol caused protein denaturation in skin, so that it behaved like a porous membrane which is unable to distinguish drug polarity.

The pore pathway is relatively impermeable to drugs. The ethanol-water mixed system, however, increased contribution of the convective flow through the pathway to the total skin permeability. Potts and Guy (1992) reported that prediction of skin permeation of drugs could be done using a simple one-layer model. In case of a high contribution of solvent flow (convective flow), however,

Table 2
Transmission FTIR spectra of skin pertreated with EtOH-H_2O systems

EtOD concentration	C–H[a] (cm^{-1})	C–H[b] (cm^{-1})	C=O[c] (cm^{-1})	C=O[d] (cm^{-1})
0%	2917.81	2850.31	1737.57	1643.07
20%	2923.59	2852.24	1743.36	1641.15
40%	2925.52	2854.17	1743.36	1648.86
60%	2927.45	2854.17	1743.36	1641.15
80%	2917.81	2850.31	1737.57	1631.51
100%	2921.66	2852.24	1739.50	1635.36

[a]C–H asymmetric stretching band.
[b]C–H symmetric stretching band.
[c]C=O stretching band from lipid.
[d]C=O stretching band from amide.

a two-layered model of skin was preferable to analyze the permeation profiles of ISDN and ANP when using the penetration enhancer, ethanol.

Acknowledgements

The authors wish to thank Dr. Tomomi Hatanaka, Toyama Medical and Pharmaceutical University for her valuable suggestions.

References

Akhter, S.A. and Barry, B.W., Absorption through human skin of ibuprofen and flurbiprofen. Effect of dose variation, deposited drug films, occlusion and the penetration enhancer *N*-methyl-2-pyrrolidone. *J. Pharm. Pharmacol.*, 37 (1985) 27–37.

Aungest, B.J., Rogers, N.J. and Shefte, E., Enhancement of naloxone penetration through human skin in vitro using fatty acids, fatty alchols, surfactants, sulfoxides and amides. *Int. J. Pharm.*, 33 (1986) 225–234.

Barry, B.W., Mode of action of penetration enhancers in human skin. *J. Controlled Release*, 6 (1987) 85–97.

Berner, B., Juang, R. and Mazzenga, G.C., Ethanol and water sorption into stratum corneum and model system. *J. Pharm. Sci.*, 78 (1989a) 472–476.

Berner, B., Mazzenga, G.C., Otte, J.H., Steffens, R.J., Juang, R. and Ebert, C.D., Ethanol:water mutually enhanced transdermal therapeutic system. II. Skin permeation of ethanol and nitroglycerin. *J. Pharm. Sci.*, 78 (1989b) 402–407.

Bommannan, D., Potts, R.O. and Guy, R.H., Examination of effect of ethanol on human stratum corneum in vivo using infrared spectroscopy. *J. Controlled Release*, 16 (1991) 299–304.

Campbell, P.S. and Chandrasekaran, S.K., *U.S. Patent* 4379454 (1983).

Chin, J.H. and Goldstein, D.B., Effect of low concentration of ethanol on the fluidity of spin labeled erythrocyte and brain membranes. *Mol. Pharmacol.*, 13 (1977) 435–441.

Friend, D., Catz, P., Heller, J., Reid, J. and Baker, R., Transdermal delivery of levonorgestrel. II. Effect of prodrug structure on skin permeability in vitro. *J. Controlled Release*, 7 (1988) 251–261.

Gale, R.M. and Berggren, R.G., *U.S. Patent* 4615699 (1986).

Gale, R.M. and Berggren, R.G., *U.S. Patent* 4681584 (1987).

Gale, R.M., Lee, E.S., Taskovich, L.T. and Yum, S.I., *U.S. Patent* 4588580 (1986).

Ghanem, A.H., Mahmoud, H., Rohr, U., Higuchi, W.I., Borsadin, S., Fox, J.L. and Good, W.R., Mechanism of action of ethanol in enhancing the transport of estradiol and other permeants in hairless mouse skin. *Pharm. Res.*, 3 (1986) S56.

Ghanem, A.H., Rohr, U., Higuchi, W.I., Liu, P., Borsadin, S. and Fox, J.L., Quantitation of lipid pathway and pore transport enhancing effect of ethanol in hairless mouse stratum corneum. *Pharm. Res.*, 14 (1987a) S70.

