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In Vitro and In Vivo Evaluation of a Novel Nonscrotal Matrix-Type Transdermal Delivery System of Testosterone

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ABSTRACT A matrix-type testosterone (TS) transdermal delivery system for nonscrotal skin was developed with DuroTak® 87-2510 as an adhesive polymer. When 3% dodecylamine was used in combination with 10% span 80, the in vitro rat permeation rate increased from 2.29 μg/cm^2/hr to 6.51 μg/cm^2/hr as the TS loading dose increased from 2% to 6%. The maximum flux of experimental patch was about 14-fold higher than that of Testoderm®. Release kinetics of TS from the patches was proportional to $t^{1/2}$ following the Higuchi equation, and the release rate of TS increased as TS loading dose increased. Also, a good linear relationship between the skin permeation rate and the release rate was observed, which implies that the release rate is the rate-limiting process of the skin permeation. In vivo study showed that the plasma concentration of TS promptly increased and reached the peak level within 3–6 hours of application of the experimental patch. Area under the curve ($AUC_{0\sim48}$) and C_{max} also linearly increased in a dose-dependent manner up to 6% of TS. These results demonstrate the feasibility of developing a nonscrotal matrix-type transdermal delivery system of TS.

KEYWORDS Testosterone, Matrix-type patch, Transdermal delivery

INTRODUCTION

Testosterone (TS) replacement therapy for men with hypogonadism has been traditionally conducted with oral, injection, or transdermal preparations. Among them, transdermal delivery system (TDS) of TS is considered to be safer and more effective than the injection method (Misra et al., 1995, 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 noninvasive zero-order delivery are the well documented advantages of transdermal route of administration (Williams & Bary, 1992).

There are two commercially available patch-type TDS of TS in the market: the reservoir-type non-scrotal TDS (Androderm®) and matrix-type scrotal TDS

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(Testoderm®). Matrix-type scrotal TDS must be applied on the scrotal skin because of its low skin permeation rate (Jordan, 1997). Also, it undergoes transdermal first-pass metabolism due to the high level of 5α-reductase present in scrotal skin, which may also result in enlargement of the prostate (Tamura et al., 1996; Wayne et al., 1996). Nonscrotal reservoir-type TDS often causes skin irritation due to high ethanol concentration as well as other irritants (Jordan, 1997), and its preparation process is costly and complex. Thus, the development of a nonscrotal matrix-type transdermal system of TS is expected to achieve an effective, safe, and convenient TS replacement therapy.

The purpose of this study was to formulate a new matrix-type transdermal system of TS that can be applied on the nonscrotal skin. Using DuroTak® 87-2510 as an adhesive polymer, span 80 and dodecylamine were added as a solubilizer and an enhancer, respectively, based on our previous studies (Kim et al., 2001; Zhao et al., 2002). In vitro and in vivo studies were conducted in the rat model, and compared with Testoderm®, to investigate the feasibility of the matrix-type nonscrotal transdermal delivery system of TS.

EXPERIMENTAL

Materials and Animals

TS and dodecylamine were purchased by Sigma (St. Louis, MO), and span 80 was purchased from Yakuri Pure Chemical Industry Co. (Osaka, Japan). DuroTak® 87-2510 was kindly supplied by National Starch & Chemical Company (Bridgewater, NJ) as a gift. Release liner Scotch Brand Tape was obtained from 3M (St. Paul, MN). Polyurethane backing laminate was obtained as a gift from Il-Yang Pharm Ltd. (Seoul, Korea). All other chemicals were reagent grade or better, and were used as received.

The animals used for the in vitro and in vivo studies were male Sprague Dawley (220–250 g) rats purchased from Dae-Han Laboratory Animal Research Center Co. (Dae-Jeon, Korea). The rats had free access to food and water until they were used for experiments.

Preparation of Matrix-Type Transdermal Systems

Various amounts of TS (0.2–0.6 g) and dodecylamine (0.3 g) were first dissolved in the minimum volume of ethanol, and then mixed with 8.7 g of adhesive polymer (DuroTak® 87-2510) and 1.0 g of span 80 using a mechanical stirrer at 800 rpm for 15 minutes under occluded condition until a clear solution was obtained. After degassing the mixture using an ultrasonicator, the mixture was cast on the release liner using a micrometer adjustable casting knife (R.K. Coat Instruments LTD., U.K.), which was set at 100 μm. After drying for 24 hours at ambient temperature, the adhesive was covered with the backing laminate. Finally, the patches were cut into 4 cm × 5 cm sizes and sealed in a pouch until used for future evaluation.

