Effect of Vehicle Properties on Skin Penetration of Emedastine

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We investigated sorption and permeation of emedastine with 11 different vehicles, composed of single or binary solvents, in excised rat skin. In the sorption study, partition parameters of the drug with each vehicle were obtained by dividing the drug amount in skin at equilibrium by its donor concentration. When the logarithm of the partition parameters for the stratum corneum/vehicle partitioning (K_s') was plotted against the dielectric constants of the vehicles, a bi-linear relationship was obtained. The skin flux of emedastine largely differ among the vehicles. A quasi-steady-state flux of emedastine exhibited a good linear relationship with K_s' , except for ethanol (EtOH)/isopropyl myristate (IPM) systems, indicating that the partitioning process is critical in determining the permeation rate. Delineation of the EtOH/IPM systems would be due to an increase in the diffusivity of the drug in the stratum corneum, as indicated by the analysis using a two-layer diffusion model. Thus, differential evaluation of partitioning and diffusion processes by both sorption and permeation studies would give further insights into the effects of vehicles on skin permeation of drugs.

Key words emedastine; skin penetration; vehicle effect; dielectric constant; sorption; partitioning

Emedastine, 1-(2-ethoxyethyl)-2-(hexahydro-4-methyl-1H-1,4-diazepin-1-yl)-1H-benzimidazole, has been used for the prophylaxis and treatment of diseases caused by histamine such as allergic dermatitis and allergic rhinitis.^{1,2)} An oral dosage form of emedastine difumarate has been proved effective and useful in humans³⁾ and approved for clinical usage.

The transdermal route would be an attractive alternative for systemic delivery of emedastine, because it would provide sustained delivery to the systemic circulation and circumvent the first-pass metabolism^{4,5)} in the gastrointestinal tract and liver. In our preliminary study,⁶⁾ emedastine free base exhibited high skin permeability, especially when applied with hydrophobic vehicles such as isopropyl myristate and octanol. This study also demonstrated that skin permeation of emedastine is highly dependent on the polarity of the vehicles.

The present study was initiated to determine the effect of various vehicles on diffusivity and partitioning of emedastine from physicochemical point of view. We examined the *in vitro* skin permeation of emedastine with 11 different vehicles composed of single or binary solvents. A sorption study was also conducted to evaluate the effect on the partitioning directly. In this experiment, the amount of emedastine in skin equilibrated with the drug-containing vehicles was measured. In addition, penetration profiles of the drug was analyzed based on the two-layer skin diffusion model.^{7,8)} Based on these results, we delineated the relationship between penetration of emedastine and the physicochemical properties of the vehicles.

MATERIALS AND METHODS

Materials Emedastine (M.W.: 302.42) was synthesized according to a routine protocol.⁹⁾ Isopropyl myristate (IPM), geraniol (GeOH), ethanol (EtOH), and glycerin were obtained commercially from Wako Pure Chemicals, Osaka,

Japan. All reagents used were of analytical grade.

Determination of Dielectric Constants of the Vehicles

Electrical capacitances of the vehicles were measured using a TR-10C type conductance analyzer AS4245 (Ando Electric Co., Ltd., Tokyo, Japan) and LE-21 type electrode AS-20457 (Ando Electric Co., Ltd.) at 1 MHz, 25 °C. The dielectric constants (ϵ) of the vehicles were calculated as,

$$\epsilon = C_v / C_0$$

where C_v and C_0 are the capacitance of the cell with vehicle and the capacitance of the dry cell, respectively.

