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In vitro and *In vivo* Evaluation of Novel Gel Formulations of Testosterone for Transdermal Delivery

Sung-Koun Heo*, Young-Seok Cho, Sang Dae Han**, Jin Kang Chang**, Eun Ju Yoon**, Dae Woong Ko**, Chang-Baeg Lim**, Suk-Jae Chung***, Chang-Koo Shim*** and Dae-Duk Kim***†**

**College of Pharmacy, Pusan National University, Busan 609-735, Korea*
***Dong Sung Pharm. Co., Ltd., Asan 337-870, Korea*
****College of Pharmacy and Research Institute of Pharmaceutical Sciences, Seoul National University, Seoul 151-742, Korea*
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ABSTRACT – HPMC-based novel gel formulations for the transdermal delivery of testosterone (TS) were developed, and the effect of various skin permeation enhancers was studied *in vitro* and *in vivo*. *In vitro* hairless mouse skin permeation of TS from the gel was investigated using Keshary-Chien diffusion cells for 8 hours at 37°C. *In vivo* plasma concentration profiles of TS after applying the gel on the abdominal skin of rat were determined using a commercial radioimmunoassay kit. Hairless mouse skin permeation of TS increased with the addition of permeation enhancers both *in vitro* and *in vivo*. Combination of diethanolamine (2%) and N-methylpyrrolidone (NMP, 6%) was the most effective among tested. Plasma concentration of TS significantly increased for at least 24 hours with the addition of diethanolamine and NMP. These results suggest the feasibility of the development of a HPMC-based gel formulation for the transdermal delivery of TS.

Key words – Testosterone, Permeation enhancer, Pharmacokinetics, Transdermal delivery, Gel

Testosterone (TS) is the primary androgenic hormone secreted by the testis in men, while only small amounts of TS are synthesized in the ovary and adrenal in women.[1,2] It is responsible for the normal growth and development of the male sex organs and for maintenance of secondary sex characteristics. As a therapeutic agent for men, TS is used for substitutional therapy for climacteric symptoms or for hypogonadism, which comes from a primary defect of the testes or from a disorder of the hypothalamus or anterior pituitary resulting in inadequate gonadotropic stimulation of the testes. For women, TS therapy may be efficacious when administered with estrogens in the treatment of menopause. TS also finds widespread application in the palliative treatment of breast cancer in women.[3]

Until recently, researchers have studied a variety of different methods for TS administration, including transdermal delivery system,[4] topical spray and topical aerosol,[5,6] sublingual tablets,[7] and subcutaneous implants.[8] Among them, the transdermal route is considered to be safer and more effective than injection methods. Avoidance of hepatic first-pass elimination, decrease in side effect, 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.[9] Nevertheless, transdermal drug delivery has always been challenged by the formidable barrier property of the intercellular lipid bilayer in the stratum corneum.[10] Skin permeation enhancers, such as ethanol, are one of the most convenient methods and have shown to be relatively highly effective. However, high content of ethanol (*i.e.*, 70%) used as a part of the cosolvent system and as a enhancer in the commercial gel formulation of TS sometimes caused skin irritation.

In this study, hydroxylpropylmethylcellulose(HPMC)-based gel containing only 25% ethanol was developed as a novel formulation of TS for transdermal drug delivery. Since it contains only 25% of ethanol, skin irritation can be minimized compared to a commercial product which contains about 70% of ethanol. Based on our previous study to formulate a PVA-based hydrogel,[11] herein the effect of skin permeation enhancers in HPMC-based gels was investigated both *in vitro* and *in vivo*.

Experimental

Material

TS was purchased from TCI (Tokyo, Japan). HPMC 2910 was obtained from Shin-Etsu (Tokyo, Japan). N-methylpryyolidone (NMP), diethanolamine, propylene glycol (PG), butylene glycol (BG) and DMSO were purchased from Sigma

†본 논문에 관한 문의는 이 저자에게로
Tel : 02)880-7870, E-mail : ddkim@snu.ac.kr

Table I–*Compositions of Gel Formulations Investigated in This Study*

Composition	Amount (g) in 100 g of gel		
	Rx 1	Rx 1	Rx 3
Testosterone	1.0	1.0	1.0
HPMC 2910	1.15	1.15	1.15
Ethanol	25	25	25
Propylene glycol	19.8	19.8	19.8
Butylene glycol	13	13	13
DMSO	6	3	--
NMP	--	3	6
Diethanolamine	2	2	2
Other Ingredients	4.2	4.2	4.2
Water	27.85	27.85	27.85

Chemical Co. (St. Louis, MO, USA). All the reagents were of analytical grade or higher.

The animals used for the *in vitro* and *in vivo* studies were male Sprague Dawley (230-270 g) rats and male hairless mice (5-6 weeks) purchased from Orient Co., Ltd. (Gyunggi-do, Korea). They had free access to food and water until they were used for experiments.

