J. Kor. Pharm. Sci., Vol.19, No.1, 39-46(1989)

Mechanism of Clonidine Permeation through Skin Based on Heterogeneous Structure

Young Ro Byun, Young Ha Kim and Seo Young Jeong†

Polymer Chemistry Laboratory, Division of Chemical Engineering and Polymer Science, Korea Advanced Institute of Science and Technology, Seoul 136-791, Korea

(Received February 22, 1989)

이형질적 이중구조로 분석한 피부에서의 클로니딘 투과기전

변영로·김영하·정서영†

한국과학기술원 고분자화학연구실

(1989년 2월 22일 접수)

The clonidine transport mechanism through the skin was investigated with assumptions that epidermis is heterogeneous and two-layer membrane. Immobilization of clonidine was not found in stratum corneum but in viable epidermis. The sorption in the viable epidermis agreed with the dual sorption theory. Diffusion coefficient in stratum corneum was five order magnitude less than that in viable epidermis. In viable epidermis, the ratio of true diffusivity to apparent diffusivity increased initially then decreased as a function of clonidine concentration, and the true diffusivity was always larger than the apparent diffusivity.

Keywords—stratum corneum, viable epidermis, clonidine, immobilization, two-layer model

The heterogeneous multilayered structure of skin is very complicated, thus the resulting transport phenomena are hardly analyzed by simple diffusional equations[1]. The epidermis consists of five histological layers (multilayer structure), among them stratum corneum, the outermost layer, is believed to be the rate-limiting barrier for transdermal diffusion[2]. The stratum corneum is a heterogeneous structure containing about 40% of protein, 40% of water and approximately 15 to 20% of lipid[3].

Several reviews addressing the principles of diffusion model through the skin have been published. Most of the developed model systems are based on the Fickian diffusion which deals with a homogeneous membrane[4,5]. However, the immobilization of the drug onto the skin significantly delays the steady state diffusion owing to the heterogeneous structure of the skin[6]. Thus the simple data treatment which assumes the skin be a homogeneous membrane has often led some confusion in understanding the diffusion experiment *in vitro*. Even though several methods, such as a heat treatment[7] and enzyme treatment[8], have been developed for preparing stratum corneum sample. There are some difficulties in dealing with stratum corneum itself in real experimental situation; those are the pinhole formation during preparation and the very thin character of the stratum corneum[4,7,9]. Unfortunately, the prevention aganist pinhole formation in stratum corneum cannot be guaranteed by above mentioned methods.

†To whom correspondence should be addressed.

Therefore, in this present study it was attempted to calculate the diffusional parameters of stratum corneum not by performing the experiment with stratum corneum, but on the basis of the diffusional data of whole epidermis (with stratum corneum) and viable epidermis (without stratum corneum).

The purposes of this study were to examine whether the diffusional parameters of clonidine, an antihypertensive drug, through stratum corneum can be calculated from heterogeneous structure assumption and to understand more precisely the mechanism of clonidine transdermal diffusion. The clonidine has been chosen for a model drug compound since there have been no reports dealing with clonidine diffusional mechanism through skin, even though the clonidine transdermal patch is commercially available in the market[10-12].

EXPERIMENTAL METHODS

Material

The tritium labelled clonidine HCl ([phenyl-4-^{3}H] clonidine hydrochloride) was purchased from Amersham Corporation (Arlington Heights, IL) and was supplied in a solution of ethanol:0.01 M hydrochloric acid (1:1). The specific activity was 24.1 curies/mmol and the radioactive concentration was 1mCi/m*l*. Analytical grade of clonidine HCl (Sigma Chemical Co., St. Louis, MO) was also used. Stock solution was freshly made by diluting labelled clonidine with unlabelled for 0.2% before each experiment. The diffusion media were prepared with pH 7.4 phosphated buffered saline (PBS). Insta gel (Amersham Co.) was used for scintillation cocktail.