In Vitro Skin Permeation Study Full-thickness abdominal skin was excised from a male Wistar strain rat weighing about 200 g (7—8 weeks) after removal of hairs and adipose tissues. The skin was punched out into a 3-cm diameter disk and mounted on flow-through type diffusion cells with the epidermal side facing the donor chamber (exposed area, 3.14 cm²).⁷⁾ The receiver chamber was perfused with phosphate-buffered saline (PBS, pH 7.4) containing 50 mg/l streptomycin sulfate and 30 mg/l penicillin G potassium salt at a flow rate of 6 ml/h. After 6-h treatment of the epidermal side with a 1-ml vehicle without the drug, the solvents were replaced with a 1-ml aliquot of the vehicle containing 5.657 mg/ml emedastine. Emedastine is a liquid compound and freely miscible with all the vehicles used. Skin penetration studies was performed at the concentration of emedastine which would be low enough for its activity coefficient to be constant. The receptor fluid was collected every 45 min for 12 h. The apparatus was thermostated at 37 °C in an incubator throughout the experiment. The tested vehicles were IPM, GeOH, EtOH, glycerin, water, EtOH/IPM (37:63 and 73:27), EtOH/water (62:38 and 32:68), and water/glycerin (30:70 and 70:30), where the ratio of the mixtures were implied by volume/volume. The ratios of the mixed solvents were determined based on solubility parameters to obtain solvents with moderate polarity. The quasi-steady state fluxes of emedastine were determined using a linear portion of

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these penetration profiles.

In vitro permeation through tape-stripped skin was also evaluated. After removal of hairs, the abdominal was stripped 15 times with an adhesive tape (Scotch tape, Sumitomo 3M Co., Japan), excised, punched out and mounted on diffusion cells. After 6-h preincubation with pH 7.4 PBS, a 1-ml PBS containing the drug was administered. The rest of the experimental procedures were the same as for intact skin.

Sorption Study Full-thickness abdominal skin was mounted in the diffusion cells, with the dermal side in contact with a sheet of aluminum foil. The epidermal side of the skin was exposed to the drug solution (5.657 mg/ml) at 37 °C for 24 h. Then, excess drug on the skin surface was gently wiped off with alcohol swabs. The stratum corneum was tape-stripped, and the tapes were immersed in 20 ml hexane and 10 ml 0.1 N hydrochloric acid with vigorous agitation in a shaker. After centrifugation at 3500×*g*, the aqueous phase was collected for HPLC assay. The skin remaining after tape stripping was homogenized in 20 ml 0.1 N hydrochloric acid. After addition of hexane to the homogenates, the mixture was well agitated and then centrifuged at 3500×*g*. The aqueous phase was subjected to HPLC. Partition parameters for stratum corneum/the underlying layer were calculated as the amount of drug in the layers divided by its donor concentration.

Measurement of Emedastine and Ethanol Measurement of emedastine was conducted using a HPLC-series 1050 (Hewlett Packard, Wilmington, DE, U.S.A.) equipped with a UV detector operated at 280 nm. The inertsil ODS-2 column (5 μm, 5 i.d.×150 mm, GL Science, Tokyo, Japan) was used in a reversed phase mode. The mobile phase was a 1:1 (v/v) mixture of acetonitrile and 0.025 M phosphate buffer (pH 2.4) containing 0.25% sodium lauryl sulfate and was flowed at 1.2 ml/min. The standard calibration curves were obtained using five different concentration of emedastine from 1 to 100 μg/ml (*r*²=0.9998). The limit of quantification was 0.5 ng of emedastine per microliter injection. The repeatability (*n*=5) was 97.3% for 1 μg/ml standard solution.

The amount of ethanol in receptor fluids was determined using a gas liquid chromatography system (Hewlett Packard model 5890A) combined with a flame ionization detector. The column used was a 60 m fused silica capillary column (coated with 1.0 μm carbowax) with an inside diameter of 0.75 mm (Supelco, Inc. Bellefonte, PA, U.S.A.). The injector port, column oven and detector port were maintained at 150, 80, and 200 °C, respectively. Helium was used as the carrier gas at a flow rate of 8 ml/min. The standard calibration curves were obtained using five different concentration of ethanol in water from 10 to 200 μg/ml (*r*²=0.9997). The limit of quantification was 2 ng of ethanol per microliter injection. The repeatability (*n*=3) was 98.1% for 10-μg/ml standard solution.

