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Mechanism of Urea Effect on Percutaneous Absorption of Clonidine

Young Rho Byun, Seo Young Jeong§ and Young Ha Kim
Polymer Chemistry Lab., Division of Polymer Science, KAIST, P.O. Box 131, Cheongryang, Seoul, Korea
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Abstract □ The urea effect on skin permeation of clonidine was investigated to reduce a log time and to increase a permeability. ICR mouse skin and human skin were used and were assumed to be a two-layer membrane consisted of stratum corneum and viable epidermis. The urea acted as a skin denaturant and humectant in the whole epidermis. Also it enhanced the skin permeability of clonidine about 3.5 times. On the other hand, it enhanced the skin permeability by acting as a humectant in the viable epidermis. But the urea effect on the whole epidermis was shown to be greater than that on the viable epidermis. Therefore, it was found that the effect of urea was greater on the stratum corneum than the viable epidermis. Variation of enhancing effect according to the concentration of urea was not found in the range of 1% to 20%.

Keywords □ clonidine, urea, enhancing effect, humectant, skin denaturant

The skin permeability of a drug is the most important factor in designing transdermal formulation. Thus it is very difficult to design a transdermal patch for the drug which requires a high therapeutic plasma level. Therefore, it is necessary to develop an enhancer to expand the applicability of transdermal drug delievery.

The transdermal drug delivery system can be divided into a system control and a skin control based upon whether the rete determining layer is polymeric or skin membrane. The rate determining layer is a polymeric membrane in the system control, thus drug permeability is mainly controlled by the drug diffusivity through polymer membrane. On the other hand, a skin, more precisely the stratum corneum[1], is the rate-determining layer in the skin control. The system control is more desirable to manage a precise permeability. To apply the system control, however, the skin permeability of drug should be much higher than that of achieving a minimum effective plasma level. Therefore, theoretically should the skin permeability of drug increase greatly by enhancer, most drug can be delivered by the system control. Furthermore, both burst and enhancing effect are prerequisie to reach the effective plasma level rapidly since the drug permeates into the blood stream after saturating the skin site at the initial stage of transdermal delivery.

Commercially available Catapres-TTS® (clonidine, antihypertensive agent) requires approximately 48 hours to reach the steady state plasma concentration[2,5]. Hence it seems that enhancers should be added to reduce this long lag time.

Until recently, many substances have been investigated as an enchancer for transdermal drug delivery. Unfortunately, the long term human toxicity and skin irritation problems have not been solved yet. The urea has been used in cream or cosmetics as a humectant[6], and there has been no report about serious human toxicity of urea[7,8].

Therefore the feasibility of the urea enhancing effect on clonidine percutaneous absorption was investigated in this study.

There are some differences in published reports about the mechanism of urea Wehlrab[9] *et al.* reported that urea enhanced skin permeation of drug by increasing the water-binding capacity in skin. Feldmann *et al.*[10] found that urea changed the structure of stratum corneum. On the other hand, Montgna[11] reported that urea induced the structual changes only in the cellular architecture, beneath the stratum granulosum, and the stratum corneum structure was not affected by urea.

Therefore, in this study, the mechanism of urea enhancing effect was examined by analyzing diffusivity and partition coefficient. Most of reports

§To whom correspondence should be addressed

concerning urea were based on analysis with full-thickness skin. But the characteristics of transport and the components are quite different between the straum corneum and the viable epidermis[12]. In this study, the skin was treated as a two layer membrane consisting of the stratum corneum and the viable epidermis.

EXPERIMENTAL METHODS

Materials

The radio-labelled clonidine ([phenyl-4-^{3}H]clonidine hydrochloride) was supplied as a solution in ethanol: 0.01M hydrochloride acid(1:1). The specific activity was 34:1 Curies/mmol and the radioactive concentration was lmCi/m*l*. Analytical grades of unlabelled clonidine (SIGMA) was also used. Stock solution was freshly made by diluting labelled clonidine with unlabelled for 0.2% before each experiment. The diffusion media were phosphate buffered saline (PBS) at pH 7.4. Insta gel(Amersham Co.) was used for scintillation cocktial. Each sample was mixed with Insta gel for 10 times in volume.

