Noninvasive Characterization of Regional Variation in Drug Transport into Human Stratum Corneum *in Vivo*

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Purpose. To investigate the mechanisms underlying the regional variations in drug transport into human stratum corneum (SC) of two model compounds of different lipophilicity and molecular size, 4-cyanophenol (CP) and cimetidine (CM), *in vivo* by non-invasive, quantitative attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy.

Methods. Saturated solutions of CP and CM were applied to the skin surface of eleven Chinese men, at five anatomical sites, including forearm, back, thigh, leg, and abdomen, for 10–15 min and 3–5 h, respectively. After the skin surface was cleansed of remaining chemicals, the SC was tape-stripped sequentially up to 20 times, and the drug concentration profiles in the tape-stripped SC were determined using ATR-FTIR spectroscopy. Thickness of the SC was estimated simultaneously using two-point measurements of transepidermal water loss before and after completion of tape stripping. Estimation of partition, diffusion, and permeability coefficients was achieved by analysis of the data using the unsteady-state diffusion equation.

Results. The rank orders of regional variation in partition and diffusion coefficients of CP and CM were different. The rank order of regional variation in permeability coefficients was similar for both drugs and decreased in the order of back > forearm > thigh > leg ≥ abdomen, but the variation was more prominent for CM.

Conclusions. Regional variation in SC transport of CP was mainly influenced by its intrinsic diffusivity across the SC, whereas variation in transport of CM could be attributed to both thermodynamic and kinetic differences among different anatomical skin sites.

KEY WORDS: regional variation; stratum corneum; drug transport; ATR-FTIR; transepidermal water loss.

INTRODUCTION

The human skin displays remarkable regional variation in percutaneous absorption of various molecules. Feldmann and Maibach (1) demonstrated that the skin absorption of hydrocortisone was the highest in the scrotum, followed by the forehead, axilla, scalp, and abdomen, and was lowest in the foot area. Scheuplein and Blank (2) have suggested the following order: plantar > palm > dorsum of the hand > scrotum and postauricular > axilla and scalp > arm, leg, and trunk, for the diffusion of simple, small molecules through the skin. Other studies (3–6) have repeatedly demonstrated regional differences with respect to the extent of percutaneous absorption of chemicals, such as pesticides, polycyclic aromatic hydrocarbons, benzoic acid and sodium salt, caffeine, and acetylsalicylic acid, in somewhat different orders. Given that the permeability barrier for both hydrophilic and amphipathic molecules resides in the outermost stratum corneum (SC) layer of the skin (2,7), studies on drug transport characteristics into the SC at different anatomical sites should explain the mechanisms responsible for the regional differences in the percutaneous absorption observed for various molecules.

Attenuated-total-reflectance Fourier-transform-infrared (ATR-FTIR) spectroscopy has been exploited as a fast and versatile tool to characterize the molecular components of the skin surface, and to qualitatively and quantitatively measure SC uptake in humans *in vivo* by direct placement of the subject's arm onto the optics. Such procedures have been used to investigate the distribution of water, lipids and proteins in the SC (8,9), to study the action of penetration enhancers, and to track the diffusion of a model compound (4-cyanophenol) as a function of the formulation used (10–12). More recently, the techniques have been improved by monitoring the amount of chemical in sequentially tape-stripped layers of the SC after brief exposure (13). In conjunction with the general unsteady-state diffusion model developed for data analysis, the ATR-FTIR technology should permit the prediction of the rate and extent of drug absorption across the SC at different anatomical sites. The model has been further applied to evaluate and quantify SC uptake from volatile and non-volatile solvents (14) and to determine the bioavailability of terbinefine in human SC from different topical formulations (15).

This study was undertaken to analyze in humans *in vivo*, using noninvasive ATR-FTIR technology along with concomitant transepidermal water loss (TEWL) measurements to estimate SC thickness and the drug transport characteristics of the SC at five anatomical sites. Specifically, the study was designed to compare the transport parameters governing the SC permeability of two model compounds of different lipophilicity and molecular size. In addition to the model compound, 4-cyanophenol (CP, MW = 119, $\log K_{O/W} = 1.6$), which has been reported in the previous studies, cimetidine (CM, MW = 252, $\log K_{O/W} = 0.4$) was chosen for its IR signature, especially the intense C ≡ N stretching absorbance at 2170 cm^{-1}. The results provide insight into the mechanisms governing regional variation in the skin absorption of molecules with different physicochemical properties *in vivo* in humans.