Data Analysis The penetration profiles obtained in the *in vitro* diffusion experiment were analyzed based on a two-layer diffusion model, where the skin was assumed to consist of stratum corneum and an underlying layer. It was also assumed that the amount of drug in the donor decreases according to its penetration through skin and its concentration in the receiver fluid is kept zero.^{7,8)} Based on this model, the Laplace transform for the amount of drug appearing in the receptor through the intact skin is expressed as follows:

$$\bar{Q} = Z_s Z_d X_0 / s / g(s) \quad (1)$$

where *s* is the Laplace variable with respect to time; *X*₀ is the initially applied dose; and

$$d_s = L_s (s / D_s)^{1/2} \quad (2)$$

$$d_d = L_d (s / D_d)^{1/2} \quad (3)$$

$$Z_s = K_s V_s / d_s \quad (4)$$

$$Z_d = K_d V_d / d_d \quad (5)$$

$$g(s) = Z_s \sinh d_d (V_v \cosh d_s + Z_s \sinh d_s) + Z_d \cosh d_d (V_v \sinh d_s + Z_s \cosh d_s) \quad (6)$$

where *V* is the effective volume; *L* is the thickness for diffusion; *D* is the diffusion coefficient; *K* is the partition coefficient between stratum corneum or viable layer and vehicle; and subscripts v, s and d indicate the vehicle, stratum corneum and viable layer, respectively.

Because of the difficulty in determining the real diffusion length (*L*), the two parameters involving *L*, namely, the diffusion parameter (*D'*) and the partition parameter (*K'*) were defined as follows:

$$D_i' = D_i / L_i^2 \quad (7)$$

$$K_i' = K_i V_i (=K_i A L_i) \quad (i=s \text{ or } d) \quad (8)$$

where *A* expresses the effective diffusion area.

Equation (1) was fitted to the skin penetration profiles of emedastine, using a nonlinear regression program with a fast inverse Laplace transform algorithm MULTI (FILT).¹⁰⁾ Using the partition parameters (*K*_s' and *K*_d') determined in the sorption study, the diffusion parameters (*D*_s' and *D*_d') were estimated by this curve-fitting procedure. The computation was conducted on a SGI Indigo Elan 4000 workstation (Silicon Graphics, Mountain View, CA, U.S.A.).

RESULTS

In Vitro Penetration of Emedastine Figure 1 shows the penetration of emedastine through rat skin with single-component vehicles; that is, water, glycerin, EtOH, GeOH, and IPM. The penetration of emedastine through tape-stripped skin is also shown in this figure. The penetration of emedastine with water was very rapid, and was approximately a half of that through tape-stripped skin. The penetration of emedastine with IPM, which have the lowest polarity among the vehicles tested, was as great as that with water. The slowest penetration was observed with EtOH.

Similar experiments were done with 6 binary solvents: EtOH/IPM (37:63), EtOH/IPM (73:27), EtOH/water (62:38), water/glycerin (30:70), EtOH/water (32:68), and water/glycerin (70:30) mixtures. Figure 2 shows penetration profiles of emedastine with the mixed vehicles. The penetration of emedastine with EtOH/IPM (37:63 and 73:27) was larger than with the individual solvents, and comparable to that across stripped skin. In the EtOH/water (62:38 and 32:68) systems, the penetration of emedastine was better than with EtOH alone, but worse than with water. The penetration of emedastine with water/glycerin (30:70 and 70:30) increased with an increase in water content.

Sorption Study Partition parameters of emedastine for the stratum corneum and underlying viable layer were deter-

Fig. 1. *In Vitro* Penetration Profiles of Emedastine through Stripped Skin (A) or Intact Skin with Water (B), Glycerin (C), EtOH (D), GeOH (E), IPM (F). Each point represents the mean $\pm$ S.D. value of three or four experiments. The theoretical curves were drawn using the parameters listed in Table 1.

