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Transdermal Delivery of Ethinylestradiol UsingEthylene-vinyl Acetate Membrane

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Ethinylestradiol (EE)-containing matrix was fabricated with ethylene-vinyl acetate(EVA) copolymer to control the release of the drug. Effect of addition of PEG 400 as receptor solution, the stripping of skin and Azone pretreatment on skin on the permeation of EE through the excised mouse skin was also studied. The permeation rate of EE through the excised mouse skin was affected by the PEG 400 volume fraction. The Azone pretreatment on skin didn't affect on the steady state flux, however, the lag time was shortened. The permeation rate of EE through the stripped skin was much larger than that through the whole skin. It showed that the stratum corneum acts as a barrier of skin permeation. The fact that there is little difference in EE permeation between the intact skin and the stripped skin with EVA membrane shows the permeation of EE through the mouse skin is mainly controlled by the membrane.

Keywords—Ethinylestradiol, Ethylene-vinyl acetate, Transdermal, Azone®

Several technologies have been successively developed to provide a mechanism of rate control over the release and the transdermal permeation of drugs. Basic components of transdermal devices are polymer matrix, penetration enhancers and excipients[1]. The use of release controlling membrane is one method to regulate the drug release. Among many polymers, the usefulness of ethylene-vinyl acetate (EVA) copolymer as a drug delivery system for hydrocortisone[2], fluoride ion[3], 5-fluorouracil[4] and macromolecule such as proteins was described. However little reports have dealt with the release of contraceptive drugs from EVA copolymer matrices. In the previous paper, study on the release of ethinylestradiol (EE) from EVA membranes of different vinyl acetate (VA) content was carried out[5]. An increased vinyl acetate comonomer content in EVA membrane increased the drug release rate and permeability coefficient. The present investigation was undertaken to determine the amounts of EE released from EVA copolymer matrices of 40% VA content through mouse skin. Also, the use of permeation enhancer is considered to increase the permeation of EE through mouse skin. Laurocapram (1-dodecylazacycloheptane-2-one, Azone) (Fig. 1) is used for a broad spectrum of drugs[6] as a penetration enhancer. It was reported that Azone affects mainly on the stratum corneum, the skin barrier[7]. In the present study, the effects of Azone on the skin permeation of EE were examined.

Experimentals

Materials

Ethylene-vinyl acetate copolymer of 40%(w/w) VA content was purchased from Aldrich Chemical Co., Inc. (U.S.A.). Ethinylestradiol was kindly supplied by Hanhwa Pharm. Co., Ltd. (Korea) and Azone was a gift from Whitby Research Inc. (U.S.A.). Acetonitrile was HPLC grade from J.T.Baker Inc. (U.S.A.). All chemicals were reagent grade

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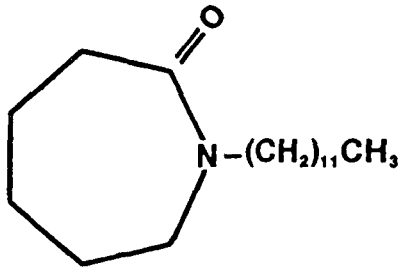

Figure 1—Chemical structure of Azone.

and used as received.

Drug-containing Matrix Preparation

Devices of EVA and EE were prepared by solvent casting process. The weighed amount of EE was dissolved in 20 m*l* of methylene chloride (adding cyclohexane, if necessary). The bead of EVA copolymer was dissolved in the drug/solvent mixture with stirring. This mixture was poured onto a teflon coated plate and the solvent was allowed to evaporate off at room temperature overnight. The membrane was removed from the plate and dried for 2 days at room temperature in vacuo. Then, a piece of matrix was cut from the membrane and weighed accurately.

Skin Preparation

Male mice (ICR strain) were sacrificed by snapping the spinal cord at the neck. The hair of abdominal area was carefully removed with an electric clipper. A square section of the abdominal skin was excised. After incision, the adhering fat and other visceral debris in the skin were carefully removed from the undersurface with tweezers. The excised skin was used immediately.

In order to prepare the stripped skin, the abdominal surface of the mouse skin was stripped with a cellophane tape for 20 times[8]. It was ca rried out by securing the animal skin on a table and the abdominal skin was stripped by placing the tape on the stratum corneum surface. A fresh piece of the tape was used for each stripping.