Ghanem, A., Mahmoud, H., Higuchi, W.I., Rohr, U.D., Borsadia, S., Liu, P., Fox, J.L. and Good, W.R., The effect of ethanol on the transport of β-estradiol and other permeants in hairless mouse skin. II. A new quantitative approach. *J. Controlled Release*, 6 (1987b) 75–83.

Good, W.R., Powers, M.S., Campbell, P. and Schenkel, L., A new transdermal delivery system for estradiol. *J. Controlled Release*, 2 (1985) 89–97.

Granger, D.N. and Taylor, A.E., Permeability of intestinal capillaries to endogenous macromolecules. *Am. J. Physiol.*, 238 (1987) H457.

Hatanaka, T., Manabe, E., Sugibayashi, K. and Morimoto, Y., An application of the hydrodynamic pore theory to percutaneous absorption of drugs. *Pharm. Res.*, 11 (1994) 654–658.

Hatanaka, T., Inuma, M., Sugibayashi, K. and Morimoto, Y., Prediction of skin permeability of drugs. I. Comparison with artificial membrane. *Chem. Pharm. Bull.*, 38 (1990) 3452–3459.

Higuchi, W.I., Liu, P., Ghanem, A.H. and Song, W., Mechanism of ethanol effect on the simultaneous transport and metabolism of β-estradiol in hairless mouse skin. *Proc. Int. Symp. Control Rel. Bioact. Mater.*, 14 (1987) 101–102.

Kedem, O. and Katchalsky, A., Thermodynamic analysis of the permeability of biological membrans to non-electrolyte. *Biochem. Biophys. Acta*, 27(1958) 229–246.

Knutson, K., Potts, R.O., Guzek, D.B., Golden, G.M., McKie, J.E., Lambert, W.J. and Higuchi, W.I., Macro and molecular physical chemical considerations in understanding drug transport in the stratum corneum. *J. Controlled Release*, 2 (1985) 67–87.

Knutson, K., Krill, S.L. and Zang, J., Solvent mediates alternation of the stratum corneum. *J. Controlled Release*, 11 (1990) 93–103.

Kurihara-Bergstrom, T., Knutson, K., Denoble, L.J. and Goates, C.Y., Percutaneous absorption enhancement of an ionic molecular by ethanol-water system in human skin. *Pharm. Res.*, 7 (1990) 762–766.

Levitt, D.G., General continuum analysis of transport through pores. I. *Biophys. J.*, 15 (1975a) 533–551.

Levitt, D.G., General continuum analysis of transport through pores. II. *Biophys. J.*, 15 (1975b) 553–578.

Morimoto, Y., Sugibayashi, K., Hosoya, K. and Higuchi, W.I., Penetration enhancing effect of Azone on the transport of 5-fluorouracil across the hairless rat skin. *Int. J. Pharm.*, 32 (1986) 31–38.

Oertel, R.P., Protein conformation changes induced in human stratum corneum by organic sulfoxides: an infrared spectroscopic investigation. *Biopolymers*, 16 (1977) 2329–2345.

Ongpipattanakul, B., Burnnette, R.R., Potts, R.O. and Francoeur, M.L., Evidence that oleic acid exists in a separate phase within stratum corneum lipids. *Pharm. Res.*, 8 (1991) 350–355.

Park, A.C. and Baddiel, C.B., Rheology of stratum corneum. II. A physico-chemical investigation of factors influencing the water content of the corneum. *J. Soc. Cosmet. Chem.*, 23, (1972) 13–21.

Potts, R.O. and Guy, R.H., Predicting skin permeation. *Pharm. Res.*, 9 (1992) 663–669.

Sasaki, H., Kojima, M., Nakamura, J. and Shibasaki, J., Enhancing effect of combining two pyrrolidone vehicles on transdermal drug delivery. *J. Pharm. Pharmacol.*, 42 (1990) 196–199.

Silvieri, L.A. and DeAngelis, N.J., Isosorbide dinitrate. In Florey, K. (Ed.) *Analytical Profiles of Drug Substances*, Academic Press, New York, Vol. 4, 1975, pp. 225–244

White, S.H., Mirejovsky, D. and King, G.I., Structure of lamella lipid domains and corneocyte envelopes of murine stratum corneum. An X-ray diffraction study. *Biochemistry*, 27 (1988) 3725–3732.