Fig. 2. *In Vitro* Penetration Profiles of Emedastine through Intact Skin with Binary Component Vehicles; EtOH:IPM (37:63) (A), EtOH:IPM (73:27) (B), EtOH:Water (62:38) (C), Water:Glycerin (30:70) (D), EtOH:Water (32:68) (E), and Water:Glycerin (70:30) (F).

Each point represents the mean $\pm$ S.D. value of three or four experiments. The theoretical curves were drawn using the parameters listed in Table 1.

mined by measuring the drug amount in the skin at equilibrium. The partition parameter (K_s' and K_d') values varied according to the vehicle tested (Table 1). K_s' for IPM or water was largest, whilst K_s' for EtOH was smallest. When the logarithm of (K_s') was plotted against the dielectric constant (ϵ) of vehicle, the profile could be characterized by two linear phases (Fig. 3). The regression curve was,

$$K_s' = 0.05813e^{-0.2464\epsilon} + 0.00004663e^{0.08530\epsilon}$$

By contrast, the relationship between K_d' and the dielectric constant of vehicle was not so clear.

When the quasi-steady state flux was plotted against

K_s' value, a good correlation was obtained, except for the EtOH/IPM systems (Fig. 4).

Model Analysis Table 1 also includes the diffusion parameters obtained by the analysis based on a two-layer skin diffusion model. For EtOH/IPM mixtures, remarkably higher diffusivity in the stratum corneum was obtained.

Measurement of Ethanol Fluxes The skin penetration of ethanol from ethanol-containing vehicles were investigated. The fluxes of ethanol with these vehicles were relatively large, being largest in EtOH:IPM (73:27), followed by EtOH:water (62:38), EtOH:water (32:68), and pure EtOH (Table 1).

Table 1. Partition Parameter Determined Experimentally and Diffusion Parameters for Penetration of Emedastine through Skin Estimated by Fitting the Penetration Profile to the Equation Derived from Two-Layer Skin Model

Vehicle	Dielectric constant of vehicle ^{a)}	Flux of emedastine ^{b)} (%·h ⁻¹)	Flux of EtOH (mg·h ⁻¹) ^{b)}	Partition parameter ^{c)} ×10 ² (cm ³)		Diffusion parameter ^{d)} (h ⁻¹)	
				Stratum corneum K'_s	Viable layer K'_d	Stratum corneum D'_s	Viable layer D'_d
IPM	3.31	3.23±0.27		2.57±0.30	41.8±3.5	2.58	0.301
EtOH: IPM (37: 63)	9.13	4.16±0.58		0.71±0.27	14.7±3.54	43.7	0.856
Geraniol	7.81	1.58±0.10		0.68±0.23	2.86±0.54	7.60	0.928
EtOH: IPM (73: 27)	18.36	7.17±0.87	67.4±0.7	0.16±0.038	3.75±0.35	54.3	30.0
EtOH	24.13	0.10±0.02	8.4±2.1	0.039±0.01	1.49±0.36	3.90	0.156
Glycerin	42.68	0.31±0.04		0.20±0.03	2.56±0.36	5.50	0.171
EtOH: Water (62: 38)	46.12	1.11±0.22	29.7±7.3	0.51±0.21	10.3±1.14	7.10	0.121
Water: Glycerin (30: 70)	57.75	0.41±0.05		0.67±0.15	9.09±1.48	0.838	0.212
EtOH: Water (32: 68)	64.11	2.51±0.35	11.2±0.8	1.34±0.21	14.0±3.37	6.00	0.269
Water: Glycerin (70: 30)	71.14	1.69±0.22		1.95±0.34	12.7±1.03	2.90	0.221
Water	78.02	3.43±0.46		3.07±0.57	11.5±1.4	3.30	0.509
(Tape-stripped skin)							
Water		4.85±0.51			31.1 ^{e)}		0.329 ^{e)}

a) Dielectric constant was measured at 1 MHz. b) The fluxes were calculated from the liner portion of the penetration profile. c) Partition parameters were determined by sorption experiments. Data represent experimental means ± S.D. d) Diffusion parameters were obtained by curve-fitting to the penetration profiles. e) These parameters were obtained by curve-fitting to the penetration profile across tape-stripped skin.

