An Application of the Hydrodynamic Pore Theory to Percutaneous Absorption of Drugs

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Received May 27, 1993; accepted November 8, 1993

Skin permeability of drugs was evaluated based on the hydrodynamic pore theory. Four polar solutes were used, with differing molecular sizes—ethylene glycol, 1,3-butylene glycol, antipyrine and sucrose—and isosorbide dinitrate was also selected as a lipophilic drug. The skin permeations of solvent (D_2O) and one of these drugs were measured simultaneously under various osmotic pressures to calculate the reflection coefficient. The clearance of isosorbide dinitrate was independent of the solvent flux, whereas a linear relationship was obtained between the solvent flux and the clearance of each hydrophilic drug except for sucrose. The reflection coefficient of the hydrophilic drugs increased with increasing molecular radius. These results suggest that the convective flow contributes significantly to the total skin permeability of hydrophilic drugs and that the extent of contribution decreases with increasing molecular size of the drugs. The pore radius of the skin barrier could be estimated from the reflection coefficient of the hydrophilic drugs and the resulting value was compared with that for the other absorption sites, jejunum, rectum, and nose. The apparent water influx was also compared to assess the volume occupied by the pores. The pore radius and apparent influx of skin were lower than those for the other absorption sites, which is apparently one reason for low skin permeability of drugs, especially hydrophilic drugs.

KEY WORDS: percutaneous absorption; skin permeability; hydrodynamic pore theory; reflection coefficient; pore size.

INTRODUCTION

A volume flow of water has an effect on the intestinal absorption of a drug. The phenomenon has been quantitatively described using hydrodynamic theories (1–3) by Kedem and Katchalsky (4). The diffusion and ultrafiltration processes in peritoneal transport have also been analyzed according to the pore theory of transcapillary exchange (5). In contrast, the contribution of volume flow to percutaneous absorption has always been ignored because of the poorer permeability of water through the skin compared with other absorption sites. Reportedly, however, the convective flow markedly contributed to ion transport across skin during iontophoresis (6). Cotransport of estradiol and ethanol was observed and this may also be due to volume flow (7). Therefore, the skin permeation of drugs should be analyzed based on the pore theory under conditions of both diffusion and convection to understand thoroughly the process of transport through the skin.

In the present study, we attempted to analyze the skin permeability of drugs by the hydrodynamic pore theory. Polar solutes with a variety of molecular radii, namely, ethylene glycol (EG), 1,3-butylene glycol (BG), antipyrine (ANP), and sucrose (SR), were used as models for hydrophilic drugs to assess the influence of molecular size on the convective transport. Isosorbide dinitrate (ISDN), a comparably lipophilic drug, was also used to elucidate the relationship between convective flow and drug lipophilicity. Deuterium oxide (D_2O) was used as an alternative to water. The skin permeations of D_2O and one of these drugs were measured simultaneously under various osmotic pressures, and data obtained were analyzed according to the hydrodynamic pore theory to give some hydrodynamic parameters. Based on the parameter values, the morphology of the skin barrier was discussed in comparison with that of other absorption sites.

THEORY

In previous reports (8–10) we proposed a parallel permeation pathway model for skin permeation of drugs. In this model, the stratum corneum is assumed to be a membrane having lipid and pore pathways. The lipid pathway is the main permeation route for lipophilic drugs and the drug permeation can be described by the solution-diffusion theory, whereas the pore pathway is the main route for hydrophilic drugs and is explained by the pore theory. Based on this model, the net flux of a drug at steady state (J) can be described as the sum of each flux via the two pathways:

$$J = J_L + J_P \tag{1}$$

where subscripts L and P indicate lipid and pore pathways.

The net flux of lipid pathway (J_L) is mathematically expressed by Eq. (2) based on Fick's first law:

$$J_L = CL_L (C_D = C_R) \tag{2}$$

where CL is the permeation clearance of drug (μL/hr), which means the product of its permeability coefficient and the application area of skin, and C is the drug concentration (mg/mL); subscripts D and R indicate donor (stratum corneum side) and receiver (dermis) so that $C_D - C_R$ represents the concentration differential of the drug across the skin. 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, Eq. (2) can be simplified to

$$J_L = CL_L C_o \tag{3}$$

where C_o is the initial concentration of drug in the donor phase.