MATERIALS AND METHODS

Materials

CP and CM were purchased from Sigma-Aldrich Fine Chemicals (St. Louis, MO). Their chemical structures and physicochemical properties are shown in Table I. The patch for topical applications of CP and CM solutions consisted of a Soft-wick IV-sponge (8 × 2 cm, Johnson and Johnson Medical, Arlington, TX), a polyester film (9 × 3 cm, Scotchpak, 3M, St. Paul, MN), and a nonocclusive transparent dressing (10 × 12 cm, Tegaderm 1626, 3M). Polypropylene tape

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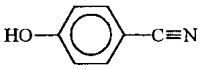
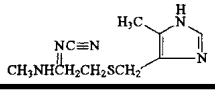
TABLE I. Chemical Structure and Physicochemical Properties of CP and CM

Molecule	Chemical structure	log $K_{O/W}$	MW	Permeability coefficient[a] (cm/h)
4-Cyanophenol		1.6	119	$4.63*10^{-3}$
Cimetidine		0.4	252	$1.0*10^{-4}$

[a] Estimated according to log P (cm/s) = −6.3 + 0.71 × log $K_{O/W}$ − 0.0061 × MW (16).

(Scotch™ brand No. 845, 3M) (17) was used for sequential removal of SC by stripping.

ATR-FTIR Spectroscopy

ATR-FTIR spectra were recorded using a Nicolet Magna 560 FTIR spectrometer (Madison, WI, USA) equipped with a Whatman Model 74-45 FTIR purge gas generator (Haverhill, MA, USA), a liquid nitrogen-cooled mercury-cadmium-telluride detector, and OMNIC E.S.P. software for data acquisition and spectral analysis. The ATR sampling device (Contact Sampler, Spectra Tech, Shelton, CT, USA) was a horizontal internal reflection accessory with a ZnSe crystal (7 × 1 cm, 45°) of a refractive index of 2.4 at 1000 cm^{-1} and a transmission range of 20,000 – 650 cm^{-1}. In this study, each spectrum obtained was the average of 128 scans. Resolution was set at 4 cm^{-1}. Under these conditions, it required 2–3 min for acquisition of a spectrum over the wavelength range of 4000 – 650 cm^{-1}. Measurements were taken under ambient laboratory conditions with 20–25°C and 50–70% relative humidity.

Assay Validation

Amount of CP and CM on the adhesive tape obtained from progressive removal of the SC was directly analyzed by ATR-FTIR spectroscopy, as adapted from the procedures described by Pirot *et al.* (13). A spectrum was recorded with the adhesive tape placed (adhesive side down) onto the ZnSe crystal, and the integration of the C ≡ N stretching vibration absorbance in the spectral region between 2240 and 2210 cm^{-1} for CP (peak absorbance around 2225 cm^{-1}) and between 2220 and 2120 cm^{-1} for CM (peak absorbance around 2170 cm^{-1}) was performed. Intraday and interday reproducibility of the analytical methods was tested by spiking the adhesive matrix with variable amounts of CP or CM, according to their individual concentration ranges in the SC, and are summarized in Table II.

The relationship between absorbance and concentration of CP or CM and the attenuating effect of the presence of SC on the ATR-FTIR signal were evaluated using tapes containing typical amounts of stripped SC over the CP and CM concentration range identified, according to the relationship:

$$PA = A \times M \times e^{-B \times M_{SC}} \quad (1)$$

where PA is the C ≡ N peak area from CP or CM, M is the mass of CP or CM (nmol/cm^2), and M_{SC} is the mass (μg/cm^2) of SC on the tape-strip, the latter two both normalized by the tape area. The parameters A and B obtained by analyzing the tapes containing both various drug concentrations (CP, 3.0–95.9 nmol/cm^2, CM, 0.11–3.62 nmol/cm^2) and typical amounts of stripped SC (14.3–114.3 μg/cm^2) from all the volunteers were 0.0035 and 0.008 for CP (coefficient of determination = 0.95), and 0.95 and 0.019 for CM (coefficient of determination = 0.92), respectively. Pirot *et al.* (13) reported the parameters A and B for CP to be 0.004 and 0.014, and validated Eq. (1) using [^{14}C]-radiolabeled CP. Similar methodology was utilized to obtain the parameters for CM, but the equation has not been previously validated using radiolabeled CM.