Fig. 3. Relationship between Partition Parameter of Emedastine for the Stratum Corneum (K'_s) and Dielectric Constant of Vehicle (ϵ)

The regression curves were,

$$K'_s = 0.05813e^{-0.2464\epsilon} + 0.00004663e^{0.08530\epsilon} \quad (R^2=0.999).$$

DISCUSSION

When the logarithm of the partition parameters for the stratum corneum was plotted against the dielectric constant (ϵ) of the vehicle, a bi-linear relationship was obtained (Fig. 3). Assuming that the vehicle does not alter the solubility of emedastine in the stratum corneum, the partition parameter of the drug would be inversely proportional to its solubility in the vehicle. Gorman and Hall¹¹⁾ demonstrated that the relationship between the logarithm of drug solubility and the dielectric constant of the solvent was bi-linear, with a positive slope in less polar solvents and a negative slope in highly polar solvents. The relationship would correspond to the partitioning/dielectric constant relationship (Fig. 3), indicating that all the vehicles tested did not alter the solubility of emedastine in the stratum corneum.

Quasi-steady-state fluxes showed a good linear relationship with the partition parameters for the stratum corneum (Fig. 4). This clearly indicates that the partitioning process is critical in determining the permeation rate. Taking together

Fig. 4. Relationship between Quasi-Steady State Flux and Partition Parameter (K'_s) of Emedastine

Open symbols represent EtOH/IPM mixtures, while closed ones represent other vehicles. When linear regression of the data except for the EtOH/IPM mixtures was conducted, the following equation was obtained:

$$\log \text{Flux} = 1.081 \log K'_s - 2.164 \quad (R^2=0.880).$$

with the partitioning/dielectric constant relationship, the permeation rate of emedastine would be controlled by the polarity of vehicles.

However, permeabilities of emedastine with the EtOH/IPM systems were outliers of the linear relationship between quasi-steady-state fluxes and partition parameters. This suggests that the vehicles alter the permeability of the skin. It has been reported that EtOH is a skin penetration enhancer, where the permeation rate of drugs is in a linear relationship with the flux of the solvent.¹²⁻¹⁴⁾ In the present investigation, however, we found that the EtOH/water systems did not exhibit an penetration-enhancing effect in spite of giving high EtOH flux which was represented in Table 1. The discrepancy with the results of other investigators¹²⁻¹⁴⁾ might be due to the difference in physicochemical properties of the compounds tested. Kim *et al.*¹⁵⁾ found that the EtOH/water systems enhanced the skin permeation rate of lipophilic drugs simply due to enhanced solubility in the vehicle by

EtOH, whereas that of hydrophilic drugs was enhanced due to increased partitioning into skin. Since emedastine is lipophilic, it would easily distribute to the stratum corneum lipids and, therefore, the cosolvation effect by EtOH¹²⁻¹⁴⁾ might not necessarily be important in enhancing the permeation of emedastine.

Rather, it should be noted that the EtOH/IPM system would increase the diffusivity of the drug in the stratum corneum, as indicated by diffusion model analysis. Hori *et al.*¹⁶⁾ classified percutaneous penetration enhancers into two distinct groups ("solvents" and "enhancers"), using an organic conceptional diagram. They indicated that the combination of vehicles from two different groups is better than one vehicle alone. Considering that EtOH and IPM belong to different groups, combination of these two solvents could exhibit a synergistic effect for penetration enhancement.

In addition, the penetrations of emedastine with EtOH:IPM (37:63) and EtOH:IPM (73:27) were almost as large as that across tape-stripped skin (Fig. 1-A), suggesting that the viable layer be rate-limiting in skin penetration rather than the stratum corneum. Therefore, for the EtOH/IPM systems, the effect of vehicle composition would not be detected.

Even for water and IPM, which did not show any effects on diffusivity in the stratum corneum (Table 1), penetration of emedastine was almost half of that across tape-stripped skin. This means that penetrability of emedastine across the stratum corneum is extremely high.

