In Vitro–in Vivo Correlation of Percutaneous Absorption: Isosorbide Dinitrate and Morphine Hydrochloride

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The potential of an *in vitro* skin preparation as a model for predicting *in vivo* percutaneous absorption of drugs was examined. *In vitro* and *in vivo* skin permeation data for two model drugs with different lipophilicity, isosorbide dinitrate (ISDN) and morphine hydrochloride (MPH), were compared using pharmacokinetic techniques. *In vitro* permeation data published previously were analyzed based on a single pathway model, and permeation parameters were obtained. The disposition parameters were estimated from the plasma concentration profiles after i.v. administration. The plasma concentrations after topical application were then simulated using the obtained permeation and disposition parameters, and the values were compared with the corresponding observed ones. Although the simulated plasma concentration curves were not greatly different from those observed, there were some differences in the time course-pattern. Causes for these *in vitro–in vivo* differences were discussed.

Keywords percutaneous absorption; skin permeability; *in vitro–in vivo* correlation; isosorbide dinitrate; morphine hydrochloride; Frandol® Tape S

In vitro preparation of skin is commonly used in studies on percutaneous absorption. Definition of the physicochemical parameters underlying absorption, enhancement of skin permeability through the use of new vehicles, new drug development and assessment of percutaneous toxicity have all been approached through the use of the *in vitro* model. Despite widespread use of the model, few data are available to support the belief that *in vitro* absorption is an accurate reflection of *in vivo* absorption. Most of the data simply compare the amount absorbed *in vitro* and *in vivo*, and little has been done to compare their time courses.[1,2] In our previous reports, the predictive methods for the *in vivo* time courses from *in vitro* ones were based on the diffusion and compartment models and convolution method.[3,4] Although these methods had relatively high predictability, the causes of discrepancies observed in some cases were not clear.

The present study aimed at evaluating the relevance of the *in vitro* model in predicting *in vivo* percutaneous absorption. Previously published permeabilities of isosorbide dinitrate (ISDN), a typical lipophilic drug, and morphine hydrochloride (MPH), a typical hydrophilic one, using excised hairless rat skin[5,6] were compared to the corresponding data in living rats by employing a pharmacokinetic technique. A diffusion model was used in the analysis of the skin permeation process because it can give some permeation parameters with physical meanings, and changes in these parameters can be related to changes in conditions between *in vitro* and *in vivo* models. The effect of formulation on the *in vitro–in vivo* correlation was also studied by comparing ISDN aqueous suspension to a commercial adhesive matrix device of ISDN, Frandol® Tape S.

MATERIALS AND METHODS

Materials ISDN was generously supplied by Toko Pharmaceutical Industries Co. (Tokyo, Japan). MPH was obtained from Takeda Pharmaceutical Industries Co. (Osaka, Japan). All other reagents used were of reagent grade and were obtained commercially.

Intravenous Administration Studies Male hairless rats (WBN/ILA-Ht, Ishikawa Laboratory Animals, Saitama, Japan) weighing 160—200 g were used. MPH and ISDN were dissolved in physiological saline and 40% ethanolic saline, respectively, at a concentration of 0.4 or 1 mg/ml. The drug solutions (2 and 5 mg/kg) were injected into the left jugular vein of a rat restrained under urethane anesthesia (1.25 g/kg i.p.). Blood samples (0.2 ml each) were withdrawn from the right jugular vein into a heparinized syringe just prior to the drug administration (blank) and at appropriate times after the dosing. Each blood sample was centrifuged at 10000 rpm for 2 min and the obtained plasma was stored at −20 °C until analysis.

***In Vivo* Skin Permeation Studies** Rats were restrained under urethane anesthesia. A glass cell with a water jacket connected to a circulating water bath at 37 °C was glued to the abdomen of each rat with cyanoacrylate adhesive, and 3 ml of ISDN or MPH aqueous suspension was applied to a 4.83 cm^2 area demarcated by the cell. Frandol® Tape S was applied to a 9.82 cm^2 area. Blood samples were obtained and treated in the same manner as in the i.v. administration studies.

Analytical Methods Plasma concentrations of ISDN were determined by the method of Sioufi and Pommier[7] with a minor modification. The plasma sample (0.1 ml) was mixed with 0.1 ml of distilled water and 5 ml of *n*-hexane containing glyceryl trinitrate as an internal standard. The mixture was shaken mechanically for 20 min and centrifuged at 3000 rpm for 5 min. The hexane layer was reextracted with 2 ml of acetonitrile by mechanical shaking for 10 min. After centrifugation at 3000 rpm for 5 min, the acetonitrile layer was transferred to a test tube and evaporated to dryness under a nitrogen atmosphere.

