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Formulation of a reservoir-type testosterone transdermal delivery system

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Abstract

A reservoir-type transdermal delivery system of testosterone (TS) was developed using an ethanol/water (70:30) cosolvent system as the vehicle. The maximum permeation rate achieved by 70% (v/v) of ethanol was further increased from 2.69 to 47.83 μg/cm^2/h with the addition of 1.0% dodecylamine as the skin permeation enhancer. The permeation rate of TS through the ethylene vinyl acetate (EVA) membrane was observed to increase as the vinyl acetate content in the copolymer increased. Addition of 1.0% (w/w) gelling agent, hydroxypropyl methlycellulose (HPMC), in the reservoir formulation resulted in desirable rheological properties with an insignificant effect on the skin permeation rate of TS. Thus, a new transdermal delivery system for TS was formulated using EVA membrane coated with a pressure-sensitive adhesive (Duro-Tak 87-2510) and HPMC as a gelling agent. This experimental patch showed comparable plasma concentration profiles in the in vivo study when compared with a commercial product, Androderm®. Moreover, the results suggested the possibility of further enhancing the permeation rate of TS by controlling the composition of the reservoir formulation.

Keywords: Testosterone; Transdermal; Reservoir-type; Formulation

1. Introduction

Testosterone (TS) deficiency is associated with symptoms that include impotence, fatigue, mood depression, and regression of secondary sexual characteristics. This male hormone deficiency may also adversely affect bone mineralization, muscle strength, immune function, and carbohydrate metabolism (Aloia and Field, 1976; Bizzaro et al., 1987; Mooradian et al., 1987; Stepan et al., 1989; Marin et al., 1992). Replacing the missing TS through injections, tablets or skin patches has been the usual treatment for this deficiency, the most common method being injections.

Recently, transdermal drug delivery systems have been introduced as suitable alternatives to the conventional TS administration due to their potential benefits (Arver et al., 1997; Misra et al.,

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1997). Avoidance of hepatic first-pass elimination, decrease in side effects, and the relative ease of drug input termination in problematic cases as well as maintaining suitable plasma concentration for longer duration through a non-invasive zero-order delivery are the well documented advantages of this route of administration (Williams and Bary, 1992). Nevertheless, transdermal drug delivery has always been challenged by the formidable barrier property of the intercellular lipid bilayer in the stratum corneum.

In a recent study, we have reported that ethanol (EtOH)/water (70:30) cosolvent system is the best vehicle combination for the transdermal delivery system of TS (Kim et al., 2000). However, it was necessary to further improve the permeation rate of TS by using suitable enhancers and/or vehicles. Enhancers such as fatty acid and fatty amine have been known to increase the skin permeation of a number of drugs including TS (Aungst et al., 1990; Yano et al., 1992; Chatterjee et al., 1997), and thus could be good candidates for TS.

Besides the enhancers and vehicles, the skin permeation of TS could be affected by the rate-controlling membrane and/or the pressure-sensitive adhesives. Thus, in the present report of this serial research to develop a reservoir-type transdermal delivery system of TS, the effects of various components of the device, such as the rate-controlling membrane, pressure-sensitive adhesive, and gelling agent, on the skin permeation rate were systemically investigated in the hope of optimizing TS delivery. Moreover, an in vivo study was conducted to compare the plasma concentration profiles of TS with those of a commercial product (Androderm®) in rats.

2. Materials and methods

2.1. Materials

TS, oleic acid, lauric acid, dodecylamine, and hydroxypropylmethyl cellulose (HPMC) were purchased from Sigma Chemical Co. (St. Louis, MO). 1-(2-(Decylthio)ethyl)-azacyclopentan-2-one (HPE-101) was obtained from Hisamitsu Pharmaceutical Co. (Tokyo, Japan). Transcutol was provided by Gattefossé (Cedex, France) as a gift. The Backing laminate (Scotchpak® Heat Sealable Polyester Film #1006), the release liner (Scotchpak® Low Adhesion Polyester Film #1022) and ethylene/vinyl acetate (EVA) copolymer membrane with varying weight fractions (%, w/w) of vinyl acetate (VAc) were gifts from 3M Co. (St. Paul, MN). Duro-Tak® adhesives were provided by National Starch and Chemical Co. (Bridgewater, NJ). HPLC grade acetonitrile and EtOH were purchased from Fisher Scientific (Fair Lawn, USA). All other chemicals were reagent grade or higher, and were used as received.