Skin Membrane Preparation[13]

The whole epidermis and viable epidermis of famale ICR mice between six and eight weeks old (25-30g) were used throughout the study. Mice were sacrificed by cervial cleavage of the spinal cord. The dorsal areas were clipped with electric clippers (number 40 blades), with taking care not to damage the skin and the full thickness skin was removed immediately after clipping. The whole epidermis was separated from the full thickness skin by placing the dermis-side down on filter paper saturated with 1% trypsin solution at 37 °C for 4 hours. Trypsin solution was prepared with PBS at pH 7.4. Trypsin solution on the epidermis was removed with light vortexing in deionized water. The viable epidermis was prepared by removing the stratum corneum from the whole epidermis by heat treatment at 50 °C for 2 min. Epidermis samples were vacuum dried under 10^{-4} Torr for 12 hr to remove remaining water and were stored in vacuo.

Diffusion Experiment

Skin permeability was measured using the Franz diffusion cell[14]. The stratum corneum was faced to the concentrated upstream compartment. Donor compartment was 420 μl and receptor compartment was 4.5 m*l* in volume. The concentration of clonidine was 15.38 mg/m*l*. The effective surface area of skin for diffusion was 0.64 cm^2. The solution of receptor compartment was stirred with a magnetic bar to reduce boundary layer effects. The diffusion cell was placed in the React-Therm (Pierce Co., heating and stirring module) for the constant temperature (37 °C). At the optimum time intervals samples (0.5 m*l*) were withdrawn from the receptor compartment and replaced with fresh isotonic PBS (pH 7.4). Then the samples were mixed with 5 m*l* of scintillation fluid (Insta gel; Amersham Co.) and the radioactivity was measured using a liquid scitillation spectrometer (Beckman LS 3801, Beckman, USA-Irvine, CA).

Calculation of Permeability

Since the sink condition (the concentration of clonidine at the receptor compartment was less than 1% to the donor compartment) was maintained throughout the experiment, the equation derived from Fick's 1st law could be applied for the calculation of the permeability[15]. The cumulative amount of clonidine through the epidermis was plotted as a function of time. Correction was made for the replacement of the fresh isotonic PBS.

In order to calculate a permeation coefficient the following equation was used;

$$J_t = PA\Delta C \quad (1)$$

where J_t (μg/sec) is the total flux calculated from the slope of plots of the cumulative amount versus time, P is the permeation coefficient (cm/sec), A is the diffusional area (cm^2), and C is the drug concentration, which was taken to be equal to the donor phase concentration ($\mu g/cm^3$). Thus, the permeation coefficient can be calculated from:

$$P=J_t/A\Delta C \tag{2}$$

since $J_t = V(dC/dt)$

$$P= V(dC/dt)/A\Delta C \tag{3}$$

where V is the volume of the receptor compartment (cm^3) and dC/dt is the steady state slope divided by volume.

Calculation of Diffusion Coefficient

A dual sorption model has been extensively utilized to explain the equilibrium data.[16,17] The model postulates that the sorption occurs by two mechanisms. The one is a simple dissolution which produces mobile or freely diffusible molecules. The mobile drug concentration (C_d) can be adequately expressed as follows:

$$C_d=K_d \cdot C \tag{4}$$

where K_d is constant and C is a solution concentration. On the other hand, the concentration of immobilized drug (C_l) can be represented by a Langmuir adsorption isotherm:

$$C_l=\frac{C_l^* bC}{1+bC} \tag{5}$$

where C_l^* and b are constants which can be calculated by plotting experimental data.

If one assumes that sink conditions exist, one can obtain the normal time lag as the following equation.[16,17]

$$\frac{t_L D}{l^2}=\frac{1}{6}+n\left\{\frac{\alpha^2/2+\alpha-(1+\alpha)\ln(1+\alpha)}{\alpha^3}\right\} \tag{6}$$

where $n = C_I^* b/K_d$, $\alpha = bC$ and l is the thickness of the membrane.

And the ratio of the true to apparant diffusivity is reduced as the following equation by Chandrasekaran[18]:

$$\frac{D_{tr}}{D_{app}}=\frac{1/6+n[\{\alpha^2/2+\alpha-(1+\alpha)\ln(1+\alpha)\}/\alpha^3]}{1+n/(1+\alpha)^2} \tag{7}$$

where D_{app} is the apparant time lag diffusion coefficient.

For a single layer membrane, diffusion coefficient can be given by the following equation:

$$D_{tr}=\frac{l^2}{6t_L} \tag{8}$$

where t_L is a lag time.