Stratum corneum sheets were prepared by the following method[9]. After the whole skin was incubated in warm distilled water at 60℃ for 30 seconds, the epidermis was separated. The resulting epidermis was placed with its epidermis side down on prewetted with 1% trypsin in pH 7.4 phosphate buffer solution at 37℃ for 4 hours. The stratum corneum was then carefully peeled from the epidermis with light vortexing in distilled water and covered with the trypsin solution for 1hr. Remaining nucleated cells were removed from the stratum corneum. The final stratum corneum was dried under the reduced pressure between two filter papers and kept in a desiccator until use.

***In vitro* Permeation of EE through the Excised Mouse Skin**

In order to determine the steady state permeation rate of EE through the excised mouse skin, two-chamber diffusion cell was used. Each half-cell had a volume of about 7 m*l* and the effective diffusional area of 0.79 cm^2. A piece of mouse skin was clamped between the two halves of the cell and the assembled cell was placed in a shaking incubator at 37℃. A drug suspension of known loading dose in a given concentration of PEG 400-saline solution was filled into the donor compartment. The same concentration of PEG 400-saline solution without the drug was added into the receptor compartment. The cell was shaken horizontally at the rate of 120 rpm to minimize the boundary effect. Total volume of the receptor solution was removed at the predetermined intervals and replaced by 7 m*l* of fresh solution. The amount of drug permeated was determined by HPLC.

Effect of Skin Pretreatment with Azone on Permeation

Whole skin was obtained from abdominal sites of male mice. Azone pretreatment was conducted with various volumes of Azone prepared on a 1.5 cm×1.5 cm cotton gauze and an occlusive condition was maintained by an adhesive tape[10]. After

24 hours, the cotton gauze was removed and the skin pretreated with Azone was mounted on a diffusion cell. Other conditions were same as described above in *in vitro* permeation experiments through the excised mouse skin.

***In vitro* Permeation of EE through the Excised Skin with EVA Membrane**

The excised skin with EVA membrane was mounted between a 2-chamber diffusion cell. Other conditions were same as described above in *in vitro* permeation experiments through the excised mouse skin.

***In vitro* Permeation from the Matrix through the Excised Skin**

The diffusion cell was used for evaluating the permeation rates of EE from matrix devices. Skin was mounted on a half-cell of two-chamber diffusion cell. The matrix devices were applied to the stratum corneum side of the skin. A half-cell was filled with 7 ml of PEG 400-saline solution. Other conditions were same as described above in *in vitro* permeation experiments through the excised mouse skin.

Quantitative Analysis of EE by HPLC

The concentration of ethinylestradiol in the samples obtained from the cells was determined by HPLC. The HPLC system was consisted of a pump (Waters 501, U.S.A.), and ultraviolet detector (Waters 484, U.S.A.), a 3.9×300 mm stainless-steel column packed with μ-Bondapak C_{18} (Waters, U.S.A.) and an integrator (D520A, Youngin scientific Co., Ltd., Korea). The mobile phase was a combination of acetonitrile and water (50: 50) and column temperature was maintained at ambient. A flow rate of 1.5 ml/min yielded an operating pressure of ~1500 psi. The UV detector was operated at the wavelength of 280 nm at a sensitivity of 0.01 AUFS. Under these conditions, EE peak appeared at the retention time of 6.5 min.

Determination of Permeability of Stratum Co rneum

The diffusional resistance across the stratum corneum (R_{sc}) can be determined, mathematically, from the diffusional resistance across the whole skin (R_{ws}) and the diffusional resistance across the viable skin (R_{vs}) by the following equation[11] :

$$R_{sc} = R_{ws} - R_{vs} \quad (1)$$

where R_{vs} is the sum of the diffusional resistances across the viable epidermis (without stratum corneum) and dermis. Eq. 1 is valid if the permeation across the whole skin or viable skin is the rate-limiting step in the course of skin permeation study. It can be accomplished by maintaining the hydrodynamic diffusion boundary layers on both sides of the skin barrier at a thickness which is negligibly small.

Theoretically, the diffusional resistance of a membrane (R) is related to the reciprocal of the permeability of the membrane (P), therefore, Eq. 1 can be expressed by the following equation :

$$\frac{1}{P_{sc}} = \frac{1}{P_{ws}} - \frac{1}{P_{vs}} \quad (2)$$

where the P_{sc}, P_{ws}, and P_{vs} stand for the permeability coefficient across the stratum corneum, whole skin and viable skin, respectively. Using Eq. 2, the permeability coefficient across the stratum corneum (P_{sc}) can be calculated from the P_{ws} and P_{vs} values.