2.2. Solubility study

The solubility of TS in an EtOH/water (70:30, v/v) cosolvent system with or without various skin permeation enhancer (i.e., oleic acid, dodecylamine, lauric acid, HPE-101, or transcutol) was measured at 37°C. An excess of TS was added to the system and mixed by vortexing. The solution was immersed in a shaking water bath at 37°C and allowed to equilibrate for 48 h. The saturated solutions were then filtered through Minisart RC four filters (0.45 μm, Sartorius, Germany). Concentrations of TS were analyzed by HPLC after appropriate dilution with methanol.

2.3. Preparation of rat skin

The animals used for the preparation of skin were male Sprague Dawley (220–250g) rats obtained from Dae-Han Laboratory Animal Research Center Co. (Taejon, Korea). They could have a free access to food and water until used for experiments, and were sacrificed in a CO_2 chamber right before the experiments. The dorsal hairs were removed with a clipper and full-thickness skin (about 16 cm^2) was surgically removed from each rat. The skin specimen was cut into appropriate sizes after carefully removing subcutaneous fat and washing with normal saline.

2.4. In vitro permeation study

In vitro skin permeation across rat skin was conducted with Keshary–Chien diffusion cells at

37°C. Freshly excised rat skin was mounted between the donor and receptor cell (stratum corneum side facing the donor). The area of diffusion for all in vitro experiments was 2.14 cm^2. EtOH/water (70:30) cosolvent system was chosen as a vehicle based on our earlier study (Kim et al., 2000) and various enhancers were added to increase the permeation rate of TS. The donor cells, which faced the stratum corneum surface, contained a saturated solution (3.0 ml) of TS in the vehicle with or without skin permeation enhancer and/or gelling agent, and were occluded with Parafilm. The receptor cells, which faced the dermis side, were filled with normal saline solution containing 40% (v/v) polyethylene glycol 400 to maintain sink condition (12.0 ml). At predetermined time intervals, 0.5 ml of the receptor solution was withdrawn and refilled with the same volume of fresh receptor solution. Samples were kept in a freezer (-20°C) until analyzed by the high performance liquid chromatography (HPLC).

The experimental conditions to study the permeation kinetics of TS across the EVA copolymer membrane were the same as those outlined above for the skin permeation studies, except that the copolymer membrane with various weight fractions of VAc was used in place of the skin sample.

The EVA membrane coated with various Duro-Tak® adhesives was mounted on the skin and the rate of permeation across the membrane/skin composite was also determined. The experimental conditions were the same as those outlined above, except that the membrane/skin composite was used in place of the EVA membrane. Finally, various concentrations of HPMC (0–5%) was added to the donor solution as a gelling agent, and the skin permeation rate of TS across the membrane/skin composite was determined.

2.5. HPLC analysis of testosterone

Concentrations of TS were determined using a HPLC system equipped with a binary pump (Gilson Model 305 and 306) and automatic injector (Gilson Model 234). A Merck C_{18} LiChroCAT 125-4 column (5 μm, 125×4 mm, Merck, Darmstadt, Germany) was used as an analytical column at ambient temperature. The mobile phase was an acetonitrile-acetate buffer (50 mM, pH 4.0) combination (60:40, v/v) at a flow rate of 1.0 ml/min. The variable wavelength ultraviolet detector (Gilson Model 118) was set at 242 nm. Injections of 20 μl were made for all solutions to be analyzed. Retention time of TS was about 3.3 min.

2.6. Determination of viscosity

Viscosity of reservoir solution of TS saturated in EtOH/water (70:30) cosolvent system with 1.0% skin permeation enhancer (i.e., dodecylamine) and various concentration of gelling agent (i.e. HPMC) was measured using a Brookfield viscometer (model LVT, Brookfield Engineering Lab., Stoughton, MA) at 60 rpm.

2.7. Fabrication of experimental reservoir-type transdermal delivery system

An experimental reservoir-type transdermal delivery system of TS was fabricated by encapsulating the TS reservoir solution within a shallow compartment molded from a drug impermeable backing laminate and a rate-controlling membrane. Reservoir solution consisted of TS saturated in EtOH/water (70:30) cosolvent system containing a skin permeation enhancer and gelling agent. Rate-controlling membrane was EVA membrane containing 28% (w/w) VAc. To ensure intimate contact of the transdermal patch to the skin, a pressure-sensitive adhesive polymer was coated onto the EVA membrane.