A diffusion model analysis is useful to depict the skin penetration of drugs from physicochemical point of view. However, if complicated boundary conditions were given, it would be practically impossible to obtain corresponding analytical solution. In this model, it was assumed that the volume of vehicle is constant during the experiment and diffusion and partition coefficients in the skin are also constant. Strictly speaking, these assumptions would not stand in some cases, because the volume and composition of the vehicle and/or barrier properties of skin may change with time due to penetration of vehicle solvent(s). Failure to fit theoretical curves to the penetration profiles in the EtOH/IPM systems would be due to these vehicle effects. Thus, the diffusion model might be useful to detect such time-dependent effects, although the parameters obtained by curve-fitting might not be useful in this case. When penetration of vehicle solvents is

negligible, however, the diffusion model analysis would be applicable to evaluate the effects of vehicles on penetration parameters.

In conclusion, the present study depicts that the partitioning of emedastine in the stratum corneum would be determined mainly by its thermodynamic activity in the vehicle. Combination of EtOH and IPM gave remarkably higher penetration of emedastine, presumably due to increased diffusivity in the skin. Thus, differential evaluation of partitioning and diffusion processes through both sorption and permeation studies would give further insights into the effects of vehicles on skin permeation of drugs.

REFERENCES

- 1) Fukuda T., Saito T., Tajima S., Shimohara K., Ito K., *Arzneim.-Forsch./Drug Res.*, **34**, 805—810 (1984).
- 2) Iemura R., Kawashima T., Fukuda T., Ito K., Nose T., Tsukamoto G., U.S. Patent No.4430343, 7 Feb. (1984).
- 3) Ishibashi Y., Harada S., Niimura M., Ueda H., Imamura S., Yamamoto S., Yoshida H., Ogawa N., Nakano S., *Rinshouiyaku*, **6**, 141—159 (1990).
- 4) Sakai T., Takahashi H., Hamada T., Awata N., Watanabe J., *J. Pharmacobio-Dyn.*, **2**, 123—131 (1987).
- 5) Sakai T., Hamada T., Awata N., Watanabe J., *J. Pharmacobio-Dyn.*, **12**, 530—536 (1989).
- 6) Harada S., Takahashi Y., Nakagawa H., *Biol. Pharm. Bull.*, **16**, 884—888 (1993).
- 7) Okamoto H., Yamashita F., Saito K., Hashida M., *Pharm. Res.*, **6**, 931—937 (1989).
- 8) Yamashita F., Yoshioka T., Koyama Y., Okamoto H., Sezaki H., Hashida M., *Biol. Pharm. Bull.*, **16**, 690—697 (1993).
- 9) Kanebo Ltd., Japan. Patent 58-79983 (1983) [*Chem. Abstr.*, **99**, 139971 (1983)].
- 10) Yano Y., Yamaoka K., Tanaka H., *Chem. Pharm. Bull.*, **37**, 1035—1038 (1989).
- 11) Gorman W. G., Hall G. D., *J. Pharm. Sci.*, **53**, 1017—1020 (1964).
- 12) Good W. R., Powers M. S., Campbell P., Schenkel L., *J. Control. Rel.*, **2**, 28—34 (1985).
- 13) Berner B., Mazzenga G. C., Otte J. H., Steffens R. J., Juang R. H., Ebert C. D., *J. Pharm. Sci.*, **78**, 402—407 (1989).
- 14) Yum S., Lee E., Taskovich L., Theeuwes F., "Drug Permeation Enhancement," ed. by Hsieh D. S., Marcel Dekker, New York, 1994, pp. 143—171.
- 15) Kim D. D., Julia L. K., Chien Y. W., *J. Pharm. Sci.*, **85**, 1191—1195 (1996).
- 16) Hori M., Satoh S., Maibach H. I., "Percutaneous Absorption," ed. by Bronaugh R. L., Maibach H. I., Marcel Dekker, New York, 1989, pp. 197—211.