Study Design

The *in vivo* studies were approved by the Committee on Human Research at the National Cheng Kung University. Eleven healthy Chinese volunteers (men, aged 22–25) with no history of dermatologic disease were enrolled following written informed consent. They were asked not to use any topically applied products after showering the night prior to the study day. Five body areas were chosen to evaluate the differences in drug transport properties: the ventral forearm, abdomen, back, thigh, and leg. Due to the hairy nature of skin

TABLE II. Assay Validation for CP and CM Content on the Tape Determined by ATR-FTIR Spectroscopy

	4-Cyanophenol			
	Intraday (n = 6)		Interday (n = 5)	
Concentration (nmole/cm^2)	Relative error (%)	CV (%)	Relative error (%)	CV (%)
3.0	18.9	23.5	−7.75	22.8
3.6	10.9	20.7	−7.95	22.4
6.0	−10.3	13.1	−5.26	11.0
12.0	−10.4	7.3	6.98	16.6
24.0	5.3	10.3	1.41	6.9
48.0	−0.7	4.9	−0.88	2.8
95.9	0.7	4.6	0.04	0.2
		Cimetidine		
	Intraday (n = 6)		Interday (n = 19)	
Concentration (nmole/cm^2)	Relative error (%)	CV (%)	Relative error (%)	CV (%)
0.11	18.90	4.9	−6.43	56.4
0.23	4.33	16.5	2.15	13.2
0.45	−8.34	26.7	−2.60	14.6
0.91	−0.86	16.8	2.37	11.9
1.81	1.43	14.8	−0.47	4.9
3.62	0.21	11.6	−0.03	0.5

on the thigh and the leg, which may interfere with the tape-stripping procedures, hair removal lotion (Nair, New York, NY) was used two days prior to the experiments to remove hairs on the two skin sites. The variations in barrier function at both sites before and two days after hair removal were within normal fluctuations as determined using TEWL measurement. On the study day, subjects were allowed to rest for at least 20 min in the laboratory prior to the start of the experiments to avoid excessive perspiration due to physical activity. After the skin surface was gently padded with sebum-absorbing tissues (Shan Hah, Japan), 500 μl of saturated aqueous solutions of CP (105 mM) and CM (19.8 mM) were applied to the skin for 10–15 min and 3–5 h, respectively, using the patch described earlier. At the end of the treatment period, the patch was removed, and the skin surface was gently cleaned and dried with gauze pad. The SC at the treated site was then quickly and progressively removed by repetitive adhesive tape-stripping up to 20 times in 2–3 min. The concentration profiles of CP or CM across the SC were determined according to the procedures described by Pirot *et al.* (13). Briefly, an ATR-FTIR spectrum of each tape-strip was immediately recorded after tape-stripping. After each reading, the ZnSe crystal was carefully cleaned with acetone to completely remove the chemicals of the previous reading. The mass of SC on each tape-strip was determined by weighing on a Mettler AE240 balance (sensitivity of 10 μg). The amount of CP or CM (nmol/cm^2) on each tape-strip was then calculated from their corresponding calibration curve. Assuming the SC density of 1 g/cm^3 and uniform distribution of SC on the tape-strips, the mass removed was converted to a volume, and thereafter an effective thickness of SC on each tape-strip was calculated. Thus, drug concentration in nmol/cm^2 on each tape-strip was transformed to units of molarity.

Estimation of Stratum Corneum Thickness

During the *in vivo* studies, both prior to the application of drug solutions and following the completion of treatment periods and tape-strippings, TEWL measurements were taken at four locations evenly distributed over the treated sites using Tewameter TM210 (Courage+Khazaka, Köln, Germany). SC thickness (L) was then calculated using two-point analysis following the equation described by Pirot *et al.* (18):

$$L = x \cdot TEWL_x/(TEWL_x - TEWL_0) \quad (2)$$

where x was the cumulative SC thickness removed by all the tape-strippings, and $TEWL_0$ was the averaged initial TEWL measured, and $TEWL_x$ corresponded to the averaged value of TEWL measured when x μm of SC have been removed.

Data Analysis

After the *in vivo* exposure of human skin at different anatomical sites to aqueous solutions of CP or CM (of known concentration C_{veh}) for 10–15 min or 3–5 h respectively, ATR-FTIR analysis of the sequentially tape-stripped SC yielded values for the drug concentration ($C(x)$) as a function of depth into the SC. Only the data points with measured drug concentration above the lowest concentrations used in the standard curves (CP: 3 nmol/cm^2, CM: 0.11 nmol/cm^2) were included in the analysis. Together with the SC thickness estimated from the above-described procedures, the data were then fitted into a solution to Fick's 2nd Law of diffusion given in Eq. 3 (13), under the boundary and initial conditions (Eqs. 4–6) of the experiments performed,

$$C(x) = KC_{veh}\left\{1 - \frac{x}{L}\right\} - \sum_{n=1}^{\infty}\frac{2}{n\pi}KC_{veh} * \sin\left(\frac{n\pi x}{L}\right)\exp\left(\frac{-Dn^2\pi^2 t}{L^2}\right) \quad (3)$$

$$C = C_0 = KC_{veh}, \text{ at } x = 0, t \geqq 0 \quad (4)$$

$$C = 0, \text{ at } x = L, t \geqq 0 \quad (5)$$

$$C = 0, \text{ at } 0 < x < L, t = 0 \quad (6)$$

where C_0 is the drug concentration at the skin surface (i.e., $x = 0$), D is the drug's diffusivity, and K is the SC/water partition coefficient of the drug, to yield values of K and D. Curve fitting was performed using a computer package (Scientist, MicroMath, Salt Lake City, UT) to obtain a best fit for the data collected. The drug permeability (P = KD/L) into the SC was then deduced.