The permeability coefficient of a skin tissue is, theoretically, related to the rate of permeation across the skin tissue; if it is the case, the rate of permeation across the stratum corneum, $(Q/t)_{sc}$, can be determined from the following equation (Eq. 3) when the rate of permeation across the whole skin, $(Q/t)_{ws}$, and that of the viable skin, $(Q/t)_{vs}$, are measured with the same drug concentration at equilibrium (C_e) :

$$\frac{C_e}{(Q/t)_{sc}} = \frac{C_e}{(Q/t)_{ws}} - \frac{C_e}{(Q/t)_{vs}} \quad (3)$$

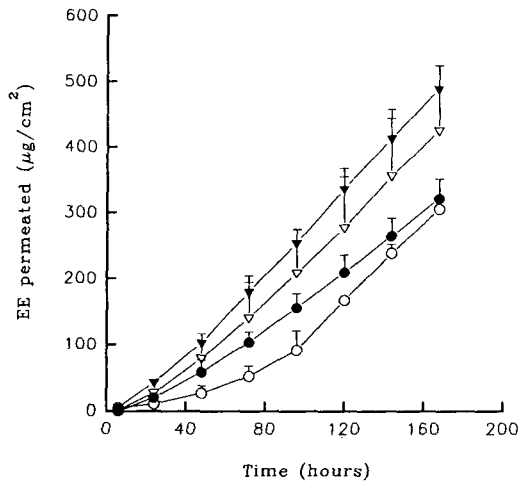

Figure 2—Effect of PEG 400 on the permeation of EE through mouse skin at constant reservoir concentration. Key : ○, saline; ●, saline containing 40% PEG 400, n=4 (Mean± S.D.).

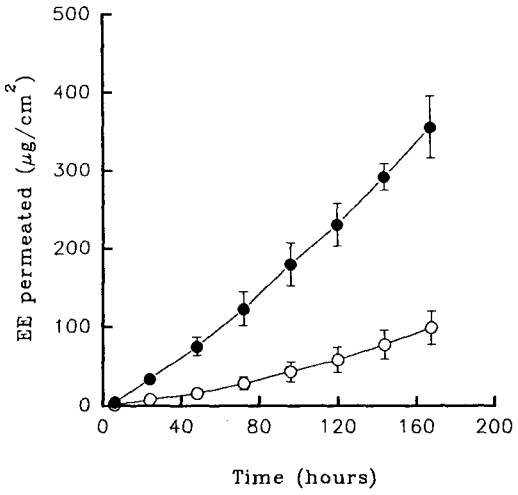

Figure 3—Effect of Azone on the EE transport across full-thickness skin. PEG 400 volume fraction was maintained at 40%(v/v).
Key : ○, 0 μl; ●, 50 μl; ▽, 100 μl; ▼, 200 μl, n=5 (Mean ± S.D.).

Results and Discussion

Effect of PEG 400 on the permeation of EE through excised mouse skin

When the EE concentration in the donor solution was maintained at a level which was greater than its equilibrium solubility, a constant skin permeation profile was achieved (Fig. 2). The permeation rates of skin (Q_s/t) in the saline or 40%(v/v) PEG 400-saline, which were measured from the slope of Qs versus t plots, were 2.03 and 6.72 ($\mu g/cm^2/sec \times 10^{-4}$), respectively. The increase in the skin permeation rate was observed to be dependent upon the equilibrium solubility (C_e) of EE in the PEG 400-saline solutions.

Effect of Azone on the Transport of EE across the Full Thickness Skin

Azone pretreatment was carried out on full-thickness skin and the effect of Azone on the transport of EE across the skin was investigated. Fig. 3 shows the time course of Q for full-thickness skin in a EVA matrix containing 5%(w/w) EE. From this plot, the steady state flux, the slope, and the lag time could be calculated. The lag time was estimated by extrapolation of the Q to zero from the plot of Q versus time[13-14]. The steady state flux of EE through the skin was similar among the pretreated Azone amounts. The lag time of EE through the skin pretreated with Azone was shorter than that of the skin not pretreated with Azone. These shorter lag times shown in the Azone pretreatment suggests that the Azone might show the enhancing effect on the skin barrier, the stratum corneum[15-16].