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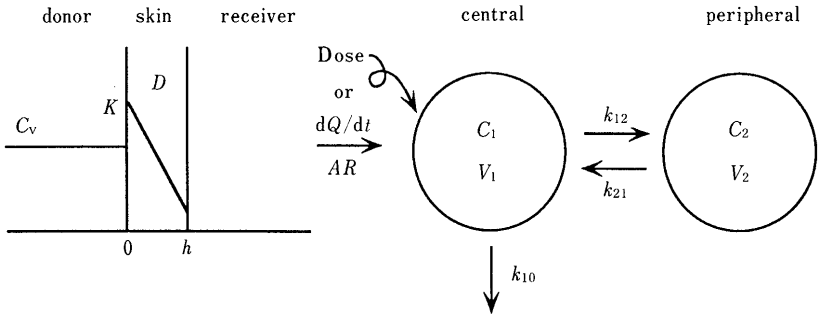

Fig. 1. Two-Compartment Model for *in Vivo* Percutaneous Absorption

The compartment model was set up with a single permeation pathway model for *in vitro* skin permeation.

The residue was dissolved in 0.1 ml of ethyl acetate, an aliquot of 1 μl was injected for gas-liquid chromatography (GLC), which was carried out using a Shimadzu GC-8A apparatus equipped with an electron-capture detector (Kyoto, Japan) and a glass column (200 cm × 3 mm i.d.) packed with 2% silicone OV-17 on Chromosorb WAW DMCS (80—100 mesh, GL Sciences, Tokyo). Chromatographic conditions were as follows: column temperature 160 °C; injection port temperature 250 °C; and carrier gas (nitrogen) flow rate 40 ml/min. The coefficient of variation was 14.1%.

Plasma MPH concentrations were determined by HPLC. A Shimadzu LC-6A apparatus equipped with an L-ECD-6A electrochemical detector, and a CTO-6A column oven were used. HPLC was performed on a column (150 × 4 mm i.d.) packed with Nucleosil 5C18 (Machery Nagel, Germany) with acetonitrile: 10 mM phosphate buffer, pH 2.5 (30 : 70) including 5 mM of sodium dodecylsulfate and 27 μM of disodium ethylenediaminetetraacetate as a mobile phase at a flow rate of 1.5 ml/min. The coefficient of variation was 17.1%.

Data Analysis Data analysis was done by a non-linear least squares regression program based on a fast inverse Laplace transform algorithm (MULTI(FILT))[8] which was run on a personal computer (PC-9801DA, NEC, Tokyo).

In vitro skin permeation data were analyzed according to a single permeation pathway model as shown in Fig. 1. When an aqueous suspension of a drug is applied to the donor side of skin and a sink condition is maintained on the receiver side, the Laplace transform for the cumulative amounts of the drug passing through a unit area of skin ($\bar{Q}$) is expressed as[9]:

$$\bar{Q}=\frac{KC_v}{q\cdot s\cdot \sinh(q\cdot h)} \tag{1}$$

where K and C_v are the skin/donor vehicle partition coefficient and aqueous solubility of the drug, h is thickness of the skin barrier, s is the Laplace variable with respect to time, and q is defined as a function of the diffusion coefficient of the drug in skin (D) and s:

$$q=(s/D)^{1/2} \tag{2}$$

The skin permeation profiles of ISDN and MPH described previously[5,6] were fitted to Eq. 1 to obtain the permeation parameters, flux ($=D\cdot K\cdot C_v/h$) and lag time ($=h^2/6D$).

In order to predict the percutaneous absorption of ISDN and MPH in living hairless rats, their disposition parameters were estimated from the plasma concentration profiles after i.v. administration. The analysis was performed based on the two-compartment model in Fig. 1. The differential equations describing the central and peripheral compartments are:

$$V_1\frac{dC_1}{dt}=V_2k_{21}C_2-V_1(k_{12}+k_{10})C_1 \tag{3}$$

$$V_2\frac{dC_2}{dt}=V_1k_{12}C_1-V_2k_{21}C_2 \tag{4}$$

where C and V are the drug concentration and apparent volume of distribution, subscripts 1 and 2 refer to central and peripheral compartments, and k_{12}, k_{21} and k_{10} are the first-order rate constants. From Eqs. 3 and 4, the Laplace transform for plasma concentrations of the drug ($\bar{C}_1$) after i.v. administration is determined as:

$$\bar{C}_1=\frac{\text{Dose}\ (s+k_{21})}{V_1(s+\alpha)(s+\beta)} \tag{5}$$

where

$$\alpha+\beta=k_{10}+k_{12}+k_{21} \tag{6}$$

$$\alpha\beta+k_{10}k_{21} \tag{7}$$

The disposition parameters of ISDN and MPH were obtained by simultaneously fitting the plasma concentration–time data in two doses to Eq. 5.