Statistical Analysis

Statistical analysis of regional variation in the transport characteristics of CP and CM into human SC among the five anatomical sites was performed by Friedman's non-parametric analysis of variance by ranks using StatView software (Abacus Concepts, Berkeley, CA). Mann Whitney test was performed to compare the differences for the parameters between all pairs of sites.

RESULTS

Figure 1 A shows the concentration profiles of CP in the SC at five different anatomical sites from subject No. 1 after exposures to a saturated aqueous solution for 15 min. Each curve represents the best fit of Eq. 3 to the data obtained from the individual anatomical sites. Similarly, the CM data at the five sites from subject No. 6 after 5 h-exposures to a saturated aqueous solution are illustrated in Fig. 1 B. However, the first data point from each site in Fig. 1 B was not included in the regression analysis as significantly more amounts of SC were removed by the first tape-strip due to SC hydration after prolonged occlusion of the skin sites. Removal of the first data point significantly improved the goodness of fit in the regression analysis. Data are plotted as a function of depth within the SC (x) normalized by the total thickness of the SC (L) determined by the TEWL estimation. Assuming uniform distribution of the SC mass removed on each tape strip, the depth within the SC following removal of the nth tape strip was determined by cumulative SC thickness removed by all n tape-strips. The parameters K and D of CP and CM through the SC were determined by nonlinear regression of Eq. 3 to the data obtained at each anatomical site. Together with the SC thickness estimated using the TEWL method, the results of CP for the 8 subjects with data from all the sites successfully fitted are summarized in Table III. The other 3 subjects had reached steady-state transport of CP in 15 min at least one SC site, and hence were excluded from the data analysis. With the exception of K_{CP} ($p = 0.147$), the parameters determined, including D_{CP}, P_{CP} and L_{CP}, exhibit significant re-

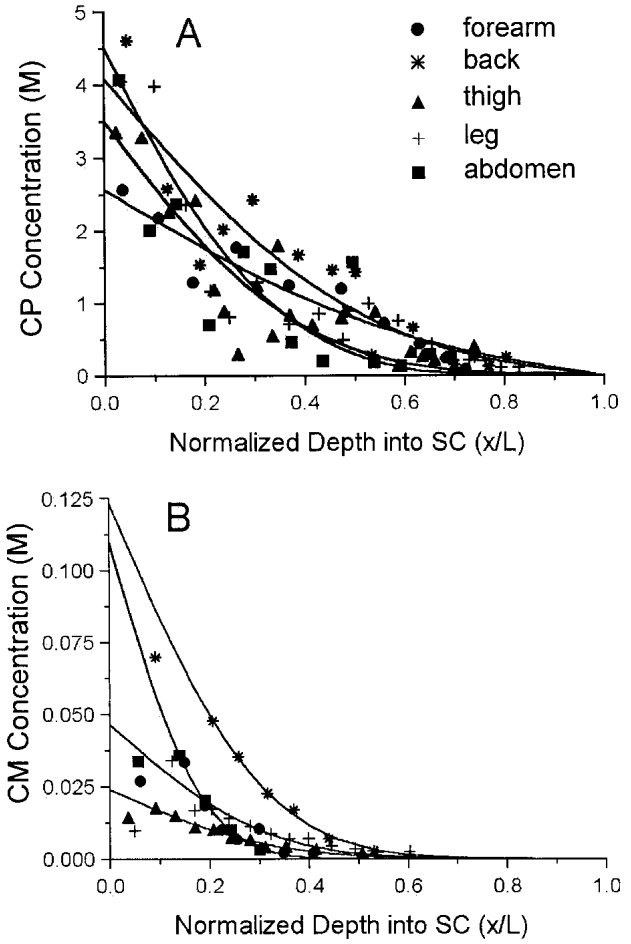


Fig. 1. Concentration profile of (A) CP and (B) CM as a function of normalized depth (x/L) into the SC at five skin sites after exposures of 15 min (CP) in subject No. 1 and 5 h (CM) in subject No. 6 to saturated aqueous solutions of the chemicals. The data points were experimentally determined. The lines through the results represent the best fits of Eq. (3) for each site.