Fabrication of the gel

Compositions of various HPMC-based gel formulations containing 1.0% (w/w) TS are shown in Table I. HPMC was first hydrated in the proper quantity of water, and then added with TS in ethanol solution containing PG and BG. After adding enhancer(s), solubilizer and other ingredients, the mixture was thoroughly dissolved using a mechanic stirrer at 4000 rpm under occluded condition until a clear solution was obtained. Gels were sealed in a Felcon tube until used for future evaluation.

***In vitro* skin permeation study**

In vitro skin permeation of TS across the hairless mouse skin was conducted using Keshary-Chien permeation cells (surface area of 2.14 cm^2) at 37°C. Mice were humanely sacrificed in a CO_2 chamber, and then full-thickness skin (about 4 cm^2) was surgically removed from the dorsal site of each mouse. After carefully removing the subcutaneous fat and washing with normal saline, the skin specimen was cut into appropriate sizes. Gel of various compositions (1.0 g) was applied to the stratum corneum side of the skin, and then mounted between the donor and receptor cells (stratum corneum side facing the donor cells). The donor cells were occluded with parafilm to prevent the invasion of other materials and vehicle evaporation. The receptor half-cells were filled with phosphate buffered saline (PBS) solution containing 40% (v/v) PEG 400 to maintain sink condition (12.0 mL). At predetermined time intervals, 1.0 mL of 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 HPLC.

HPLC analysis of testosterone

TS concentrations were determined using a HPLC system (Shimadzu LC-10AD) with a UV detector (Shimadzu SPD-10AV). A J'sphere ODS-H80 column (S-4 μm, 150 × 4.6 mm, YMC Co., Ltd., Japan) was used as the analytical column at ambient temperature. An acetonitrile-water combination (40:60) was used as the mobile phase at a flow rate of 1.0 mL/min. The wavelength of the UV detector was set at 242 nm. Injections of 20 μL were made for all solutions to be analyzed. Retention time of TS was about 11 minutes.

***In vivo* pharmacokinetics study**

Male Sprague-Dawley rats (230-270 g) were lightly ether anesthetized and abdominal hair was removed with a hair clipper. After one day, rats were fixed at supine position under light ether anesthesia, and femoral artery of the rats was cannulated with polyethylene tubing (PE-50, Intramedic, Clay Adams, USA) for blood sampling. After complete recovery (1 hr) from the anesthesia, each gel (2.0 g) was applied on the abdominal skin of rat (surface area = 6 × 4 cm^2). At predetermined time intervals, blood samples (about 150 μL) were withdrawn from the femoral artery for 24 hours, and were immediately centrifuged for 5 min at 8000 rpm. Plasma was kept at -20°C until analyzed.

Plasma concentration of TS was measured by radioimmunoassay (RIA) using reagents and protocol supplied by the Diagnostic Products Corporation (DPC, Los Angeles, California). Fifty microliters of plasma was used for assay. The lower limit of detection was 0.2 ng/mL.

Data analysis

From the plasma-concentration profiles of TS after administration in rats, AUC_{24hr} was calculated from the trapezoidal rule. Time (T_{max}) to reach the maximum plasma concentration (C_{max}) was directly read from the profiles. The differences between the pharmacokinetic parameters of gels were compared by the Student's t-test at the $p < 0.05$ level.

Results and Discussion

In our preliminary studies, diethanolamine was the most effective skin permeation enhancer among tested, thus its syn-

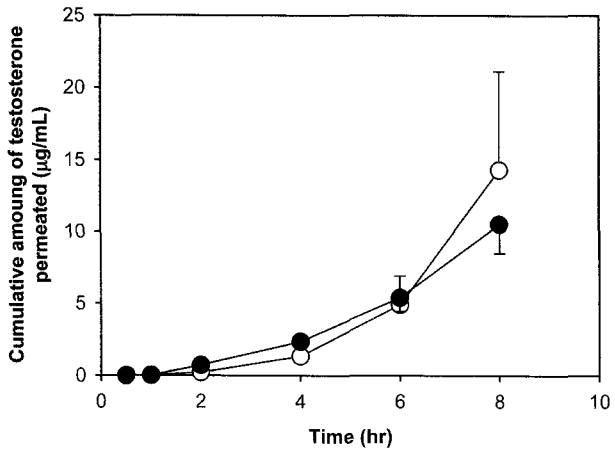

Figure 1–*In vitro* hairless mouse skin permeation profiles of testosterone after applying 1.0 g of HPMC-based gel (Rx 3, ●) or a commercial product (○) using Keshary-Chien permeation cells (surface area of 2.14 cm^2) at 37°C (mean ± standard deviation, n=3~4).