When a drug is administered transdermally, Eq. 3 is changed to:

$$V_1\frac{dC_1}{dt}=AR\frac{dQ}{dt}+V_2k_{21}C_2-V_1(k_{12}+k_{10})C_1 \tag{8}$$

where AR and dQ/dt represent the application area and flux per unit area of skin. Therefore, the Laplace transform for plasma concentration ($\bar{C}_1$) after topical application is:

$$\bar{C}_1=\frac{\bar{Q}\cdot AR\cdot s(s+k_{21})}{V_1(s+\alpha)(s+\beta)} \tag{9}$$

The plasma concentration–time curves for ISDN and MPH after topical application were simulated by Eqs. 1 and 9 using their permeation and disposition parameters obtained in previous analyses. Further, the *in vivo* flux and lag time were estimated by computer-fitting of the observed plasma concentration profiles after topical application to

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Eqs. 1 and 9 by fixing only the disposition parameters.

Statistical analysis was carried out by the paired t-test, and a p-value of 0.05 or less was considered significant.

RESULTS AND DISCUSSION

Analysis of *in Vitro* Skin Permeation Data Figure 2 shows previously reported data on the permeation of ISDN and MPH through excised hairless rat skin.[5,6] The drugs were applied to the skin as an aqueous suspension or adhesive matrix device with drug crystalline (Frandol® Tape S), and the amount which permeated across the skin into water in the receiver compartment of a two-chamber diffusion cell (0.95 cm^2 of effective diffusion area) was measured at 37 °C. By computer-fitting the data to Eq. 1, the permeation parameters listed in Table I were obtained. The best-fit curves are also shown in Fig. 2. The flux and lag time of ISDN from aqueous suspension were the same as those from Frandol® Tape S, revealing that both formulations have the same thermodynamic activity of ISDN due to the presence of crystalline.[6] The values of flux and lag time for ISDN were lower than those for MPH.

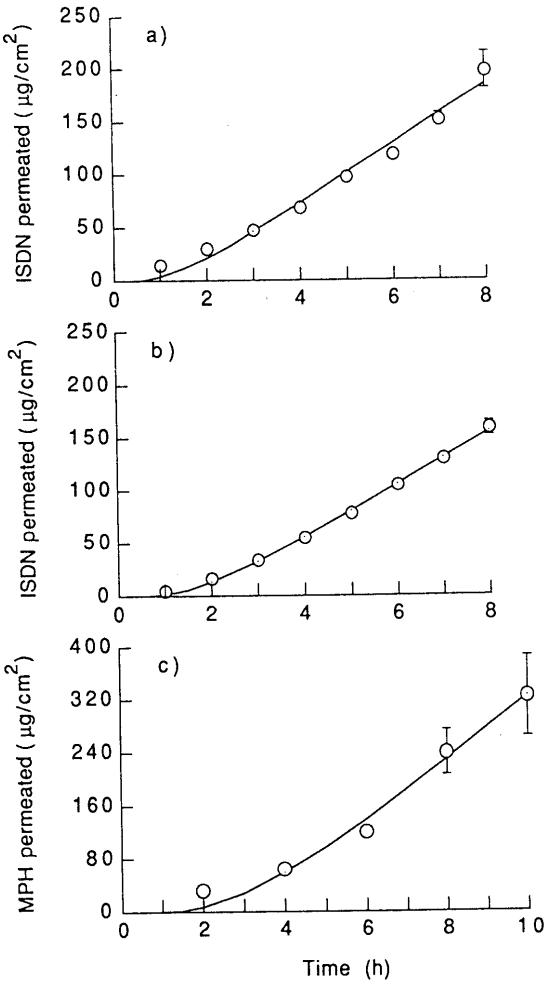

Fig. 2. Permeation Profiles of Drugs through Excised Hairless Rat Skin

a) ISDN from aqueous suspension. b) ISDN from Frandol® Tape S. c) MPH from aqueous suspension. Each point represents the mean ± S.E. of 3 experiments. The data were reported previously.[3,4] Solid curves are those best fitting Eq. 1.