gional variation ($p < 0.05$) among the five sites. Their rank orders are as follows:

K_{CP}: **back** > abdomen > thigh > forearm > *leg*
D_{CP}: **leg** > **forearm** > back > **thigh** > *abdomen*
L_{CP}: forearm = leg = thigh > back = abdomen
P_{CP}: **back** > forearm > thigh > *leg* > *abdomen*

where significant difference ($p < 0.05$) existed between any two sites shown in the **bold**, *italic* or ***bold plus italic*** fonts, while no significant difference ($p > 0.05$) was found between any two sites indicated with the same font or as the underlined vs. any other fonts. Differences in D_{CP} among the five sites varied 3-fold, and regional variation originates mainly from the abdomen, where its D_{CP} is statistically lower than the leg, forearm and thigh. Variation in P_{CP} among the five sites is less than 2-fold and arises mainly from the higher permeability at the back relative to the other four sites. Only the differences between the back and leg or the abdomen are statistically significant. The highest P_{CP} on the back was mainly due to a high K_{CP}, while the lowest P_{CP} on the abdomen was due to a low D_{CP} despite its K_{CP} not being the lowest and its SC being the thinnest.

The CM results for all the 11 subjects are summarized in Table IV. All the parameters determined, including K_{CM}, D_{CM}, P_{CM} and L_{CM}, exhibit significant regional variation ($p < 0.05$) among the five sites. Their rank orders are summarized as follows:

K_{CM}: **back** > *forearm* > *abdomen* > ***leg*** > ***thigh***
D_{CM}: **back** > **thigh** > **forearm** > **leg** > *abdomen*
L_{CM}: forearm = thigh = leg > back > *abdomen*
P_{CM}: **back** > *forearm* > *thigh* > *leg* = *abdomen*

where the fonts used to identify the significance of differences are the same as explained earlier. Differences in the K_{CM} were as large as 4-fold, being the highest on the back and the lowest on the thigh. All the differences in K_{CM} between any two sites were statistically significant except between forearm vs. abdomen and leg vs. thigh. Differences in D_{CM} were less than 3-fold, with the abdominal D_{CM} being statistically smaller than the other four sites, while D_{CM} was not statistically different among the four sites. Regional variation in P_{CM} ranged 5-fold among the five sites, with significantly greater permeability on the back in comparison with the other four sites, while P_{CM} was not statistically different among the four sites. The greatest permeability to CM on the back was attributed to the largest K_{CM} and the highest D_{CM} at this site.

Comparisons of regional variation in K, D and P in the SC between CP and CM are depicted in Fig. 2. The rank orders of regional variation in K_{CP}, D_{CP} and L_{CP} were different from K_{CM}, D_{CM} and L_{CM} respectively. The rank order of regional variation in P was similar for both compounds and their permeability decreased in the order of back > forearm > thigh > leg ≥ abdomen, but the variation was more prominent for CM ($p = 0.008$ for CP versus $p < 0.001$ for CM).

DISCUSSION

Regional variation in the transport of two model compounds into human stratum corneum was investigated using procedures slightly modified from a facile and minimally invasive methodology previously described (13). The total thickness of SC of different anatomical sites was calculated by two-points measurements of TEWL before and after the tape-stripping process (18). As shown in Table V, both data sets obtained from the CP and CM treatments on contralateral sites agreed with each other, except for the SC thickness at the back. Sebum level in the back region has been reported to be more than 10-fold higher than in the thigh and volar forearm, and is twice as high as that at the abdomen (19). On the skin surface, sebum spreads and forms a thin and structureless sheet, and its thickness is greater in sebum-rich areas (20). Since the sites of CM treatment have been occluded for 5 h in contrast to less than 15 min for CP treatment, it can be speculated that SC thickness on the back may be overestimated due to prolonged SC hydration in this sebum-rich region. Comparisons of these data with previously reported results employing different techniques are shown in Table V, and fair agreement is found despite the possibility of racial differences involved. Both the SC thickness at the abdomen and the back were thinner than the SC thickness at the forearm, thigh and leg. The cumulative SC thickness removed by tape-strippings ranged from 48–92% of total SC thickness in our studies. In contrast to the previous estimates of SC thickness based on the cumulative SC mass removed by 20 tape-

TABLE III. Characteristics of CP Transport into Human SC *in Vivo* at Different Anatomical Sites (n = 8)