The partition coefficient was calculated from the following equation:

$$K=\frac{Pl}{D} \tag{9}$$

For two layer model, the steady state flux of drug through whole epidermis can be expressed by Eq. (10).

$$\frac{1}{J_w}=\frac{1}{J_{st}}+\frac{1}{J_{via}}=\frac{l_{st}}{D_{st}K_{st}C}+\frac{l_{via}}{D_{via}K_{via}C}$$

$$=\frac{l_{via}(1+1/(E-1))}{D_{via}K_{via}C} \tag{10}$$

where E is the ratio of flux through the viable epidermis to that through the whole epidermis. Subscripts *st*, *via* and *w* refer to stratum corneum, viable epidermis and whole epidermis, respectively. If the diffusional boundary layer in both donor and receptor compartments is negligible, the overall time lag elapsed before reaching steady state permeation under a perfect sink condition can be given by the following equation:

$$t_w=\frac{\frac{l_{via}^2}{D_{via}}\left(\frac{l_{via}}{6D_{via}K_{via}}+\frac{l_{st}}{2D_{st}K_{st}}\right)+\frac{l_{st}^2}{D_{st}}\left(\frac{l_{via}}{2D_{via}K_{via}}+\frac{l_{st}}{6D_{st}K_{st}}\right)}{\frac{l_{via}}{D_{via}K_{via}}+\frac{l_{st}}{D_{st}K_{st}}} \tag{11}$$

RESULTS AND DISCUSSION

The steady state flux of clonidine through whole epidermis and viable epidermis as a function of clonidine concentration are shown in Fig. 1. The clonidine flux *in vitro* was proportional to the concentration. At the saturated concentration of clonidine (76.92 mg/m*l*), flux through the whole epidermis and the viable epidermis were 20.08 μg/cm^2·min and 156 $\mu g/cm^2$·min, respectively.

The concentration of clonidine in the epidermis

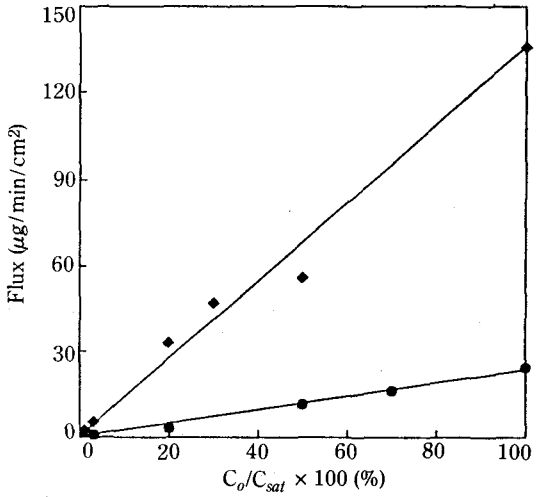

Figure 1—The clonidine flux through epidermis as a function of concentration.
Key: ●, whole epidermis **(n = 4)**; ◆, viable epidermis (n = 4)

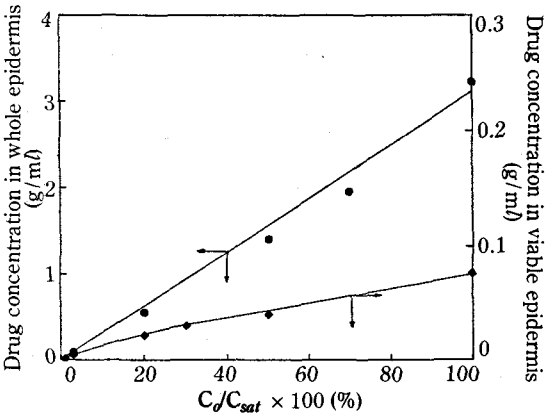

Figure 2—The clonidine concentration in epidermis as a function of solution concentration.
Key: ●, whole epidermis **(n = 4)**; ◆, viable epidermis (n = 4)

at the steady state was determined by equation KC, where K is the partition coefficient which was determined by lag time method and C is the solution concentration. The results of these measurements for the epidermis are shown in Fig. 2. The sorption profile of clonidine in the whole epidermis was different from that in the viable epidermis. In the whole epidermis, the sorption curve exhibited a linear relationship with drug concentration, but the non-linearity appeared below 20%