On the other hand, Kedem and Katchalsky (4) 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 as follows:

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0724-8741/94/0500-0654$07.00/0

$$J_P = CL_P (C_D - C_R) + (1 - \delta)J_V \quad (C_D + C_R)/2 \quad (4)$$

where δ and J_V are the reflection coefficient of the drug and the net flux, respectively. In this equation, however, C_D should be very close to C_R. Then we have assumed the J_P by referencing their theory as follows:

$$J_P = CL_P C_o + (1 - \delta)J_{solvent} C_o \quad (5)$$

where $J_{solvent}$ is the solvent flux (μL/hr). The reflection coefficient is an index of the ability of the barrier to discriminate between solute and solvent. If the barrier is impermeable to the solute, then $\delta = 1$; if the barrier is equally permeable to solute and solvent, then $\delta = 0$. All intermediate cases are $0 < \delta < 1$. Clearly, the reflection coefficient reflects the contribution of convective flow to the total permeability of solute through the barrier.

From Eqs. (1), (3), and (5), the following equation can be obtained:

$$J = CL_L C_o \pm CL_P C_o + (1 - \delta)J_{solvent} C_o \quad (6)$$

If a parameter, CL_{drug} (cm^3/min), is assumed to be J/C_o, Eq. (6) becomes

$$CL_{drug} = CL_L + CL_P + (1 - \delta)J_{solvent} \quad (7)$$

Equation (7) indicated that the relationship between CL_{drug} and $J_{solvent}$ is linear and that δ can be calculated from the slope of the regression line. D_2O and H_2O were used as the donor and the receiver solvent, respectively, in the experiment, so J_{D_2O} was used instead of $J_{solvent}$ as below. A few more assumptions were made to use Eq. (7) for analysis: a sink condition of D_2O in the receiver and negligible efflux of H_2O.

EXPERIMENTAL

Materials

EG, BG, ANP and SR were purchased from Tokyo Chemical Industry Co. (Tokyo). ISDN was kindly supplied by Toko Pharmaceutical Industries Co. (Tokyo). D_2O and inulin were obtained from Merck Co. (Darmstadt, Germany) and Wako Pure Chemical Industrial Co. (Osaka, Japan), respectively. All these drugs and chemicals were used without further purification. Other chemicals and solvents were of reagent grade and obtained commercially.

Skin Permeation Procedure

Abdominal skin was freshly excised from male WBN/ILA-Ht rats (Ishikawa Laboratory Animals, Saitama, Japan), aged 6 to 7 weeks, and mounted between two half-diffusion cells with a water jacket connected to a water bath at 37°C, each having a 2.5-mL volume and a 0.966-cm^2 effective diffusion area (11). The receiver compartment was filled with distilled water and the donor compartment with D_2O containing a drug and inulin. Application amounts were 1 mg/mL for ANP and ISDN, 10 mg/mL for EG and BG, and 100 mg/mL for SR. Inulin was used as a volume marker, at 0.2 mg/mL. The osmotic-pressure differential was adjusted to 3.08, 0, and −3.08 osmol/L by the addition of NaCl to donor or receiver compartments. The donor and receiver solutions were stirred with star-head bars driven in each compartment by a constant-speed synchronous motor throughout the permeation experiment (11). Twelve hours after the start of the experiment, the donor and receiver solutions were replaced with fresh solutions. After 6 hr, a 2.2-mL aliquot of each solution was withdrawn, and the concentrations of drug, D_2O, and inulin were determined. In the other experiments, a 0.2-mL sample was withdrawn from the receiver at 1-hr intervals and the same volume of fresh receiver solution was added to keep the volume constant.

Analytical Methods

ANP and ISDN were assayed by HPLC as described previously (8). EG and BG concentrations were determined by gas chromatograph (GC) according to the method of Nadeau and Oaks (12) with a minor modification. Determination of SR was carried out by GC-FID after trimethylsilylation (13). D_2O was quantified by measuring the intensity of the O–D stretching vibrational band at 2512 cm^{-1} in infrared spectroscopic spectra (14). The modified coloring method of Harrison was used for analysis of inulin (15). Each assay method was sensitive enough for the following assay.