Parameter	Site	Forearm	Back	Thigh	Leg	Abdomen	[a]p-value
K	Mean ± SD	29.9 ± 7.7	42.0 ± 11.6	31.8 ± 8.6	25.8 ± 9.4	34.3 ± 9.4	0.147
	Mean Rank	2.75	4.00	3.00	2.00	3.25	
L (μm)	Mean ± SD	11.1 ± 1.5	7.9 ± 1.6	10.4 ± 2.6	10.9 ± 1.8	7.6 ± 1.5	0.002
	Mean Rank	3.88	1.88	3.25	4.25	1.75	
D ($\times 10^7$ cm^2/h)	Mean ± SD	3.40 ± 1.97	2.43 ± 2.02	2.52 ± 1.94	2.63 ± 2.08	1.07 ± 0.61	0.022
	Mean Rank	3.56	3.25	3.06	3.75	1.38	
P ($\times 10^3$ cm/h)	Mean ± SD	8.65 ± 5.80	11.74 ± 8.13	6.46 ± 2.78	5.08 ± 1.84	4.49 ± 2.16	0.008
	Mean Rank	3.38	4.50	3.00	2.38	1.75	
Coefficient of determination from curve fitting (range)		0.73–0.94	0.86–0.95	0.60–0.98	0.71–0.96	0.73–0.96	

[a] p-value determined by Friedman non-parametric analysis of variance of the parameters among five sites.

strippings, these estimates lead to an increase in the accuracy for both parameters, K^*C_{veh} and D/L^2, derived from the curve-fitting. As shown in Table VI, the transport characteristics of CP in human SC of the ventral forearm in this study were compared with parameters reported in other studies. In general, those parameters agreed fairly well with one another. The largest discrepancy appeared for the K estimate, which was near 3-fold higher than that reported by Pirot *et al.* (13). For SC transport of CM in the ventral forearm, greater variation in $K_{SC/W}$ was found. Nonetheless, the obtained SC permeability ($3.40 \pm (2.53) \times 10^{-5}$ cm/h) was close to the theoretical prediction (1^*10^{-4} cm/h). The accuracy and precision of the ATR-FTIR approach for the assay of CM in the tape-stripped SC need to be checked in the future study to confirm those parameters obtained.

A recent study of Reddy *et al.* (23) suggested by mathematical simulations that tape-stripping procedures should preferably be conducted at least two different exposure times to prevent artifacts resulting from continued diffusion. Exposure times of 15 and 7 min to CP on contralateral arms of a single subject were performed, and parameters were compared with those determined using a single exposure time (15 min). It was found, when tape stripping procedures were accomplished under the authors experimental settings (<2 min), the rank order remained the same for P_{CP} with deviations up to 30% appearing for the values of K_{CP}, D_{CP}, and P_{CP}. Only the rank orders of leg, thigh, and forearm for K_{CP} and D_{CP} changed. The differences among the three sites for these parameters have been described earlier as not statistically significant. Consequently, conclusions drawn on the regional variation of drug transport characteristics in the SC in this study using a single exposure time should be valid and reliable.

Regional variations in SC lipid composition and TEWL measurements have been described (24,25). Holbrook and Odland (21) also found that the mean thickness and number of cell layers of the SC vary inter- and intra-individually with the anatomic sites. More recently, Schwindt *et al.* (22) demonstrated by TEWL techniques and tape stripping that water diffusion coefficient ($\times 10^9$D, cm^2/s) in the SC ranked in the order: back (2.34 ± 0.82) = forearm (2.54 ± 1.2) > thigh (2.27 ± 1.2) > abdomen (1.38 ± 0.42). They used a K of 0.162 in the calculation regardless of possible variation due to different anatomical sites. As a result, the rank order of water diffusion coefficient they obtained was exactly the same as the rank orders of P_{CP} and P_{CM} reported here. The diffusion coefficients of water, CP, and CM appeared to be the lowest at the abdomen, the same site.

The permeability barrier of the skin is regulated by a series of lipid multilayers segregated within the SC interstices (26). To account for regional variations in percutaneous transport, Elias *et al.* (27) correlated the thickness, the number of cell layers, and the lipid content of leg vs. abdominal SC samples with penetration of water and salicylic acid. They demonstrated an inverse correlation between penetration of water, and to a lesser extent of salicylic acid, and lipid content of the leg (3.0%) vs. abdominal (6.8%) SC (neither the number of cell layers nor the thickness of the SC correlates with the observed differences in permeability). Our results also confirmed *in vivo* that the abdomen is the least permeable site to both molecules despite its SC being the thinnest among the five sites, and the influence of SC lipid content on the percu-

TABLE IV. Characteristics of CM Transport into Human SC *in Vivo* at Different Anatomical sites (n = 11)