Skin membrane preparations[13]

The whole epidermis and the viable epidermis of female ICR mice between six and eight weeks old (25-30 g) were used throughout studies. Mice were sacrificed by cervial cleavage of the spinal cord. The dorsal areas were clipped with electric clippers (number 40 blades), taking care not to damage the skin and the full thickness skin removed immediately after clipping. The whole epidermis was separated from the full-thickness skin by placing the dermis-side down on filtered 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 by light vortexing in deionized water. The viable epidermis was prepared by removing the stratum corneum from the whole epidermis. The whole epidermis was immersed in water at 50 °C for 20 seconds. Epidermis samples were vacuum dried under 10^{-4} Torr for 12 hours to remove remaining water and stored in vacuo.

The breast skin of female persons between fourty and fourtyfive years old were also used. In the case of human skin, only whole epidermis were used. Preparation method of human skin was same as that of mouse skin. But the human epiderms were treated with hexane at 4 °C to eliminate lipid after trypsin treatment.

Experimental Produres

Skin permeability of clonidine was measured in the Franz diffusion cells[14]. The stratum corneum was always exposed to the concentrated upstream compartment. Donor compartment was 420 u*l* and receptor compartment was 4.5 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. Cells were placed in the React-Therm (Pierce Co., heating & stirring module) for the constant temperature at 37 °C. At the optimum time intervals samples were withdrawn from the receptor compartment and replaced by fresh isotonic PBS buffer (pH 7.4). Then the samples were mixed with 5 m*l* of scintillation fluids (insta gel; Amersham Co.) and the concentrations were determined using a liquid scintillation spectrometer. (Beckman LS 3801)

Calculations of the transport parameters

Since the sink condition was maintained throught each experiment, the equation derived from Fick's 1st law could be applied for the calculation of the permeability coefficients. 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 permeability coefficient the following equation was used[15];

$$J_t = PA\Delta C \tag{1}$$

where, J_t(ug/sec) is the total flux calculated from the slope of the cumulative amount vs. time; P is the permeability coefficient (cm/sec); A is the diffusional area (cm^2); and C is the diffusional concentration across the skin, which was taken to be equal to the donor phase concentration (ug/m*l*).

Thus, the permeability coefficient can be calculated from;

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

$$\text{since } J_t = V(dC/dt)$$

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

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

Diffusion coefficient can be given by the following equation[1-6];

$$D = \frac{l^2}{6t_L} \tag{4}$$

where t_L is a lag time, l is a thickness of skin.

The partition coefficient (lag time method) was calculated from the following equations;

$$K=\frac{Pl}{D} \tag{5}$$

RESULTS AND DISCUSSION

Fig. 1,2 show the urea enhancing effect on clonidine percutaneous absorption through the whole epidermis and viable epidermis of mice, respectively. In these experiments, clonidine concentration in donor compartment was 1.545% (equivalent 20% to the saturation concentration of clondine) and the urea was 1% in solution. As can be seen in Fig. 1, urea reduced the time lag of clonidine approximately 65% to the original and the total amount of clonidine absorption through the whole

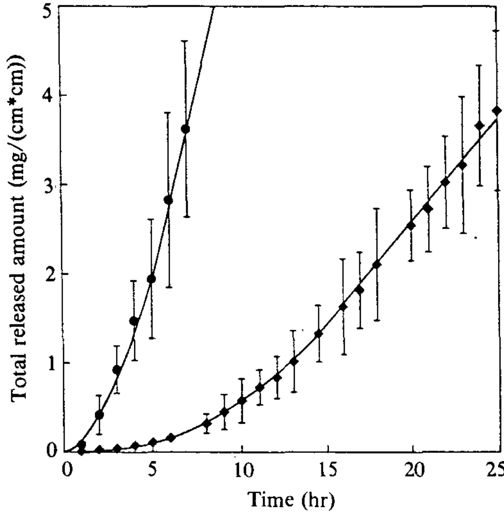

Fig. 1. Comparison of clonidine diffusion across whole epidermis in urea and fresh solution. ●: in urea solution,◆: in fresh solution(n = 4, means ± SD).