Analysis of Intravenous Administration Data Time courses of plasma concentration of ISDN and MPH after i.v. administration at doses of 2 and 5 mg/kg are shown in Fig. 3. For both drugs, the plasma concentrations indicated a two-exponential decline for each dose, and the slope of the terminal phase was almost the same among doses. Such profiles can be described by a linear two-compartment model as shown in Fig. 1. The disposition parameters calculated by simultaneously fitting the plasma concentration for all doses to Eq. 5 are listed in Table II, and the fitting curves are shown in Fig. 3. The obtained parameters for each drug were in agreement with those in Sprague–Dawley rats, as reported previously.[10,11]

Analysis of *in Vivo* Percutaneous Absorption Data The plasma concentrations of ISDN and MPH after transdermal application were simulated by Eqs. 1 and 9 using the permeation and disposition parameters in Tables I and II. The concentrations were also measured after the application of aqueous suspensions (4.83 cm^2) and Frandol® Tape S (9.82 cm^2). As can be seen from Fig. 4,

Table I. *In Vitro* Skin Permeability of ISDN and MPH

	Flux ($\mu g/cm^2/h$)	Lag time (h)
ISDN from aqueous suspension	28.05 ± 2.328	1.401 ± 0.3701
ISDN from Frandol® Tape S	25.55 ± 0.7345	1.872 ± 0.1268
MPH from aqueous suspension	48.57 ± 7.702	3.342 ± 0.8767

Each value represents the mean ± S.D.

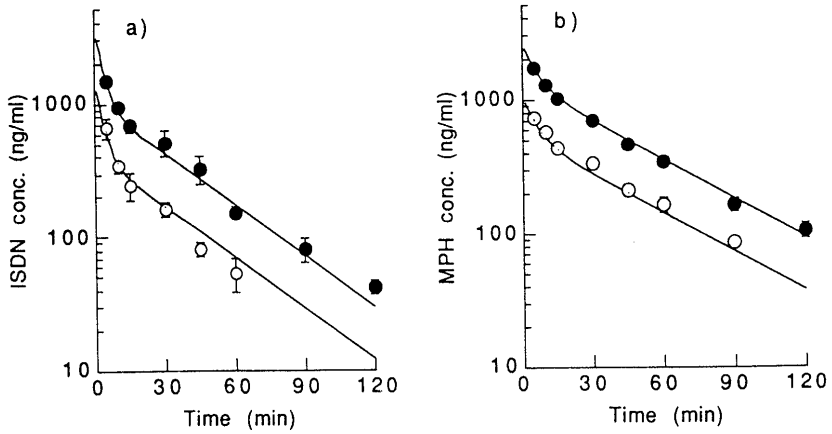

Fig. 3. Plasma Concentrations of Drugs after Intravenous Administration to Hairless Rats

a) ISDN. b) MPH. ○, 2 mg/kg; ●, 5 mg/kg. Each value represents the mean ± S.E. of 3 animals. Solid curves are those best fitting Eq. 5.

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TABLE II. Pharmacokinetic Parameters of ISDN and MPH in Hairless Rats

	ISDN	MPH
α (h^{-1})	19.26 ±9.805	10.81 ±3.953
β (h^{-1})	1.767±0.1859	1.333±0.09404
k_{21} (h^{-1})	6.175±1.113	6.149±1.765
V_1 (l/kg)	1.213±0.6756	1.847±0.2800

Each value represents the mean±S.D.