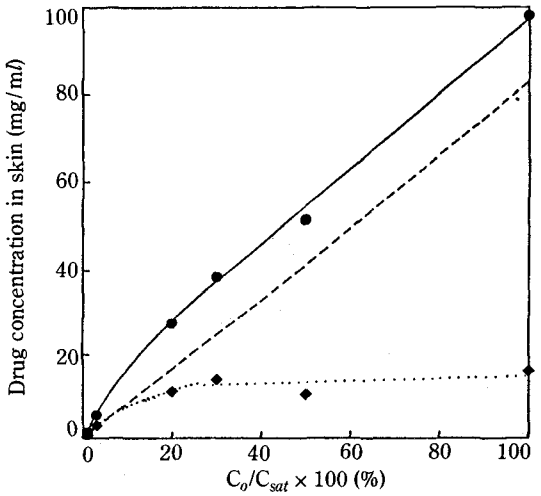

Figure 3—Sorption isotherm of clonidine in viable epidermis.
Key: ●, sorption isotherm;---, dissolution compartment; ◆, immobilized compartment

of saturated concentration in the viable epidermis. This non-linearity indicates that the immobilization occurs only in the viable epidermis. The steady state diffusivity in the viable epidermis should be essentially independent of clonidine concentration above 20% of saturation since flux was proportional to the amount of clonidine. On the other hand, in the whole epidermis the sorption curve was propotional to the whole range of clonidine concentration and the sorbed amount of drug was much larger than that of the viable epidermis. This can be thought the clonidine partition to the stratum corneum is much higher than to the viable epidermis and the immobilization effect of drug in the stratum corneum is negligible. Therefore, the apparent time lag diffusivity (D_{app}) should be essentially independent of clonidine concentration in the whole epidermis. This suggests that only one measurement of D_{app} at any drug concentration be enough to estimate the diffusivity and this D_{app} be equal to the true diffusivity (D_{tr}). As a result, diffusion and permeability coefficients in whole epidermis were $2.94(\pm 0.54) \times 10^{-9}$cm/sec and $4.35(\pm 0.81) \times 10^{-6}$cm/sec, respectively.

It is interesting to note that the partition of ionizable clonidine salt form was much higher in the

Table I—*Transport Parameters in Viable Epidermis.*

Clonidine solution concentration (C) (mg/ml)	Dissolved clonidine concentration (C_d) (mg/ml)	Immobilized clonidine concentration (C_I) (mg/ml)	True diffusion coefficient (D_{tr}) ($\times 10^7 cm^2/sec$)	Apparent diffusion coefficient (D_{app}) ($\times 10^7 cm^2/sec$)	Experimental ratio (D_{tr}/D_{app})	Theoretical ratio (D_{tr}/D_{app})
0.77 (1%/sat)	0.63	0.43	18.79	9.19	2.05	1.57
2.30 (3%/sat)	1.89	2.28	14.31	5.31	2.69	2.13
15.38 (20%/sat)	12.63	8.55	13.30	6.50	2.05	1.76
23.08 (30%/sat)	18.94	10.70	10.05	5.29	1.90	1.57
38.46 (50%/sat)	31.57	8.00	9.06	5.88	1.54	1.38
76.92 (100%/sat)	63.14	12.31	10.77	7.38	1.46	1.21

stratum corneum than that in the viable epidermis. It should also be noted that the stratum corneum (phospholipid content: 0%[22]) itself is not much hydrophobic compared to the viable epidermis (phospholipid content: 25-62%[22]), even the whole epidermis is generally accepted as a hydrophobic membrane. Therefore, it is feasible that the ionizable drug can be highly partitioned in stratum corneum rather than in viable epidermis.

Fig. 3 represents the equilibrium sorption isotherm in the viable epidermis (solid line). This can be divided into a mobile drug (dashed line) and an immobilized drug isotherm (dotted line). The partition coefficient of mobile drug [K in Eq. (4)] was calculated from the slope where the sorption curve showed linearity (>20% in Fig. 2), then free drug isotherm was obtained from Eq. (4). Additionally, the immobilized drug isotherm was obtained by plotting the differences between sorption curve and mobile drug isotherm. As can be seen in Fig. 3, immobilization (dotted line) followed the Langmuir adsorption pattern. Therefore, Langmuir constants C_I^* and b were calculated by Eq. (5). The respective values of K_d, C_I^* and b were 0.82, 5.0 mg/ml and 0.70 ml/mg.