Skin Permeation Profile of EE across a Stripped Skin

The effect of stratum corneum on the skin permeability of EE was evaluated by studying the skin permeation across a stripped skin of mouse. Results indicated that the skin permeation profiles of EE across the stripped skin (no stratum corneum) also follow the same linear relationship, as defined by Eq. 2, as does the whole skin (with stratum corneum) (Fig. 4). Compared to the data generated earlier in the whole skin (Table I), stripping process appears to promote substantially the skin permeability of the rather

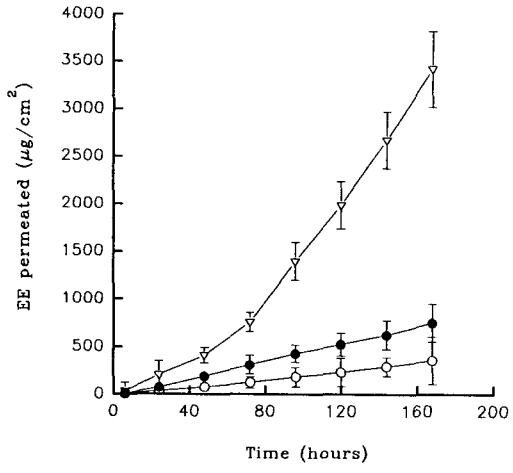

Figure 4—Effect of skin stripping on the permeation of EE mouse through mouse skin. PEG 400 volume fraction was maintained at 40%(v/v).
Key : ○; Full skin, ●; Stratum corneum, ▽; Stripped skin. n=5 (Mean± S.D.).

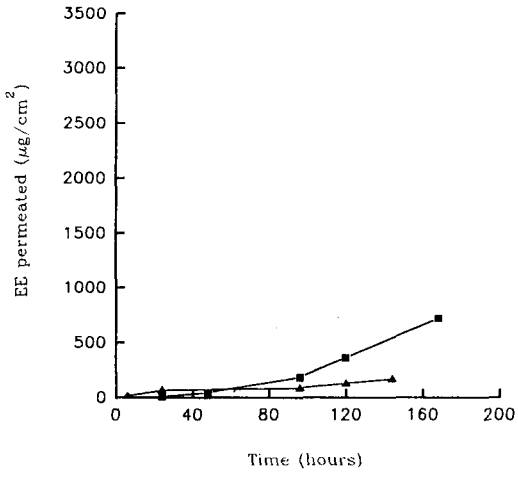

Figure 5—Permeation profile of EE through excised stripped mouse skin with EVA copolymer membrane. PEG 400 volume fraction was maintained at 40%(v/v).
Key : ▲; EVA Full skin, ■; EVA + Stripped mouse skin. n=5.

Table I—Rate of Permeation and Permeability Coefficient of EE across Stratum Corneum

	Rate of permeation μg/cm²/hr(± S.D.)	Permeability coefficient cm/hr×10⁻⁴(± S.D.)
Full skin	2.604 (0.08)	8.25 (1.1)
Stripped skin	27.749 (0.42)	124.00 (5.9)
Stratum corneum		
(observed)	4.594 (0.13)	12.75 (1.7)
(calculated)	2.874	8.61

Table II—Steady State Permeation Rate of EE through the Mouse Skin Alone or Piled Layer of Skin and EVA Membrane Composed of 40%w/w VA

	F_t values (±S.D.) (μg/cm²/hr)
EVA membrane	33.848 (2.93)
Full skin alone	2.604 (0.08)
Stripped skin alone	27.749 (0.42)
EVA+Full skin	1.757 (0.14)
EVA+Stripped skin	7.490 (0.57)

impermeable EE by elimination of the rate-limiting stratum corneum. The further observation suggests that the stratum corneum presents a great diffusional resistance to the permeation of EE across the skin.

The results are shown in Table I. As expected, the rate of permeation across the stratum corneum, $(Q/t)_{sc}$, is much smaller than the magnitude of $(Q/t)_{vs}$, the rate of permeation across the viable skin. As compared with the experiments using stratum corneum prepared before, the permeability coefficient and the rate of permeation are approximate to those which are calculated as previously mentioned. The results demonstrate that the stratum corneum acts as the major barrier in the permeation of EE across the skin.

Effect of EVA membrane on the permeation of EE through the excised mouse skin

The effect of a rate controlling membrane on the permeation of EE from suspension in 40% (v/v) PEG 400-saline solution through intact (full thickness) or stripped skin was evaluated. EVA membrane of 40% VA content was selected as a rate controlling layer because of its relatively high permeability described in previous paper[5].

Fig. 5 shows the time course of Q through a piled layer of the EVA membrane and intact or stripped skin. The flux is shown in Table II. The

EE permeation through the stripped skin was similar to that through the intact skin.

Compared with Fig. 4 showing that the EE pe rmeation through the stripped skin was about 10.6 times larger than that through the intact skin, the fact that there is little difference of EE permeation between the intact skin and the stripped skin when piled with EVA membrane shows the permeation of EE through the mouse skin is highly controlled by the controlling membrane.