Parameter	Site	Forearm	Back	Thigh	Leg	Abdomen	[a]p-value
K	Mean ± SD	6.49 ± 4.63	10.08 ± 5.45	2.60 ± 1.11	3.33 ± 2.55	5.37 ± 2.51	<0.001
	Mean Rank	3.55	4.64	1.73	1.91	3.18	
L (μm)	Mean ± SD	11.4 ± 2.6	9.3 ± 1.3	10.8 ± 1.7	10.1 ± 1.6	7.8 ± 1.0	<0.001
	Mean Rank	4.09	2.59	3.96	3	1.36	
D ($\times 10^9$ cm^2/h)	Mean ± SD	6.87 ± 4.80	7.49 ± 4.54	7.58 ± 3.79	5.48 ± 2.85	2.80 ± 1.38	<0.006
	Mean Rank	3.18	3.82	3.64	2.82	1.55	
P ($\times 10^3$ cm/h)	Mean ± SD	3.42 ± 2.52	7.49 ± 4.88	1.78 ± 0.98	1.51 ± 0.76	1.80 ± 0.95	<0.001
	Mean Rank	3.46	4.73	2.46	2.18	2.18	
Coefficient of determination from curve fitting (range)		0.76–0.99	0.77–1.00	0.87–0.99	0.85–0.99	0.92–1.00	

[a] p-value determined by Friedman non-parametric analysis of variance of the parameters among five sites.

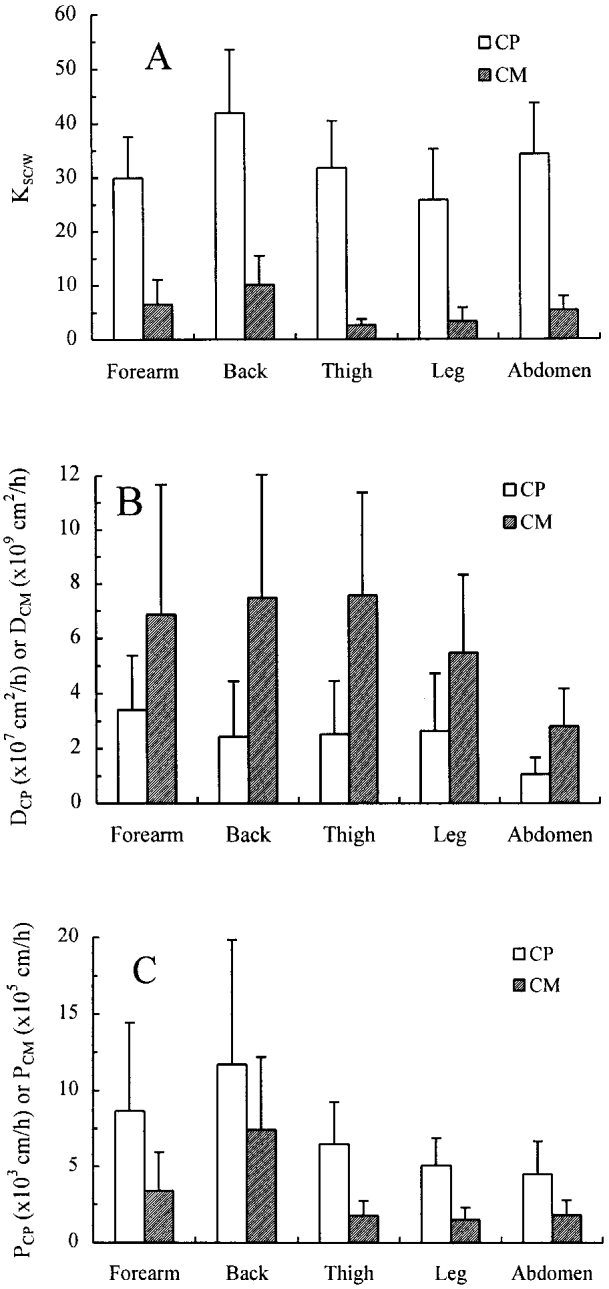


Fig. 2. Transport characteristics of CP (n = 8) and CM (n = 11) into human SC *in vivo* at different anatomical sites. (A) $K_{SC/W}$: $p < 0.05$ only for back vs. leg for CP; $p < 0.05$ except for forearm vs. abdomen and thigh vs. leg for CM. (B) diffusion coefficient: $p < 0.05$ for abdomen vs. the other sites, except back, for CP; $p < 0.05$ for abdomen vs. the other sites for CM. (C) permeability: $p < 0.05$ for back vs. leg and abdomen for CP; $p < 0.05$ for back vs. the other sites for CM. Data represents mean ± SD.