In Vitro Skin Permeation Study

The rats were sacrificed in a CO_2 chamber right before the experiments. The dorsal hair was removed with a clipper and full-thickness skin (about 16 cm^2) was surgically removed from each rat. The fat and connective tissue were removed from skin. The skin specimens were cut into appropriate sizes after carefully washing with normal saline.

In vitro skin permeation of TS was investigated using the Valia-Chien skin permeation system (diffusion area of 0.64 cm^2 and volume of 3.5 mL) at 37°C. The receptor solution was composed of 40% PEG 400 in isotonic phosphate buffer (pH 7.4) solution to maintain the sink condition. After applying a patch on the stratum corneum side of the skin, aliquots (400 μL) of receptor solutions were withdrawn at predetermined time intervals and immediately replaced with an equal volume of fresh solution. The TS content in the samples was determined by high-performance liquid chromatography (HPLC).

In Vitro Release Study

Keshary-Chien diffusion cells (diffusion area of 2.27 cm^2 and volume of 15 mL) were used at 37°C to study the release profiles of TS from the adhesives. Each TS patch was mounted between the donor and receptor compartments, facing the adhesive layer with the receptor solution composed of 40% PEG 400 in isotonic phosphate buffer (pH 7.4) solution. At predetermined time intervals, the receptor solution (400 μL) was withdrawn, and the same volume of fresh solution was immediately replaced. The amount of drug released was determined by HPLC.

HPLC Analysis of TS

An HPLC system equipped with a binary pump system (Gilson Model 305 and 306) and an auto-injector (Gilson Model 234) was used to determine the concentration of TS. A Merck C_{18} LiChroCART 125×4 column (5 μm particle size, 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) at a flow rate of 1.0 mL/min. The variable wavelength UV detector (Gilson Model 118) was set at 242 nm. All solutions to be analyzed were injected at a volume of 20 μL. There was no interference from the matrix or skin, and TS yielded a single peak at approximately 6 minutes.

In Vivo Study

Abdominal hair of male Sprague Dawley rats (220–250 g) was carefully removed using a clipper one day before the experiments. The rats were fixed in a supine position under light ether anesthesia, and the femoral artery was cannulated with polyethylene tube (PE-45, Intramedic, Clay Adams, USA) for blood sampling. An experimental patch or Testoderm® was cut to 4 cm × 5 cm size, and was applied on the abdominal skin of each rat. Blood samples (150 μL) were withdrawn at predetermined time intervals for 48 hour. Blood samples without TS patch application were also taken to determine the basal TS concentration. Plasma was immediately separated by centrifugation, and was stored at −20°C until analyzed by radioimmunoassay (RIA).

The plasma concentration of TS was determined by RIA using a commercially available kit supplied by the Diagnostic Products Corporation (DPC, Los Angeles, California). Fifty microliters of plasma was used for assay. The lower limit of detection was 2 ng/mL.

TABLE 1 **The Effect of Testosterone Concentration on the Rat Skin Permeation Rate and Lag Time[a]**

Testosterone concentration (%)	Permeation rate (μg/cm²/hr)	Lag time (hr)
2	2.29 (±0.10)	6.96 (±3.50)
4	4.07 (±0.86)	8.44 (±1.41)
6	6.51 (±1.05)	7.02 (±1.20)
Testoderm®	0.46 (±0.14)	3.52 (±0.74)

Each system contains 10% Span 80, 3% dodecylamine, and various concentration of TS in DuroTak® 87-2510.

[a]Each value is the mean ± SD (n = 3).

RESULTS

Effect of TS Loading Dose on the In Vitro Skin Permeation

Our previous report Zhao et al., 2002 showed that span 80 and dodecylamine synergistically increased the solubility of TS in the adhesive polymers, thereby enhancing the skin permeation rate of TS. Since the maximum permeation rate was achieved when 3% dodecylamine was used in combination with 10% span 80, various loading doses of TS was incorporated