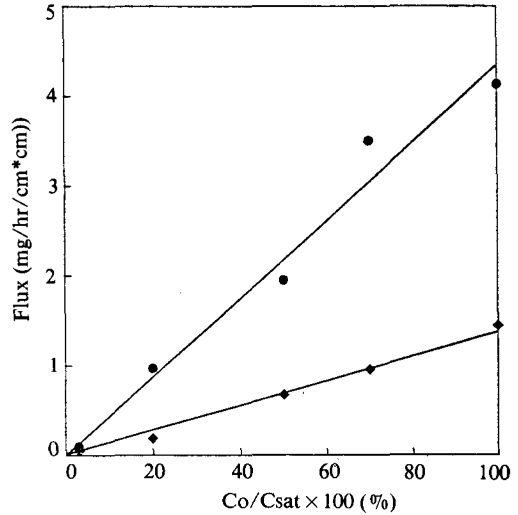

Fig. 3. Effect of urea on clonidine flux through whole epidermis. ●: in urea solution,◆: infresh solution

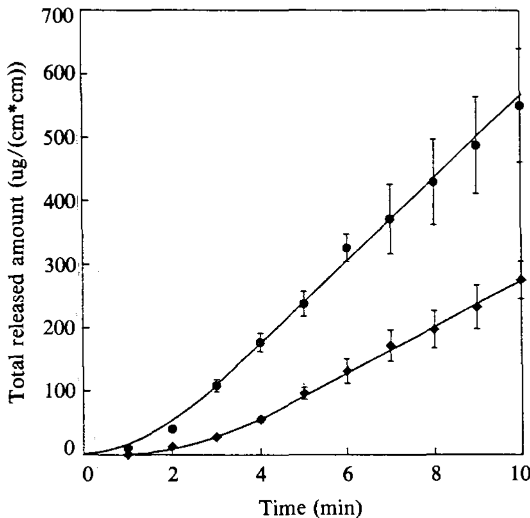

Fig. 2. Comparison of clonidine diffusion acrose viable epidermis in urea and fresh solution. ● : in urea solution, ◆ : in fresh solution (n = 4, means ± SD).

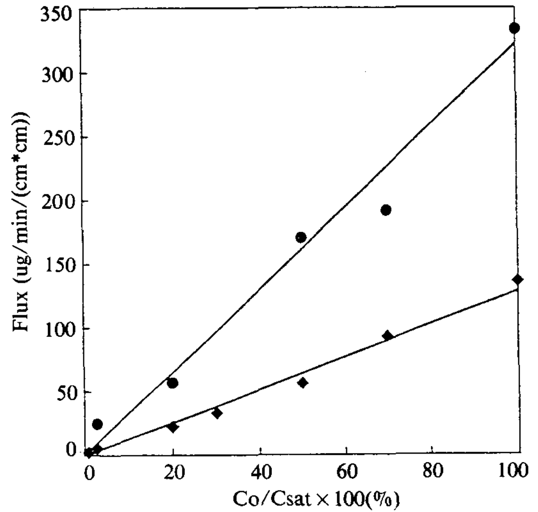

Fig. 4. Effect of urea on clonidine flux through viable epidermis. ●: in urea solution,◆ : in fresh solution

epidermis was significantly increased. Fig. 2 also represents the urea enhancing effect on clonidine absorption through the viable epidermis. In this case (Fig. 2), the time lag was cut by approximately 30% with urea. Therefore, it is obvious that the reducing time lag by urea was greater in the whole epidermis compared to the case of viable epidermis, i.e., diffusivity of clonidine through whole epidermis was more highly affected by the urea than that through viable epidermis.

In order to determine whether the changes in clonidine concentration influence the urea enhancing effect in percutaneous absorption, the changes of steady-state flux of clonidine according to the clonidine concentration were measured, as shown in Fig. 3 and 4. In the case of whole epidermis (Fig. 3), the steady-state flux of clonidine was proportional to the concentration of clonidine and the addition of urea proportionally increased steady-state flux about 3.5 times in whole concentration range. Thus it was evident that the urea enhancing effect was independent of the concentration of clonidine and urea would not affect the physical properties of clonidine but the nature of epidermis. Fig. 4 also shows the changes of steady-state flux of clonidine in the viable epidermis. This result (Fig. 4) was similar to the case of whole epidermis (Fig. 3), except the urea enhancing power, which was 2.2 times.

From these data (Fig. 1-4), transport parameters (diffusion coefficient, permeability and partition coefficient) of clonidine were calculated (see Table I). As can be seen in Table I, the diffusivity and partition coefficient were increased approximately 3 and 1.3 times, respectively, by addition of urea in the whole epidermis of mice. A significant change in the diffusivity of clonidine by addition of urea suggested that the urea denature the structure of epidermis in some-way(ultrastructural or morphorological study is beyond the scope of our experiment). Also the small increase in partition coefficient of clonidine might be due to the increase of water-binding capacity in the whole epidermis by urea acted as a humectant. In the case of the viable epidermis of mice, the diffusivity and partition coefficient increased approximately 1.4 and 1.63 times, respectively, by addition of urea, that is, the urea mainly acted as a humectant in the viable epidermis. Hence it was found that the urea enhanced the clonidine permeation both by denaturation of stratum corneum and increase in a water-binding capacity of viable epidermis.