Data Analysis

The permeation clearance (CL) was calculated using the following equation:

$$CL = (dC_R/dt)V_R/C_D \quad (8)$$

where V_R is the volume of the receiver solution and t is time.

The significance of each correlation was analyzed by the correlation analysis. The statistic significance was evaluated by one-tail t test. Probability values of <0.05 were considered significant. The reflection coefficient of the drug was calculated from the slope of the regression line.

The pore size of the pore pathway was estimated following the Levitt equation (16):

$$= 16/3\ (a/R)^2 - 20/3\ (a/R)^3 + 7/3\ (a/R)^4 - 0.354\ (a/R)^5 \quad (9)$$

where a and R are drug and pore radius, respectively. This equation can be used to calculate the pore radius of several mucosae (17).

RESULTS AND DISCUSSION

Effect of Water Volume Flux on Skin Permeability of Drugs

Table I shows the molecular weight, molecular radius, and solubility in water of the model drugs used in the present study. To evaluate the skin permeability of D_2O and drug at a steady state, the time dependence of the permeation was investigated. Time courses of flux of D_2O and permeation clearance of ANP from 12 to 18 hr after the start of the permeation experiments under various osmotic pressures are illustrated in Fig. 1. These values under each osmotic pressure did not change with time, suggesting that steady-state permeation was achieved during this period. The amounts of D_2O and ANP permeated were so low that a sink condition of these compounds was maintained in this experimental period. Volume change by measuring the inulin con-

Table I. Physicochemical Properties of Model Compounds Used in This Study

	MW	MR[a] (nm)	Solubility in water
Isosorbide dinitrate (ISDN)	236.14	0.23–0.43	Slightly soluble
Ethylene glycol (EG)	62.07	0.14–0.23	Freely soluble
1,3-Butylene glycol (BG)	90.02	0.22–0.26	Freely soluble
Antipyrine (ANP)	188.23	0.26–0.52	Freely soluble
Sucrose (SR)	342.30	0.26–0.63	Freely soluble

[a] Molecular radius (maximum − minimum) was calculated by molecular model.

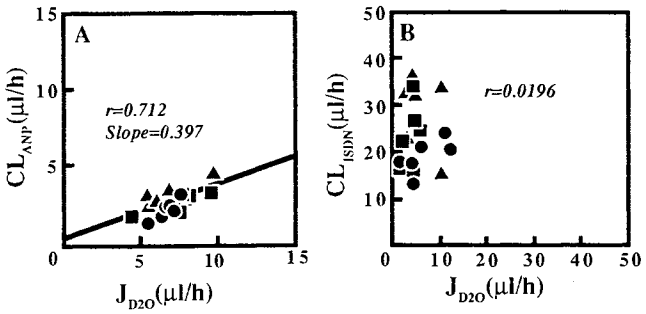

Fig. 2. Relationship between permeation clearance of ANP (CL_{ANP}) (A) and ISDN (CL_{ISDN}) (B) together with influx of D_2O (J_{D_2O}): (●) 3.08 osmol/L; (▲) 0 osmol/L; (■) −3.08 osmol/L. The line was obtained by linear regression analysis.

centration in donor compartment was negligible. Then the following experiments were carried out using the mean values of permeation clearance from 12 to 18 hr. No clear difference of $J_{solvent}$ and CL_{ANP} among three osmotic-pressure differentials was found, probably due to the relatively high interspecies difference, which suggests that osmotic-pressure change did not impair the barrier function of skin.