taneous absorption of the smaller and slightly more lipophilic CP was not statistically as significant as the effect on CM permeation. This further substantiates the findings by Elias *et al.* For the transport characteristics in the SC, the magnitude of regional variation in diffusion coefficient was comparable between CP and CM. On the other hand, with respect to the sensitivity of SC/water partitioning, our results demonstrated greater regional variation in CM than CP, presumably due to variations in the SC lipid content. This does not support the findings of Raykar *et al.* (28). In their *in vitro* studies using 21-esters of hydrocortisone varying in acyl-chain structure, they showed that the SC uptake of hydrophilic solutes is dominated by the protein domain, while lipid domain dominates the uptake of lipophilic solutes. They concluded that SC lipid content is an important determinant of the affinity of the SC for lipophilic solutes, but has no effect on the uptake of hydrophilic solutes. The greatest uptake of both CP and CM occurred on the back in this study. As the surface of the back skin is known to be abundant in sebum, whether this influenced the *in vivo* SC uptake of different molecules remained to be clarified. Taking into consideration both the thermodynamic and kinetic determinants for skin permeability, the overall permeability to CP and CM demonstrated a similar rank order among the five sites. The more pronounced regional variation in P_{CM} relative to P_{CP} was accounted for by the additional variation in SC/water partitioning for CM.

The uptake and transit through SC being the first step in percutaneous absorption, drug absorption from topical and transdermal applications also involves a sequence of events, including partitioning into the viable epidermis, diffusion through the epidermis and upper dermis, and capillary uptake (29). Other than the differences in SC permeability at various regions of the body, percutaneous absorption is known to be influenced by changes in skin integrity which may occur with gender, age, race, presence of disease, skin condition (e.g., occlusion, abrasion, temperature), and blood flow etc. (29,30). The observed magnitude of regional variation in SC permeability may not be equally reflected in systemic absorption. However, for transdermally delivered medications, it may be

TABLE V. Comparisons of Human SC Thickness at Different Anatomical Sites Measured in This Study with Literature data

Site	This study CP (n = 8)	This study CM (n = 11)	Ref. 1	Ref. 2 (n = 6)	Ref. 3 (n = 6)
Forearm	11.1 ± 1.5	11.4 ± 2.6	16	12.9 ± 3.8	12.3 ± 3.6
Back	7.9 ± 1.6	9.3 ± 1.3	10.5	9.4 ± 2.2	11.2 ± 2.6
Thigh	10.4 ± 2.6	10.8 ± 1.7		10.9 ± 3.1	13.1 ± 4.7
Leg	10.9 ± 1.8	10.1 ± 1.6			
Abdomen	7.6 ± 1.5	7.8 ± 1.0	15	8.2 ± 1.9	7.7 ± 1.7

Note: Ref. 1: Scheuplein and Blank (2). Ref. 2: Holbrook and Odland (21). Ref. 3: Schwindt *et al.* (22).

TABLE VI. Comparisons of CP Transport Characteristics into Human Forearm SC *in Vivo* Measured in This Study and Literature Data

Parameter	This study (n = 8)	Ref. 1 (n = 4)	Ref. 2 (n = 3)
K	29.9 ± 7.7	11.6 ± 7.8	8.4 ± 3.6
D/L^2 (h^{-1})	0.28 ± 0.17	1.7 ± 1.0	0.30 ± 0.05
L (μm)	11.1 ± 1.5	7.0 ± 2.8[a]	10–20[a]
D (×10^7 cm^2/h)	3.40 ± 1.97		3.13, 6.84, 12.6[b]
P = K*D/L (×10^3 cm/h)	8.65 ± 5.80	11 ± 4[a]	3.74 ± 0.94[c]

Note: Ref. 1: Stinchcomb *et al.* (14), Ref. 2: Pirot *et al.* (13).
[a] Determined as total SC thickness removed by 20 tape-strippings.
[b] Derived using L values of 10, 15, and 20 μm, respectively.
[c] Derived using L = 15 μm.

wise to avoid applications to the back region unless delivery rate through back skin has been provided. For topical medications, it is likely that the magnitude of differences may be further enlarged in patients under pathological skin conditions associated with impaired barrier function, such as atopic dermatitis, psoriasis, erythroderma, generalized eczema, and UV irradiation etc., especially when an extensive body area is involved in the back (30). It is recommended that care should be taken to adjust dosage of topically applied medication under such conditions to avoid intoxication.

In conclusion, this study demonstrated that ATR-FTIR is a useful technique to elucidate the transport mechanisms underlying the regional variation in drug transport through human SC *in vivo*. The data analysis differentiated the diffusion and partition components contributing to the observed penetration results, indicating that regional variation in SC transport of CP was mainly influenced by its intrinsic diffusivity in the SC, whereas the variation in SC transport of CM was attributed to both thermodynamic and kinetic differences among different anatomical skin sites.

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