The EVA membrane was placed over on the adhesive-coated release liner, and then the backing laminate was placed on it. The composite was heat sealed and cut to the appropriate size. The TS reservoir solution (2.0 ml) was dispersed into the device using a disposable syringe. The device was heat sealed again to close the unsealed side of the device ensuring no reservoir leaking out of the device. Then, each patch was kept in a sealed aluminum pouch to minimize the loss of ethanol.

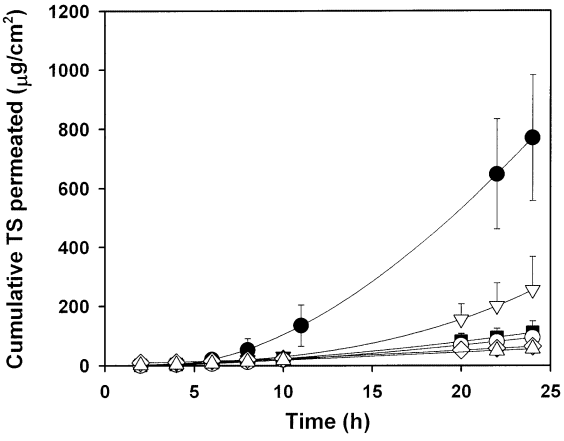

Fig. 1. Effect of skin permeation enhancers (1.0% w/v) on the permeation of TS saturated in ethanol/water (70:30) cosolvent system: (●) dodecylamine; (▽) oleic acid; (■) lauric acid; (○) HPE-101; (◇) transcutol; (△) no enhancer. The bars represent SD, $n = 3$.

2.8. In vivo evaluation of the experimental patch

Rats were lightly ether anesthetized and abdominal hairs were removed with a hair clipper. After 1 day, rats were fixed at supine position and under light ether anesthesia, femoral arteries of the rats were cannulated with a polyethylene tube (SP-45, Natume Co., Tokyo, Japan) for blood sampling. Experimental patch (surface area = 3.5×3.5 cm^2) or Androderm® (surface area = 7.5 cm^2) were applied on the abdominal skin of rat. Blood samples (150 μl) were withdrawn at predetermined time intervals for 24 h, and were centrifuged for 5 min at 3000 rpm. The plasma was separated and kept at −20°C until analyzed by radioimmunoassay (RIA).

2.9. Radioimmunoassay of testosterone

Plasma concentration of TS was measured by RIA using the reagents and protocol supplied by Diagnostic Products Corporation (Los Angeles, CA). Fifty microliters of plasma was used for assay. The lower limit of detection was 2 ng/ml.

3. Results and discussion

3.1. Effect of permeation enhancers on the skin permeation of TS

The feasibility of using dodecylamine, oleic acid, lauric acid, HPE-101, or transcutol to further improve the skin permeation rate of TS beyond what was achieved by using EtOH/water (70:30) cosolvent system, was investigated. A synergistic effect on the skin permeation rate of TS was observed by adding 1.0% (w/v) of various enhancers to the cosolvent system as shown in Fig. 1. Among the five different enhancers, dodecylamine was the most effective, increasing the permeation rate of TS from 2.68 to 47.83 μg/cm^2/h at a concentration of 1.0% (w/v). Since there was no significant difference in the solubility of TS by adding dodecylamine (Table 1), this result implies that dodecylamine increased the permeation rate of TS by increasing the permeability coefficient of TS about 25 times.

Table 1
Rat skin permeation parameters of testosterone saturated in ethanol/water (70:30) cosolvent system with 1.0%(w/v) of various enhancers at 37°C[a]

Enhancer	Permeation rate (μg/cm^2/h)	Solubility (mg/ml)	Permeability coefficient (cm/h) × 10^3	Lag time (h)
Dodecylamine	47.83 ± 5.39	66.71 ± 0.76	0.72 ± 0.08	7.09 ± 3.64
Oleic acid	13.98 ± 6.23	68.71 ± 7.61	0.20 ± 0.09	7.04 ± 1.87
Lauric acid	5.72 ± 2.03	66.35 ± 2.87	0.09 ± 0.03	5.50 ± 0.71
HPE-101	4.93 ± 2.07	64.11 ± 5.29	0.08 ± 0.03	5.68 ± 1.93
Transcutol	2.86 ± 0.41	69.05 ± 6.52	0.04 ± 0.01	1.96 ± 1.04
No enhancer	2.68 ± 0.68	68.31 ± 2.92	0.04 ± 0.01	2.88 ± 0.84

[a] Each data is the mean ± SD of three determinations.