In viable epidermis the D_{app} was dependent on the clonidine concentration (Table I). Therefore, it was attempted to calculate the true diffusion coefficient (D_{app}). The experimentally measured values of the diffusion coefficient (D_{app}) can be expressed as $D_{app} = D_{tr}(\partial C_d/\partial C)$ from the definition

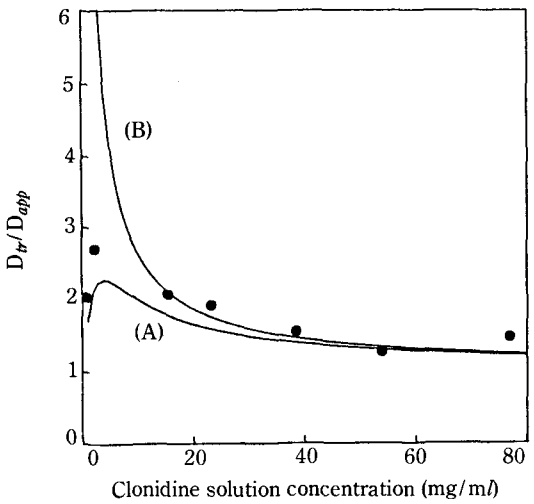

Figure 4—Effect of clonidine concentration on D_{tr}/D_{app}. Key: (A), function defined by Eq. (A-1); (B), function defined by Eq. (A-1) substituting for ln

$J = DK(\partial C/\partial x)$, where C denotes the concentration of free-to-diffuse clonidine molecules.[19] It is interesting to note that the D_{tr} is not constant in diluted clonidine solution (Table I), which presumably be due to a variation in activity of clonidine.[20,21] At high concentration D_{tr} seemed to be independent of clonidine concentration.

As shown in Fig. 4, D_{tr}/D_{app} increased initially then decreased as a function of clonidine concentration. The theoretical curve which was generated by dual sorption theory [Eq. (7)] using the obtained values of the constants K_d, C_I^* and b was also plotted in Fig. 4 (curve A). It is apparent

Table II—*Calculated Transport Parameters in Stratum Corneum.*

Epidermis	Permeability $(dQ/dt)/\Delta C$ (cm/sec)	Time lag (t_L) (hr)	$\frac{(dQ/dt)_{whole}}{(dQ/dt)_{viable}}$	Diffusion coefficient (cm^2·sec)
Whole epidermis	$4.35(\pm 0.81)\times 10^{-6}$	10.49		$2.94(\pm 0.31)\times 10^{-9}$
Viable epidermis	$27.34(\pm 0.37)\times 10^{-6}$	0.0275	7.77	$1.01(\pm 0.12)\times 10^{-6}$
Stratum corneum				2.24×10^{-11}

that the agreement between theory and experiment is quite acceptable, suggesting the validity of the dual sorption model in the analysis of clonidine permeation through viable epidermis, it should be noted that the dual sorption theory has been used to explain the transdermal scopolamine permeation in Chandrasekaran's study.[18] Even they used the same data treatment as present study, the theoretical curve was very different from the present study. It was found that if one used the log instead of ln in Eq. (7), totally different theoretical curce could be obtained as illustrated in Fig. 4(B) (see more details in Appendix).

In order to calculate transport parameters in the stratum corneum using obtained true transport parameters of whole epidermis and viable epidermis, the Eqs. (10) and (11) were used. As a result, calculated diffusion coefficient of the stratum corneum is $2.44\times 10^{-11}cm^2/sec$. This indicates that the stratum corneum is a rate-limiting barrier for clonidine transdermal permeation since the diffusivity was five order magnitude less than the viable epidermis.