In order to ascertain the enhancing effect of urea on the human epidermis, same experiment as described above was performed using female breast skin (Fig. 5). The urea enhancing effect was almost similar to the case of mice skin, as shown in Fig. 5. The diffusivity and partition coefficient of clonidine through human whole epidermis were increased 2.28 and 1.34 times, respectively, by urea (Table I).

Fig. 6 shows the urea enhancing effect on clonidine permeation through whole epidermis of mice according to the concentration of urea. The range of urea concentration was from 1 to 20% . As can

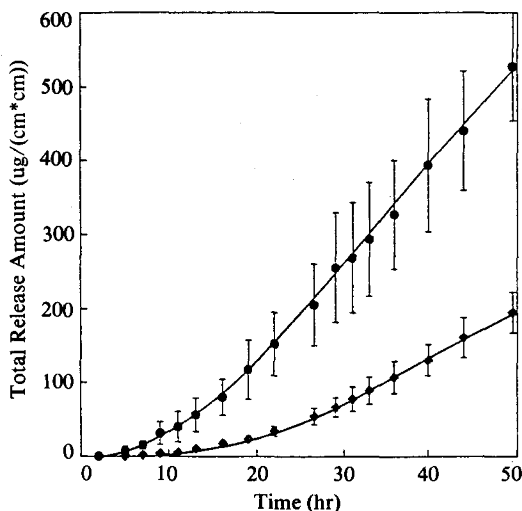

Fig. 5. Enhancing effect of urea on human epidermis.
●: in urea solution, ◆: in fresh solution (n=4, mean ± SD)

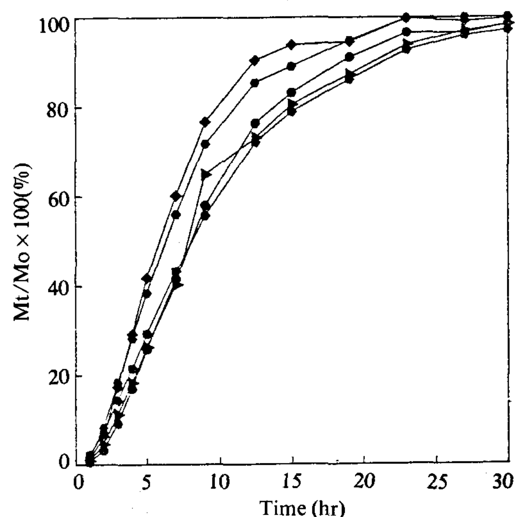

Fig. 6. Comparison of diffusion and urea concentration in whole epidermis.
●: 1%, ●: 5%, ◆: 10%, ▲: 15%, ●: 20%

Table I. Comparison of transport parameters in fresh solution and in urea solution.

	Permeability (cm/sec)	Diffusion coefficient (cm^2sec)	Partition coefficient
In fresh solution			
Whole epidermis (mouse skin)	4.35×10^{-6}	2.94×10^{-9}	37.4
Viable epidermis (mouse skin)	2.73×10^{-5}	5.31×10^{-7}	1.28
Whole epidermis (human skin)	1.09×10^{-6}	1.53×10^{-9}	17.9
In Urea solution			
Whole epidermis (mouse skin)	1.61×10^{-5}	8.53×10^{-9}	47.2
Viable epidermis (mouse skin)	6.28×10^{-5}	7.55×10^{-7}	2.08
Whole epidermis (human skin)	2.48×10^{-6}	2.50×10^{-9}	24.0

be seen in Fig. 6, it was not found that the significant differences in enhancing power could be obtained by using concentrated urea solution. That is, the urea enhancing effect was not significantly different above 1% concentration. This was because there is a limit to skin hydration or denaturation by urea, and that the urea enhancing effect does not increase any further above a certain concentration.

In conclusion, the urea evidently enhanced the clonidine percutaneous absorption by acting both as a skin denaturant and humectant. The denaturating effect of urea was more significant on the stratum corneum than on the viable epidermis since the diffusivity of clonidine was more highly influenced in the whole epidenrmis compared to the viable epidermis. And the hydration effect of urea was greater on the viable epidermis than the stratum corneum. In addition, urea could be acted as an enhancer under 1% concentration.

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