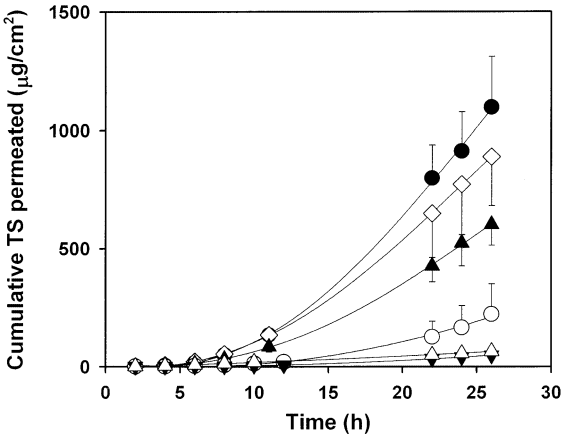

Fig. 2. Effect of dodecylamine concentration (%, w/v) on the permeation of TS saturated in ethanol/water (70:30) cosolvent system: (●) 1.2%; (◇) 1.0%; (▲) 0.7%; (○) 0.5%; (▼) 0.3%; (△) 0%. The bars represent SD, $n = 3$.

Fatty acids and amines are known to have a potent skin permeation-enhancing effect when used alone or in combination with alcohols or glycols (Cooper, 1984; Williams and Bary, 1992). These effects appear to involve the disruption of lipid bilayer that are filling the extracellular spaces of the stratum corneum. Unsaturated fatty acids particularly affect the fluidity of lipids in the intercellular layers of the stratum corneum because of their resemblance in structure to the lipids (Kim et al., 1996). Dodecylamine is a typical unsaturated fatty amine, which is known to increase the skin permeation rate of various drugs (Aungst et al., 1990). When dodecylamine was added in propylene glycol, skin permeation rate of TS significantly increased by increasing the partitioning of TS on the skin, thereby increasing the permeability coefficient (Aungst et al., 1990).

To estimate the effect of the concentrations of dodecylamine, various concentrations (0.1, 0.3, 0.5, 0.7, 1, 1.2%) were added to the EtOH/water (70:30) system. Fig. 2 shows the permeation profiles of TS saturated in 70% (v/v) of ethanol containing various concentration of dodecylamine. Permeation of TS increased proportionally as the concentration of dodecylamine increased from 0.1% up to 1.2% in EtOH/water (70:30) cosolvent system (Table 2). However, because of the possibility of irritation due to a high concentration, for further studies, 1.0% (w/v) dodecylamine was chosen for the reservoir formulation of TS saturated in EtOH/water (70:30) cosolvent system.

3.2. Effect of EVA membrane on the controlled release of TS

To control the release of TS from the reservoir compartment, EVA copolymer membrane was selected as the rate-controlling membrane. EVA copolymer membranes used in this study had various vinyl acetate content [4.5–28% (w/w)] with the average thickness of 50.10 ($\pm$2.01) μm. When TS was saturated in 70% (v/v) ethanolic solution containing 1.0% (w/v) dodecylamine, the permeation profiles of TS across the EVA copolymer membrane was as shown in Fig. 3A. The membrane permeation rate of TS varied with different weight fractions of vinyl acetate in

Table 2
Rat skin permeation parameters of testosterone saturated in ethanol/water (70:30) cosolvent system with various concentration of dodecylamine at 37°C[a]

Dodecylamine (%. w/v)	Permeation rate (μg/cm²/h)	Solubility (mg/ml)	Permeability coefficient (cm/h) × 10³	Lag time (h)
0.1	2.80 ± 0.24	64.63 ± 1.38	0.04 ± 0.00	8.05 ± 0.10
0.3	4.12 ± 0.69	66.28 ± 7.61	0.06 ± 0.01	9.64 ± 0.26
0.5	13.46 ± 3.07	64.63 ± 2.87	0.21 ± 0.05	9.03 ± 1.14
0.7	36.97 ± 0.61	74.23 ± 8.29	0.50 ± 0.01	8.98 ± 2.41
1.0	47.83 ± 5.39	66.71 ± 0.76	0.72 ± 0.08	7.09 ± 3.64
1.2	68.63 ± 8.42	69.79 ± 2.92	0.98 ± 0.12	6.90 ± 1.94

[a] Each data is the mean ± SD of three determinations.