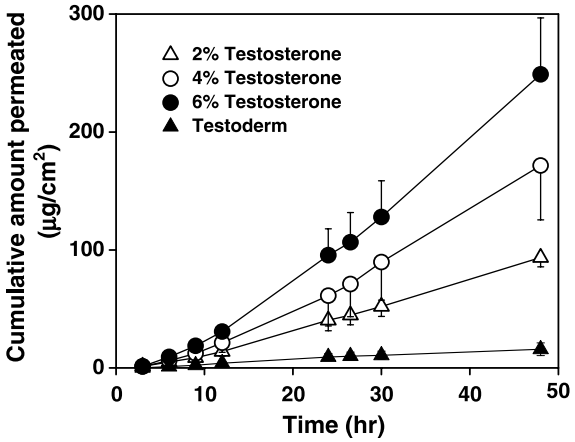

FIGURE 1 **Effect of TS Loading Doses on Rat Skin Permeation Profiles of TS Patches. Each System Contained 10% Span 80 and 3% Dodecylamine in DuroTak® 87-2510 as Adhesive. The Permeation Profile of Testoderm® was Included as a Comparison (n = 3, mean ± SD).**

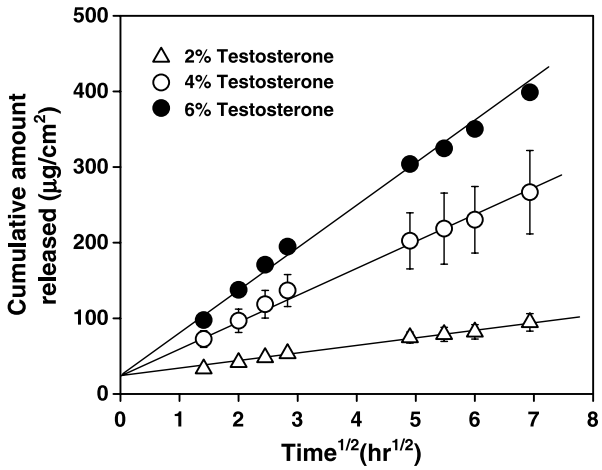

FIGURE 2 **The Release Profiles of TS from Transdermal Delivery Systems Containing Various Loading Doses of TS. Each System Contained 10% Span 80 and 3% Dodecylamine in DuroTak® 87-2510 as Adhesive (n = 3, mean ± SD).**

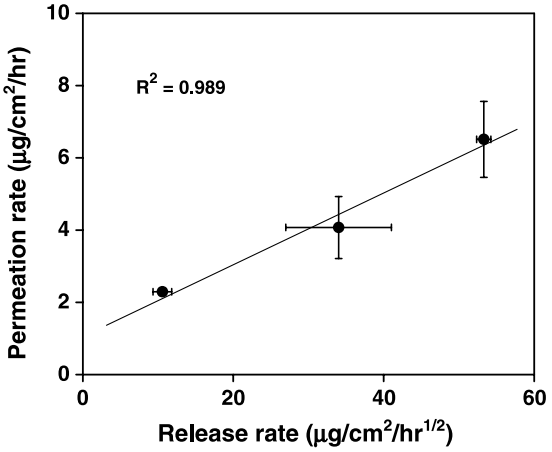

FIGURE 3 **Relationship Between the Release Rate and the Permeation Rate of TS. Each System Contained 2–6% TS, 10% Span 80, and 3% Dodecylamine in DuroTak® 87-2510 as Adhesive (n=3, mean±SD).**

in this formulations for further enhancement of the permeation rate. As shown in Fig. 1, the skin permeation rate increased from 2.29 μg/cm^2/hr to 6.51 μg/cm^2/hr as the TS loading dose increased from 2% to 6% (Table 1). The maximum permeation achieved in this study was about 14-fold higher than that of Testoderm® (0.46 μg/cm^2/hr). However, the lag time of Testoderm® (3.51 hour) was almost half of the experimental patches (about 7–8 hours).

In Vitro Release Kinetics

Figure 2 shows the release profiles of TS from the experimental patches with different TS loading doses. The cumulative amount of TS released was proportional to the square root of time, following the Higuchi equation Higuchi, 1961. As the TS loading doses increased up to 6%, the release rate also proportionally increased. It is interesting to note a good linear relationship between the release rate and the skin permeation rate (Fig. 3). This implies that the release rate of TS from the patch is the rate-limiting step of the skin permeation process.