Conclusions

The present study on the transdermal controlled release of EE through the EVA membrane and EE-containing matrix showed the following results;

1. The permeation rates of skin (Q_s/t) in the saline or 40%(v/v) PEG 400-saline were 2.03 or 6.72 ($\mu g/cm^2/sec \times 10^{-4}$), respectively. The permeation rate of EE through the excised mouse skin was affected by PEG 400.

2. Azone pretreatment affected on the steady state flux slightly and shorten the lag time probably due to the enhancing effect on the skin barrier, the stratum corneum.

3. The permeability of EE was markedly increased with stripping the mouse skin to remove the stratum corneum which is acting as a barrier of skin permeation.

4. The fact that there is little difference between the intact skin or the stripped skin when piled with EVA membrane shows that the permeation of EE through the mouse skin is highly controlled by the controlling membrane.

References

1) J. Hadgraft, "Transdermal drug delivery", Marcel Dekker, Inc., New York and Basel, 1987, pp. 298-300.
2) S. Miyazaki, K. Ishii, K. Sugibaysahi, Y. Morimoto and M. Takada, Antitumor effect of ethylene-vinyl acetate copolymer matrices co ntaining 5-fluorouracil on Ehrlich Ascites ca rcinoma in mice, *Chem. Pharm. Bull.*, **30**, 3770-3775 (1982).
3) J. C. Johnson(ed), "Sustained release medications", Noyes Data Co., Park Rkdge, 1980, pp. 96-97; S. W. Kim, R. V. Petersen and J. Feijen, "Drug design", vol X, ed. by E. J. Ariens, Academic Press, New York, pp. 217-219 (1980).
4) B. D. Halpern, O. Solomon, L. Kopec, E. Korostoff and J. L. Ackerman, "Controlled release polymeric formulations", ed D. R. Paul and F. W. Harris, American Chemical, Washington D. C., pp.135-146 (1976).
5) S. C. Shin and S. Y. Byun, Controlled release of ethinylestradiol from ethylene-vinyl acetate membrane, *Int. J. Pharm.*, accepted (1995).
6) R. B. Stoughton and W. O. McClure, Azone®: A new non-toxic enhancer of cutaneous penetration, *Drug Dev. Ind. Pharm.*, **9**, 725-744 (1983).
7) K. Sugibayashi, Y. Morimoto, K. Hōsoya and W. I. Higuchi, Effect of absorption enhancer, Azone, on the transport of 5-fluorouracil across hairless rat skin, *J. Pharm. Pharmacol.*, **37**, 578-580 (1985).
8) C. R. Behl, E. E. Linn, G. L. Flynn, C. L. Pierson, W. I. Higuchi and N. F. O. Ho, Permeation of skin and Eschar by antiseptics I: Baseline studies with phenol, *J. Pharm. Sci.*, **72**, 391-396 (1983).
9) K. Knutson, R. O. Potts, D. B. Guzek, D. M. Golden, J. E. McKie, W. J. Lamber and W. I. Higuchi, Macro- and molecular physical- chemical considerations in understanding drug transport in the stratum corneum, *J. Cont. Rel.*, **2**, 67-87 (1985).
10) T. Okano, M. Miyajima, F. Komada, G. Imanidis, S. Nishiyama, S. W. Kim and W. I. Higuchi, Control of drug concentration-time profiles *in vivo* by zero-order transdermal delivery systems, *J. Cont. Rel.*, **6**, 99-106 (1987).

11) Y. W. Chien, "Novel drug delivery systems: Fundamentals, developmental concepts and biomedical assessments", Marcel Dekker, New York, U.S.A., pp. 149-217, (1982).
12) Y. Morimoto, K. Sugibayashi, K-I. Hosoya and W. I. Higuchi, Effect of the absorption enhancer, Azone, on the transport of 5-fluorouracil across the hairless rat skin, *J. Pharm. Pharmacol.*, **37**, 578-583 (1985).
13) Y. Morimoto, K. Sugibayashi, K-I. Hosoya and W. I. Higuchi, Penetration enhancing effect of Azone on the transport of 5-fluorouracil across the hairless rat skin, *Int. J. Pharm.*, **32**, 31-40 (1986).
14) D. S-L. Chow, I. Kaka and T. I. Wang, Concentration-dependent enhancement of 1-dodecylazacycloheptan-2-one on the percutaneous penetration kinetics of triamcinolone acetonide, *J. Pharm. Sci.*, **73**, 1794-1798 (1984).
15) K. Sugibayashi, S. Nakayama, T. Seki, K-I. Hosoya and Y. Morimoto, Mechanism of skin penetration-enhancing effect by Laurocapram, *J. Pharm. Sci.*, **81**, 58-64 (1992).