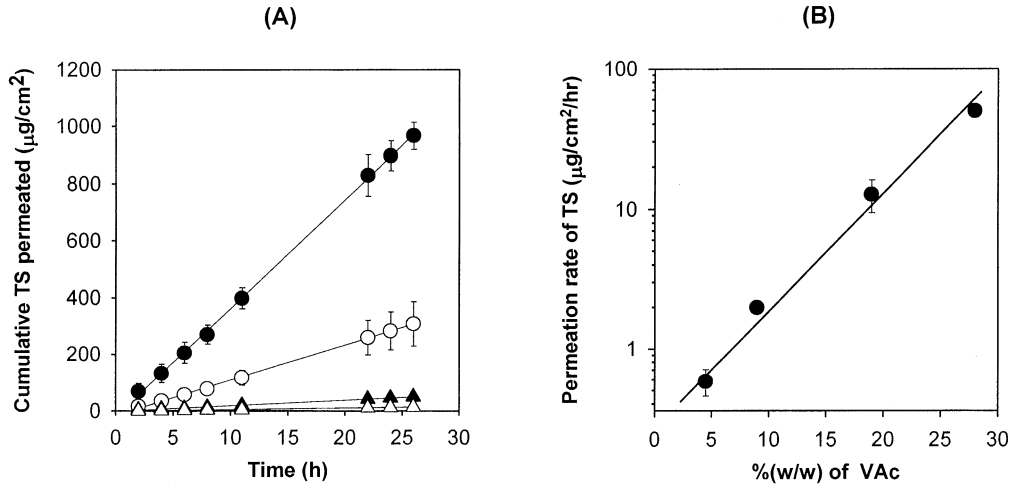

Fig. 3. (A) Permeation profiles of TS across the EVA membrane with various weight fractions (%, w/w) of vinyl acetate: (●) 28%; (○) 19%; (▲) 9%; (△) 4.5%. (B) Relationship between skin flux of TS and increasing concentration of vinyl acetate in EVA membrane. The bars represent SD, $n = 3$.

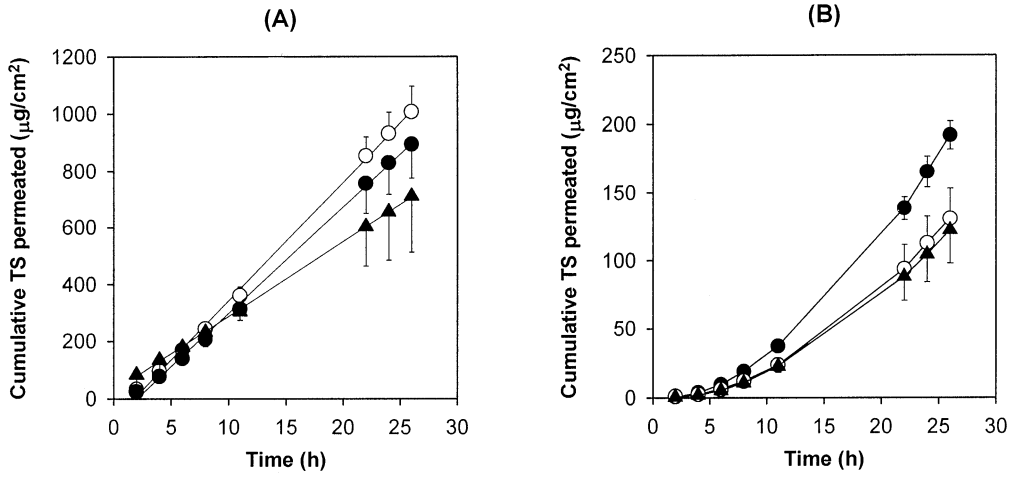

Fig. 4. (A) Permeation profiles of TS across the EVA membrane (28% VAc) coated with various adhesives: (○) Duro-Tak 73-9261; (●) Duro-Tak 87-2510; (▲) Duro-Tak 87-2516. (B) Permeation profiles of TS across the membrane/skin composite. The bars represent SD, $n = 3$.