In conclusion, the immobilization of clonidine appeared not in stratum corneum but in the viable epidermis, and the immobilization effect in the viable epidermis could be explained by the dual sorption theory. In viable epidermis, the ratio of true diffusivity to apparent diffusivity increased initially then decreased as a function of clonidine concentration, and the true diffusivity was always larger than the apparent diffusivity. More importantly, transport parameters of stratum corneum can be obtained from the experimentally obtained transport parameters of whole epidermis and viable epidermis. Thus difficulties in the experiment using only a stratum corneum can be avoided by the method as decribed in this study.

APPENDIX

Dual sorption theory was analyzed in detail in order to manifest the difference between the data which is published by Chandrasekaran[18] and the present study. The Eq. (7) can be expressed as follows;

$$y(\alpha)=\frac{D_{tr}}{D_{app}}=\frac{(1+\alpha)^2\{\alpha^3+3n\alpha^2+6n\alpha-6n(1+\alpha)\ln(1+\alpha)}{\alpha^3\{(1+\alpha)^2+n\}} \quad (A-1)$$

where $\alpha=bc$. If l'Hopital law is used in order to obtain the limiting value of Eq. (A-1) to zero, Eq. (A-1) can be derived as follows;

$$\lim_{\alpha\to 0+} y(\alpha)=1 \quad (A-2)$$

Also, l'Hopital law is used in order to obtain the limiting value of Eq. (A-1) to the infinite, the following equation can be obtained.

$$\lim_{\alpha\to\infty} y(\alpha)=1 \quad (A-3)$$

The slope of D_{tr}/D_{app} is obtained as Eq. (A-4) by differentiating Eq. (A-1)

$$y'(\alpha)=\frac{\begin{array}{c}\alpha^3\{(1+\alpha)^2+n\}[(1+\alpha)^2\{3\alpha^2+6n(\alpha-\ln(1+\alpha))\}\\+2(1+\alpha)\{\alpha^3+6n(\alpha^2/2+\alpha-(1+\alpha)+\\\ln(1+\alpha)\}\}]-(1+\alpha)^2[\alpha^3+6n\{\alpha^2/2+\alpha-(1+\alpha)\\\ln(1+\alpha)\}][3\alpha^2\{(1+\alpha)^2+n\}+2\alpha^3(1+\alpha)]\end{array}}{\alpha^6\{(1+\alpha)^2+n\}^2} \quad (A-4)$$

By the l'Hopital law, the limiting value to zero in Eq. (A-4) can be expressed;

$$\lim_{\alpha\to 0+} y'(\alpha)=\infty \quad (A-5)$$

Therefore, the typical curve of Eq. (A-1) is

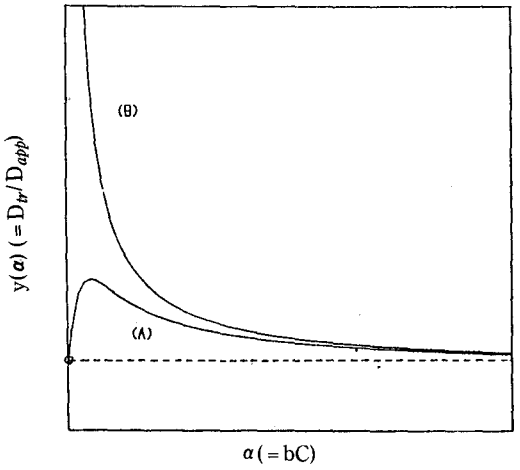

Figure A1—Theoretical cure of D_{tr}/D_{app}. (A), function defined by Eq. (A-1); (B), function defined by substituting log for ln.

shown as Fig. A1 (A).

On the other hand, if log is used in Eq. (A-1) instead of, the limiting values are obtained as following;

$$\lim_{\alpha \to 0^+} y(\alpha) = \infty \qquad \text{(A-6)}$$

$$\lim_{\alpha \to \infty} y(\alpha) = 1 \qquad \text{(A-7)}$$

Thus, the typical curve in this case is shown as Fig. A1(B). In conclusion, the theoretical curve which was published by Chandrasekaran *et al.*[18] seemed to be false. Also, the values of D_{tr}/D_{app} do not decrease continuously, but increasing first and then decreasing as a function of concentration.

ACKNOWLEDGEMENT

This study was supported by the Ministry of Science and Technology (MOST) grant number N02440.

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