Figure 2 shows the relationship between the permeation clearance of ANP and ISDN and D_2O flux. A linear relationship existed between ANP clearance and D_2O flux (γ = 0.712; $P < 0.05$), whereas ISDN clearance was independent of D_2O flux. The results cannot be explained by the extent of reflection from the pore pathway, because both drugs showed similar reflection coefficients due to their similar molecular radii. We have already reported that lipophilic drugs such as ISDN permeate the skin primarily via the lipid pathway (8,9). In general, the contribution of this pathway is higher than that of the pore pathway due to the skin's low porosity (10). The extent of permeation of ISDN via the lipid pathway is so large that the convective flow cannot be found. This was supported by the fact that the mean CL_{ISDN} was 10 times higher than the mean CL_{ANP}.

Similar permeation studies of the hydrophilic compounds, EG, BG, and SR, were performed to examine whether an effect of molecular size on convective transport was observed (Fig. 3). The permeation clearances of EG and BG significantly correlated with D_2O flux (γ = 0.877, $P < 0.05$, in EG and γ = 0.816, $P < 0.05$, in BG), suggesting some contribution of convective flow to the total skin permeability. On the other hand, no significant correlation was obtained in SR. The reflection coefficient of hydrophilic drugs was calculated from the slope of each regression line. Figure 4 shows a relationship between the coefficient and the mean molecular radius. As shown, the reflection coefficient increased with increasing molecular size. Molecular size dependence of the diffusivity of a solute in a medium is well-known (18). Convective transport also seems to be influenced by molecular size because the reflection coefficient has a tendency to increase with an increase in the molecular radius [Eq. (9)]. In SR, the biggest solute in the present

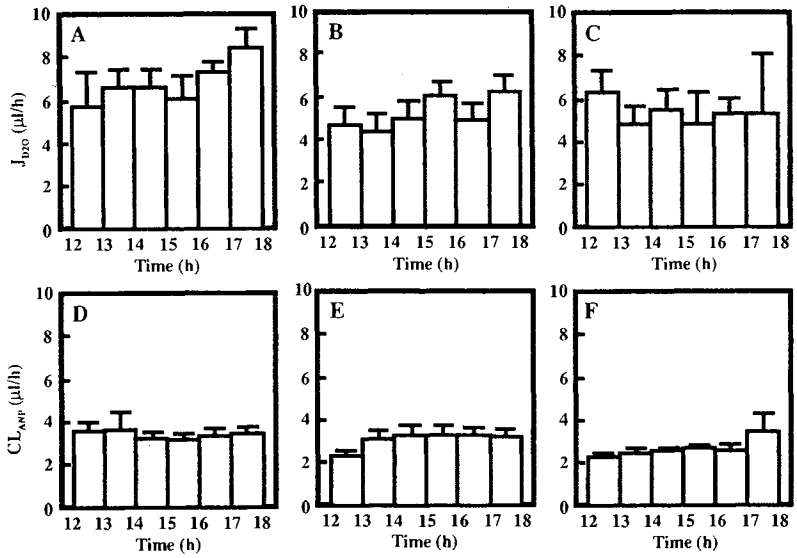

Fig. 1. Time courses of influx of D_2O (J_{D_2O}) (A–C) and permeation clearance of ANP (CL_{ANP}) (D–F) under various osmotic-pressure differentials. A and D, 3.08 osmol/L; B and E, 0 osmol/L; C and F, − 3.08 osmol/L. Each column represents the mean ± SE of three experiments.

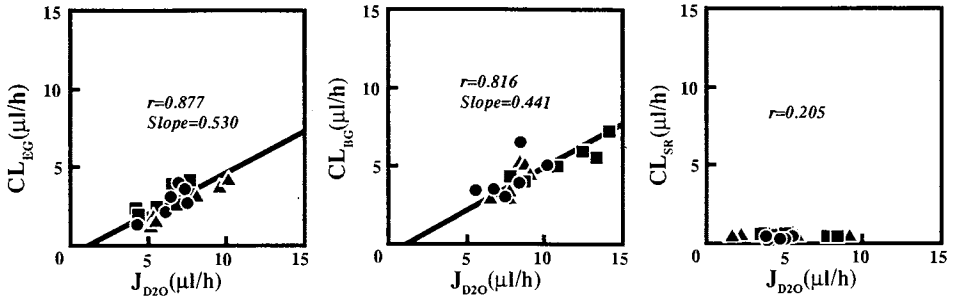

Fig. 3. Relationship between permeation clearance of EG (CL_{EG}) (A), BG (CL_{BG}) (B), and SR (CL_{SR}) (C) together with influx of D_2O (J_{D_2O}): (●) 3.08 osmol/L; (▲) 0 osmol/L; (■) −3.08 osmol/L. Each line was obtained by linear regression analysis.

study, the extent of reflection from the pore pathway may be too large to observe the convective transport.