the EVA copolymer membrane. A logarithmic relationship exists between the permeation rate of TS across the EVA membrane and the vinyl acetate content in the EVA membrane (Fig. 3B), which is consistent with a previous report of levonorgestrel (Chen et al., 1995). The steady-state permeation rate through the EVA membrane containing 28% vinyl acetate was 49.71 ($\pm$4.26) μg/cm²/h.

3.3. Permeation of TS through membrane/skin composite

The EVA copolymer containing 28% vinyl acetate was chosen for further studies, and various Duro-Tak® adhesives were coated with the thickness of 5.0 μm. Fig. 4A shows the permeation profiles of TS through the EVA membrane coated

with various adhesives, and Fig. 4B shows those through membrane/skin composite. When the EVA membrane was coated with Duro-Tak 87-2516, the permeation rate of TS was slightly lower compared to those when Duro-Tak 73-9261 or 87-2510 was coated (Fig. 4A). But, there was no significant difference in permeation rate of TS between Duro-Tak 73-9261 and 87-2510 (Table 3). However, when the EVA membrane coated with Duro-Tak 87-2510 was placed on the rat skin, the permeation rate of TS through the membrane/skin composite was significantly higher than when Duro-Tak 73-9261 or 87-2516 was coated, probably due to its higher adhesion than the others (Hong et al., 2001).

The permeation rate of TS across the membrane/skin composite barrier can be defined by:

$$(Q/t)_{m/s} = P_{m/s}C_d \quad (1)$$

where C_d is the solubility of TS in the reservoir solution and $P_{m/s}$ is the permeability coefficient of TS across the membrane/skin composite, which can be expressed by:

$$\frac{1}{P_{m/s}} = \frac{1}{P_m} + \frac{1}{P_s} \quad (2)$$

where P_m and P_s are the permeability coefficient of membrane and skin, respectively.

As shown in Table 3, permeability coefficients obtained from the experiment were almost one third of the calculated value from the Eq. (2). In the case of the Duro-Tak 87-2510, the permeability coefficient of TS through the combined layer (8.67 ± 0.67 μg /cm^2/h) was much lower than that calculated by Eq. (2) (20.35 μg/cm^2/h). This may be due to the incomplete adhesion of EVA membrane to the skin, which resulted in the barrier between the membrane and the skin. Based on these results, Duro-Tak 87-2510 was selected for further study, and was coated on the EVA membrane containing 28% VAc.

3.4. Effect of gelling agent, HPMC

In order to increase the viscosity of the reservoir solution, various concentrations of HPMC were added in the solution since it is known as the most viscous polymer among cellulose derivatives (Vazquez et al., 1992). After saturating TS in 70% ethanolic solution with 1.0% dodecylamine, the effect of various concentrations of HPMC on the permeation profiles across the EVA membrane/skin composite was studied. As shown in Fig. 5, the viscosity of the reservoir solution significantly increased showing logarithmic relationship as the concentration of HPMC increased up to 5.0% (w/v). As expected, the permeation rate of TS decreased from 8.67 μg/cm^2/h to 5.66 (0.79) μg/cm^2/h with the addition of 5% (w/v) HPMC. However, the addition of 1.0% (w/v) HPMC al-

Table 3
The effect of various Duro-Tak adhesives on the permeability coefficient [(cm/h) × 10^3] of testosterone across the EVA membrane and (membrane/skin) composite at 37°C[a]

Adhesive (Duro-Tak)	Membrane only	(Membrane/skin) composite		
		Experimental	Calculated[b]	E/C[c]
No adhesive	0.75 ± 0.06	ND[d]	ND[d]	ND[d]
73-9261	0.59 ± 0.05	0.09 ± 0.01	0.32	0.28
87-2510	0.53 ± 0.07	0.13 ± 0.01	0.31	0.42
87-2516	0.36 ± 0.18	0.08 ± 0.01	0.24	0.33

[a] Various Duro-Tak adhesives were coated (5.0 μm thickness) on the ethylene/vinyl acetate (EVA) membrane containing 28% (w/w) vinyl acetate. Solubility of testosterone saturated in the ethanol/water (70:30) cosolvent system containing 1.0% (w/v) dodecylamine was 66.71($\pm$0.76) mg/ml. Each data is the mean $\pm$ SD of three determinations.