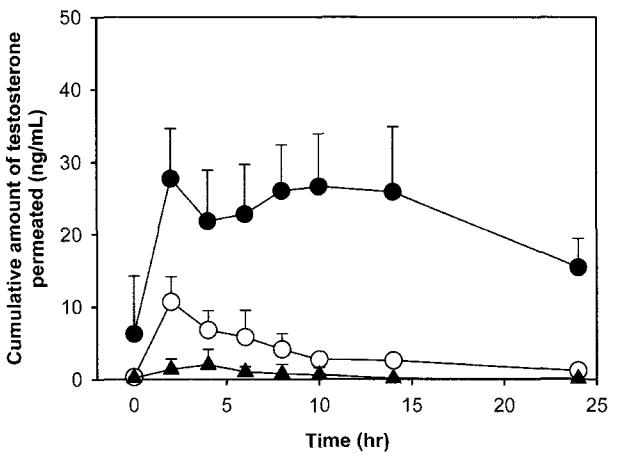

Figure 2–Plasma concentration profiles of testosterone after applying 2.0 g of HPMC-based gel (Rx 3, ●) or a commercial product (○) on the abdominal skin of male Sprague-Dawley rats (surface area = 6 × 4 cm^2) (mean ± standard deviation, n=4). Basal plasma concentration profile of testosterone (▲) was also observed as a control.

Table II–*In vitro Hairless Mouse Skin Permeation Parameters of Testosterone Gel Formulations*

Formulation	Skin permeation parameters	
	Flux (μg/cm^2/hr)	Lag time (hr)
Rx 1	1.80 ± 0.58*	3.30 ± 0.08
Rx 2	2.07 ± 0.22*	3.27 ± 0.12
Rx 3	2.56 ± 0.54	3.87 ± 0.15
Commercial gel	3.24 ± 1.59	3.86 ± 0.08

Each value is the mean ± standard deviation (n=3).
*; $p<0.05$ compared to a commercial gel

ergistic effect with other enhancers, such as DMSO and NMP, was investigated. As shown in Figure 1, addition of NMP in the HPMC-based gel formulation significantly enhanced *in vitro* hairless mouse skin permeation of TS. While DMSO was not effective in enhancing the skin permeation rate of TS, NMP increased in a dose-dependant manner (Table II). Moreover, when 6% (w/w) of NMP was added together with 2% (w/w) of diethanolamine (Rx 3), the skin permeation rate of TS was not significantly different from that of a commercial product, which contains approximately 70% of ethanol and 0.5% (w/w) of isopropyl myristate in Carbopol-based gel formulation.

It was reported that dodecylamine is the most effective in enhancing the skin permeation rate of TS from a soft hydrogel,[11] reservoir-type and matrix-type transderamal delivery systems.[12,13] Although dodecylamine is well known to increase the partitioning of TS on the skin, thereby increasing the permeability coefficient,[14] skin irritation always limited its common use in topical formulations. Thus, various amines that are listed in the United States Pharmacopeia (*i.e.*, ethylenediamine, triethanolamine, diethanolamine, and triethylamine) were selected and their skin permeation-enhancing effect was tested in a preliminary study. Since diethanolamine was the most effective among tested (data not shown), synergistic enhancing effect of DMSO and NMP was investigated. NMP is a non-toxic skin permeation enhancer which is known to increase the skin permeation rate of various drugs.[15-18] It is miscible with water and has been applied industrially in transdermal formulations.

Figure 2 shows the plasma TS concentration versus time profiles in SD rats following the application of the gel formulation (Rx 3) containing 1% TS. Basal plasma TS concentration profile of rats without the gel application was also observed as a control, which was within the normal level of 3-10 ng/mL as reported in the literature.[19] Plasma concentration of TS significantly increased after applying the HPMC-based gel formulation, and reached maximum concentration within 2 hours after the application. High plasma concentration of TS maintained for at least 24 hours, probably due to the sustained-release of TS from the gel. It is interesting to note that HPMC-based gel formulation resulted in higher TS plasma concentration than the commercial product, which is inconsistent with the *in vitro* skin permeation study. Both C_{max} and AUC_{24hr} values of HPMC-based gel were significantly higher than those of the commercial product (Table III). Since the commercial product contains about 70% of ethanol, it was easily dried after applying on the skin, which might result in the reduction of driving force of skin permeation. However, since HMPC-

Table III–*Pharmacokinetic Parameters of Testosterone Gel Formulations in Sprague Dawley Rats*

Formulation	Pharmacokinetic parameters		
	C_{max} (ng/mL)	T_{max} (hr)	AUC_{24hr} (ng·hr/mL)
Rx 3	30.93 ± 7.21**	4.0 ± 4.0	541.41 ± 144.60**
Commercial gel	11.74 ± 2.60	3.0 ± 2.0	87.32 ± 18.24

Each value is the mean ± standard deviation (n=4).
**; $p<0.01$ compared to a commercial gel

based gel formulation contains 25% of ethanol and 33% of glycols (Table I), it is not easily evaporated and thus is expected to maintain the driving force as well as minimize the skin irritation caused by ethanol. The effect of solvent evaporation may not be reflected during the *in vitro* skin permeation studies due to the occlusion of the donor cells.

Conclusions

HPMC-based novel gel formulations containing 25% (w/w) ethanol was successfully prepared for transdermal delivery of TS. Although *in vitro* skin permeation rate was not significantly different from a commercial gel formulation which contains about 70% of ethanol, significantly high plasma concentration profile of TS was achieved with the HPMC-based gel formulation in *in vivo* study. These results suggest that it is feasible to develop a novel gel formulation of TS which can minimize the skin irritation caused by high content of ethanol. However, further clinical studies need to be conducted in order to confirm these animal study results.

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