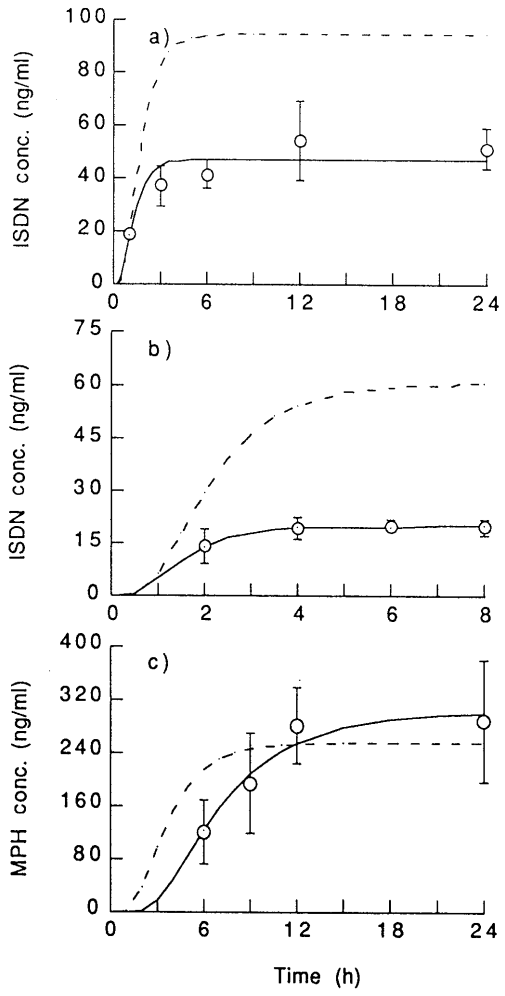

Fig. 4. Plasma Concentrations of Drugs after Topical Application to Hairless Rats

a) ISDN aqueous suspension. b) Frandol® Tape S. c) MPH aqueous suspension. Each value represents the mean±S.E. of 3 animals. Solid curves are those best fitting Eqs. 1 and 9, and the dashed ones are the predicted profiles using permeation parameter values *in vitro*.

the simulated concentrations (dashed curves) were not greatly different from the observed ones (open symbols), and the discrepancies were found in only a few of the time courses. There are some differences in the curve pattern, however: the ISDN concentrations were overestimated throughout the course of the experiment, and the predicted MPH level rose faster than the observed one.

These differences might reflect the fact that the *in vivo* flux and lag time were different from the *in vitro* values. Next, the plasma concentration data were fitted to Eqs. 1 and 9 with the disposition parameters fixed, and the obtained permeation parameters and fitting curves are shown in Table III and Fig. 4 (solid curves), respectively.

TABLE III. *In Vivo* Skin Permeability of ISDN and MPH

	Flux ($\mu g/cm^2/h$)	Lag time (h)
ISDN from aqueous suspension	13.96[a] ±1.179	0.9828 ±0.3426
ISDN from Frandol® Tape S	8.406[a] ±0.07249	1.245 ±0.04298
MPH from aqueous suspension	57.67 ±4.076	7.327[a] ±0.8480

Each value represents the mean±S.D. *a*) Significantly different from the corresponding *in vitro* value ($p<0.05$).

The obtained flux of ISDN was lower and lag time of MPH was higher than the corresponding *in vitro* values (Table I, $p<0.05$).

One important difference between *in vitro* and *in vivo* conditions is thickness of the dermis. Under general *in vitro* conditions, a drug must pass through the thicker dermis, and the permeation resistance decreases the permeability of the lipophilic drug.[12] However, the *in vitro* flux of ISDN was higher than the *in vivo* and the lag time was almost the same. The other difference is in the condition of the dermis (receiver) side of the skin, namely the presence of bulk water *in vitro*. The bulk water enhances skin hydration, and consequently the fluidity of lipids in the stratum corneum increases.[13] The difference in permeability of ISDN might thus have resulted from this factor.

The water present in the receiver cell also lowers the influx of water due to the increase in the opposite direction of osmotic pressure. It may cause a decrease in the apparent permeability of a hydrophilic drug because of low convective flow.[14] On the other hand, the affinity of skin for a hydrophilic drug should be raised by the hydration. The difference in MPH permeability might be produced by a balance between net water flux and hydration of the skin.

The reasons for *in vitro–in vivo* differences in skin permeability may exist in the pharmacokinetic model used in this study. In the model for *in vivo* percutaneous absorption, a sink condition was assumed at the dermis side of the skin. If the *in vivo* skin permeation is limited by blood flow rate, the permeability of lipophilic drugs such as ISDN is underestimated.

Further, there was an effect of formulation on the *in vitro–in vivo* correlation for percutaneous absorption: *i.e.*, the discrepancy between *in vitro* and *in vivo* for Frandol® Tape S was greater than that for ISDN aqueous suspension. This was due to the relatively low *in vivo* permeability of ISDN for the tape. Since Frandol® Tape S is a semi-solid formulation consisting of a pressure sensitive adhesive matrix, the contact surface with skin, and hence the permeability of a drug, may be sensitively changed by the conditions under which it is applied. The releasing rate of ISDN from the matrix may be affected by the skin hydration. There are more factors affecting *in vitro–in vivo* correlation for percutaneous absorption from semi-solid formulations than solutions.

In conclusion, *in vitro* preparation of skin is a good model for the prediction of *in vivo* percutaneous absorption. However, researchers on percutaneous absorption should be mindful that there are some differences between *in vitro* and *in vivo* absorption.

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