Comparison of the Morphology of Skin with that of Other Absorption Sites

The hydrodynamic parameters obtained in the present study can provide some morphological information on the skin barrier. Levitt (16) proposed an equation describing the relationship among solute radius, pore radius, and reflection coefficient [Eq. (10)]. Because the solute radius and reflection coefficient are known, the pore radius of the pore pathway can be calculated using the Levitt equation. Then the pore size of skin barrier was calculated to be 0.378–1.576 nm based on the mean and SE range of the reflection coefficient and the minimum and maximum radii of all drugs. The pore pathway may be a hydrophilic aqueous domain near the polar region of the lipid bilayer or near the keratin of cornified cells as postulated by Flynn (19) and Barry (20) due to its size (nanometer order).

The pore size in skin was further compared with that in the other absorption sites. Hayashi *et al.* (17) have reported on the pore size of the jejunum, rectum, and nose calculated based on the modified Levitt equation from *in situ* absorption data of ANP by Karino *et al.* (3), Nishihata *et al.* (21), and Hirai *et al.* (22), respectively. We again calculated the pore radius from these data based on the Levitt equation. The pore size of skin was also calculated from the reflection coefficient of ANP. Apparent water influx was compared among the absorption sites to evaluate the volume occupied by the pores in the absorption sites. The apparent water influx in isotonic perfusion was used for the jejunum, rectum, and nose, whereas the permeation clearance of D_2O without any osmotic pressure differential was used for skin. The pore size of skin (0.38–1.58 nm) and nose (0.36–0.96 nm) was smaller than that of jejunum (0.73–2.00 nm) and rectum (0.60–2.00 nm). On the other hand, apparent water influx (value per 5 cm^2 of application area) in the nose, jejunum, rectum, and skin decreased in that order (111.2 ± 15.5, 32.5 ± 2.2, 15.9 ± 2.4, and 0.606 ± 0.022 μL/min, respectively). Small pore size and volume occupied by the pores seem to result in a lower permeability of drugs through skin, especially of hydrophilic drugs, than through other absorption sites.

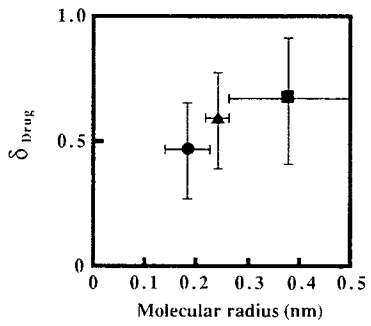

Fig. 4. Relationship between the reflection coefficient and the mean molecular radius of hydrophilic drugs. (●) EG; (▲) BG; (■) ANP.

In the present study, the skin permeability of drugs was evaluated based on the hydrodynamic pore theory. Skin is relatively impermeable to drugs, especially hydrophilic drugs, owing primarily to small pore size and volume. However, the problem may be solved by using a solvent having a high skin permeability because of the significant contribution of the convective flow to the total skin permeability of a drug. Further application of the hydrodynamic pore theory to cases with solvents other than water is desired.