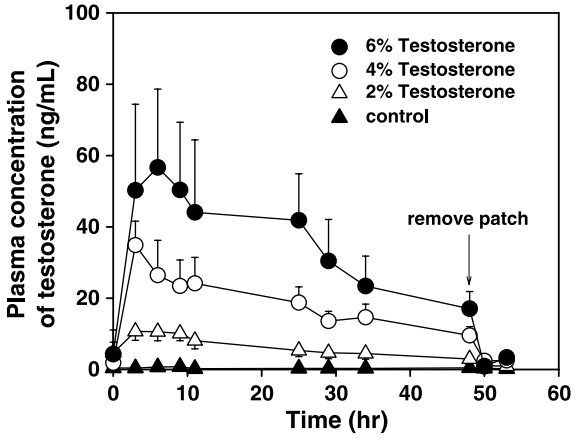

FIGURE 4 **Plasma Concentration Profiles of TS After Applying a Transdermal Delivery System (20 cm^2) of TS on the Rat Abdominal Skin. Each System Contained 2–6% TS, 10% Span 80, and 3% Dodecylamine in DuroTak® 87-2510 as Adhesive (n=4–9, mean±SD).**

TABLE 2 **The Effect of Testosterone Concentration on C_{max} and Area Under the Curve (AUC_{0-48}) When an Experimental Patch (20 cm^2) Was Applied on Rat Abdominal Skin[a]**

TS concentration (%)	C_{max} (ng/mL)	AUC_{0-48} (ng·hr/mL)
2	10.68 (±2.50)	299.68 (±84.14)
4	34.87 (±6.70)	885.68 (±207.17)
6	56.64 (±21.97)	1600.44 (±627.19)

Each system contains 10% Span 80, 3% dodecylamine, and various concentrations of TS in DuroTak® 87-2510.
[a]Each value is the mean±SD (n=4–9).

In Vivo Study

Figure 4 shows the plasma concentration profiles of TS after applying an experimental patch with various concentrations of TS on the rat abdominal skin. Basal plasma TS concentration profile of rats without patch application was also observed as a control. After applying an experimental patch, plasma concentration of TS promptly increased, and reached the peak level within 3–6 hours of application. When the patch was removed after 48 hours, plasma TS concentration rapidly fell to the basal level within 5 hours. As the TS loading dose in the experimental patch increased up to 6%, plasma TS concentration increased accordingly. Table 2 shows that C_{max} increased from 10.68 ng/mL up to 56.64 ng/mL as the TS loading dose increased from 2% to 6%. Also, $AUC_{0\sim48}$ calculated by the trapezoidal rule linearly increased as the TS loading dose in the adhesive polymer increased up to 6% (r^2=0.994).

DISCUSSION

In our previous study (Zhao et al., 2002), a matrix-type transdermal delivery system composed of 6% TS, 10% span 80, and 3% dodecylamine in DuroTak® 87-2516 could achieve the rat skin permeation rate of 4.14 (±0.34) μg/cm^2/hr. When DuroTak® 87-2510

was used in this study, the skin permeation rate of TS further increased up to 6.51 (±1.05) μg/cm^2/hr with the same formulation. DuroTak® 87-2516 is an acrylate-vinylacetate copolymer with hydroxy functional group, while DuroTak® 87-2510 is an acrylic copolymer type without functional groups. According to the information from the manufacturer, DuroTak® 87-2510 has higher adhesion force than DuroTak® 87-2516. Higher skin permeation rate was also achieved by DuroTak® 87-2510 than DuroTak® 87-2516 in the formulation of a reservoir-type TS transdermal delivery system (Kim et al., 2001). DuroTak® 87-2510 seems to enhance the skin permeation rate by higher adhesion to skin and/or by higher compatibility with TS, thereby causing higher release rate of TS than the other adhesives.

The highest skin permeation rate achieved in this study (6.51±1.05 μg/cm^2/hr) is equivalent to delivering about 6.25 mg of TS per day, supposing that a patch with the surface area of 40 cm^2 is applied. A commercially available matrix-type product (Testoderm®) is designed to administer 4 mg of TS per day when the same size patch is applied on the scrotal skin. Since the rat skin permeation rate is known to be generally higher than that through the human skin (Kim & Chien, 1996), further enhancement may still be required in order to apply to human nonscrotal skin. However, since the nonscrotal-skin application can bypass the first-pass metabolism in the scrotal skin by 5α-reductase, the therapeutic plasma concentration might be achieved by lower skin permeation rate than expected, which will be determined in future clinical studies. The significance of this study is that it supports the feasibility of developing a nonscrotal matrix-type TDS for TS that can increase the patient compliance.

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