[b] Calculated from: $(1/P_{m/s}) = [(1/P_m) + (1/P_s)]$, where, P_m and P_S are the permeability coefficient of membrane and skin, respectively.

[c] $E/C = P_{m/s}$ (experimental)/$P_{m/s}$ (calculated).

[d] Not determined.

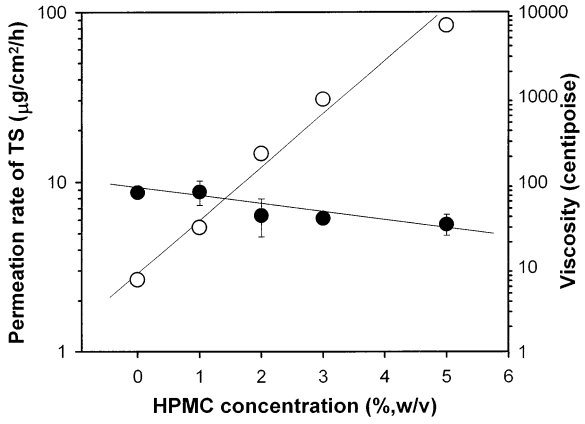

Fig. 5. Effect of gelling agent (HPMC) concentration (%, w/v) on (●) the permeation rate of TS across the membrane/skin composite and on (○) the viscosity of the reservoir solution. TS was saturated in ethanol/water (70:30) cosolvent system with 1.0% (w/v) dodecylamine. EVA membrane (28% VAc) was coated with Duro-Tak 87-2510. The bars represent SD, $n = 3$.

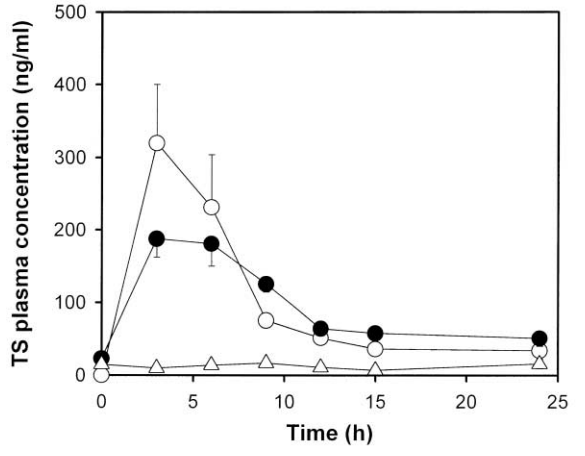

Fig. 6. Plasma concentration profiles of TS following the application of (○) the experimental patch and (●) the commercial product (Androderm®) in rats. (△) Control is the TS plasma concentration without patch application. The bars represent SE, $n = 4–9$.

ready resulted in the desirable rheological properties with the insignificant reduction in TS permeation rate (8.73 ± 1.44 μg/cm²/h). Thus, the final reservoir formulation was decided as TS saturated in EtOH/water (70:30) cosolvent system containing 1.0%(w/v) dodecylamine and 1.0% (w/v) HPMC.

3.5. In vivo evaluation of the experimental patch

Fig. 6 shows the plasma TS concentration versus time profiles following the application of the experimental patch or Androderm®. Basal plasma TS concentration profile of rats without patch application was also observed as a control. The experimental patch (12.25 cm²) and Androderm® (7.50 cm²) showed comparable plasma concentration profiles. Plasma concentration of TS significantly increased after applying the TS patch, and reached the maximum concentration within 3 h after application. Rapid increase in the plasma concentration might be partially due to the initial burst release and absorption of ethanol in the reservoir formulation. However, high plasma concentration of TS maintained only for 12 h probably due to the depletion of ethanol in the formulation afterward. We believe that the plasma concentration of TS can be maintained for longer duration by controlling the burst release of the ethanol and/or by preventing the depletion of ethanol. Further studies are under way in this laboratory to develop a reservoir-type transdermal delivery system of TS with higher permeation rate and longer duration by controlling the composition of the reservoir formulation, especially with the appropriate selection of the gelling agent(s).

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