REFERENCES

1. N. Lifson, L. M. Gruman, and D. G. Levitt. Diffusive-convective models for intestinal absorption of D_2O. *Am. J. Physiol.* 215:444–454 (1968).
2. H. Ochsenfahrt and D. Winne. The contribution of solvent drag to the intestinal absorption of tritiated water and urea from the jejunum of the rat. *Naunyn-Schmiedeberg Arch. Pharmacol.* 281:175–196 (1974).
3. A. Karino, M. Hayashi, S. Awazu, and M. Hanano. Solvent drag effect in drug intestinal absorption. II. Studies on drag effect in drug absorption clearance and water influx. *J. Pharmacobio-Dyn.* 5:670–677 (1982).
4. O. Kedem and A. Katchalsky. Thermodynamic analysis of the permeability of biological membranes to non-electrolytes. *Biochim. Biopys. Acta* 27:229–246 (1958).
5. E. Nakashima, F. Ishikawa, H. Sato, Y. Deguchi, I. Tamai, R. Matsushita, F. Ichimura, and A. Tsuji. Kinetics of peritoneal drug transport in rats. An application of the pore theory of transcapillary exchange. *J. Pharm. Sci.* 77:481–488 (1988).
6. V. Srinivasan and W. I. Higuchi. A model of convective solvent flow. *Int. J. Pharm.* 60:133–138 (1990).
7. P. Liu, T. Kurihara-Bergstrom, and W. R. Good. Cotransport of estradiol and ethanol through human skin in vitro. Understanding the permeant/enhancer flux relationship. *Pharm. Res.* 8:938–944 (1991).
8. T. Hatanaka, M. Inuma, K. Sugibayashi, and Y. Morimoto. Prediction of skin permeability of drugs. I. *Chem. Pharm. Bull.* 38:3452–3459 (1990).

9. Y. Morimoto, T. Hatanaka, K. Sugibayashi, and H. Omiya. Prediction of skin permeability of drugs: Comparison of human and hairless rat skin. *J. Pharm. Pharmacol.* **44**:634–539 (1992).
10. T. Hatanaka, K. Sugibayashi, Y. Morimoto, K. Katayama, and T. Koizumi. Prediction of skin permeability of drug based on single and parallel permeation pathway models (submitted for publication).
11. M. Okumura, K. Sugibayashi, K. Ogawa, and Y. Morimoto. Skin permeability of water-soluble drugs. *Chem. Pharm. Bull.* **37**:1404–1406 (1989).
12. H. G. Nadeau and D. M. Oaks. Determination of ethylene and propylene glycols in mixtures by gas chromatography. *Anal. Chem.* **32**:1760–1762 (1960).
13. G. G. S. Dutton, K. B. Gibney, G. D. Jensen, and P. E. Reid. The simultaneous estimation of polyhydric alcohols and sugars by gas-liquid chromatography. *J. Chromatogr.* **36**:152–162 (1968).
14. V. Thorton and F. E. Condon. Infrared spectrometric determination of deuterium oxide in water. *Anal. Chem.* **22**:690–691 (1959).
15. H. E. Harrison. A modification of the diphenylamine method for determination of inulin. *Proc. Soc. Exp. Biol. Med.* **49**:111–114 (1942).
16. D. G. Levitt. General continuum analysis of transport through pores I. Proof of Onsager's reciprocity. *Biophys. J.* **15**:533–551 (1975).
17. M. Hayashi, T. Hirasawa, T. Muraoka, M. Shiga, and S. Awazu. Comparison of water influx and sieving coefficient in rat jejunal, rectal and nasal absorptions of antipyrine. *Chem. Pharm. Bull.* **33**:2149–2152 (1985).
18. R. W. Baker and H. K. Lonsdale. Controlled release. Mechanisms and rates. In A. C. Tanquary and R. E. Lacey (eds.), *Controlled Release of Biologically Active Agents,* New York, 1974, pp. 15–72.
19. G. L. Flynn. Mechanism of percutaneous absorption from physicochemical evidence. In R. L. Bronaugh and H. I. Maibach (eds.). *Percutaneous Absorption.* Marcel Dekker. New York, 1989, pp. 27–51.
20. B. W. Barry. Lipid-protein partitioning theory of skin penetration enhancement. *J. Control. Release* **15**:237–248 (1991).
21. T. Nishihata, J. H. Rytting, and T. Higuchi. Enhanced rectal absorption of theophylline, lidocaine, cefmetazole, and levodopa by several adjuvants. *J. Pharm. Sci.* **71**:865–868 (1982).
22. S. Hiral, T. Yashiki, T. Matsuzawa, and H. Mima. Absorption of drugs from the nasal mucosa of rat. *Int. J. Pharm.* **7**:317–325 (1981).