View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Elsevier - Publisher Connector

Acta Pharmaceutica Sinica B 2013;3(6):392–399

Institute of Materia Medica, Chinese Academy of Medical Sciences
Chinese Pharmaceutical Association

Acta Pharmaceutica Sinica B

www.elsevier.com/locate/apsb
www.sciencedirect.com

ORIGINAL ARTICLE

Preparation and characterization of a metered dose transdermal spray for testosterone

Wangding Lu, Huafei Luo, Yubo Wu, Zhuangzhi Zhu, Hao Wang*

National Pharmaceutical Engineering Research Center, China State Institute of Pharmaceutical Industry, Shanghai 201203, China

Received 12 August 2013; revised 9 September 2013; accepted 3 October 2013

KEY WORDS

Metered dose transdermal spray;
Testosterone;
Transdermal vehicle;
Azone;
Permeability;
Pharmacokinetics;
Rat

Abstract The objective of the present work was to develop a metered dose transdermal spray (MDTS) formulation for transdermal delivery of testosterone and to characterize its efficacy. Testosterone release from a series of formulations was assessed *in vitro*. Skin from hairless mice was used in permeation experiments with Franz diffusion cells. The spray pattern, pump seal efficiency, average weight per metered dose and dose uniformity were evaluated. An optimized formulation containing 10% (*w/v*) testosterone, 9% (*v/v*) azone and 91% (*v/v*) ethanol was based on good skin permeation and acceptable drug concentration and permeation enhancer (PE) content. A skin irritation study indicated that the formulation was non-irritating in a rat model. An *in vivo* pharmacokinetic study indicated that the optimized formulation showed a different plasma concentration-time profile from that of the commercially available product Testopatch®. The Testopatch® product demonstrated a more sustainable drug release. The evaluation of the testosterone MDTS indicated that it could deliver reproducible amounts of the formulation per actuation. The results obtained showed that the MDTS is a potential alternative therapeutic system for transdermal testosterone delivery.

© 2013 Institute of Materia Medica, Chinese Academy of Medical Sciences and Chinese Pharmaceutical Association. Production and hosting by Elsevier B.V. All rights reserved.

*Corresponding author. Tel.: +86 21 51320211; fax: +86 21 51320728.
E-mail address: wanghao99@hotmail.com (Hao Wang).
Peer review under responsibility of Institute of Materia Medica, Chinese Academy of Medical Sciences and Chinese Pharmaceutical Association.

Production and hosting by Elsevier

2211-3835 © 2013 Institute of Materia Medica, Chinese Academy of Medical Sciences and Chinese Pharmaceutical Association. Production and hosting by Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.apsb.2013.10.003

1. Introduction

Testosterone is the major circulating male androgen[1]. Testosterone deficiency is usually associated with adverse effects on body composition, bone density, sexual function and mood, and may also increase cardiovascular risk. Numerous studies have demonstrated the benefits of testosterone replacement in men with overt hypogonadism. Amongst several possible administration routes for testosterone replacement, a transdermal drug delivery system (TDDS) offers advantages over oral and intramuscular application. Hepatic first-pass metabolism in the liver after oral administration requires high testosterone doses, and potentially painful injections may lead to supra-physiological testosterone serum concentrations[2]. The relatively low molecular weight (MW = 288) and moderate lipophilicity ($\log P_{o/w} = 3.3$; water solubility = 0.039 mg/mL at 37 °C) of testosterone are favorable factors for transdermal delivery[3,4].

Gels, creams and patches are the most commonly used dosage forms for TDDS[5]. Currently, gel and patch formulations (*e.g.*, Androgel®, Testoderm® and Androderm®) are used clinically. However, a gel formulation has to be applied over large surface areas to achieve the target plasma levels, and transfer of the drug to female partners has been recorded. Although the approved transdermal patches can avoid this problem, they have other drawbacks which significantly reduce patient acceptance and compliance because of skin irritation caused by occlusion[6]. The development of alternative formulations for the transdermal delivery of testosterone remains a desirable goal[7].

MDTS, which is quick drying and non-occlusive, is a better alternative to both the patch and gel systems for easy application and good tolerance. Moreover, the spray can form a "patchless drug reservoir" on the skin[8]. MDTS formulations are topical aerosols formulated as single phase solutions consisting of drug, penetration enhancers and polymers[9].

The aim of this work was to develop a safe MDTS formulation for testosterone. *In vitro* drug release was evaluated using skin from the hairless mouse, and the primary mechanism of drug delivery was investigated. The pharmacokinetic profile was evaluated by comparing the pharmacokinetic parameters of the optimal formulation with that of commercial product Testopatch®. The developed spray formulations were further evaluated for their performance characteristics including spray pattern, pump seal efficiency, average weight per metered dose and content per spray. A skin irritation study also was carried out using rat as an animal model.

2. Materials and methods

2.1. Materials

Testosterone was supplied by Zizhu Pharma Co., Ltd. (Beijing, China). Polyacrylic resin (Eudragit® RL PO and Eudragit® E PO) were procured from Degussa (Germany). Polyvinyl pyrrolidone/vinyl acetate (Plasdone® S-630) and *N*-methyl-2-pyrrolidone (NMP) were supplied by International Specialty Products (USA). Polyvinylpyrrolidone (PVP K30) was kindly provided by BASF (France). PEG 200, propylene glycol (PG) and ethanol were purchased from Sino Pharma Chemicals Co., Ltd. (Shanghai, China). Azone was purchased from Kejie Pharma Co., Ltd. (Tianmen, Hubei, China). Isopropyl myristate (IPM) was provided by Uniqema (UK). All other chemicals and solvents were of analytical reagent grade or chromatography reagent grades.

2.2. Solubility studies

Excess testosterone was added to different solvents[10] and the resulting suspensions were shaken at 25 ± 1.0 °C for 72 h. After removal from the shaker bath, the equilibrated samples were centrifuged for 3 min at 17,800 × *g*. The supernatant was filtered (pore size: 0.22 µm) prior to further examination. The sample was diluted such that the concentration was within the detection range of the instrument. Saturated concentrations were determined for each solution by HPLC using the method described below.

2.3. Formulation preparation

The MDTS formulations were developed as topical solutions made up of volatile and non-volatile vehicles containing the drug dissolved in a single phase. The non-volatile vehicle contains only permeation enhancer (PE) or combination of PE and film forming polymer (FFP). The spray system was prepared by incorporating FFP and PE into the solvent system. We used ethanol as the volatile vehicle in this study. The drug application system (Wantong Fixed Quantity Valve System Co., Ltd., Suzhou, China) consisted of a 10 mL container and an actuator with an actuating volume of 100 µL. Formulations were prepared with a series of batches using different PEs or FFPs (Table 1). The selection of optimum FFP was based on drying time, cosmetical attractiveness and outward stickiness of the film formed[11]. And the selection of PE was based on transparency and non-volatility.

2.4. Characterization of the developed MDTS formulation

The characterization performed for the MDTS formulations included the evaluation of the spray pattern, effectiveness of the pump seal, average weight per metered dose and content uniformity[12]. The spray pattern was assessed by delivering the spray through the MDTS onto paper. The formulation was held at a distance of 5 cm from the paper. The wet part formed was outlined, then the outlined part was clipped from the paper. The area of the pattern was determined. Effectiveness of the pump seal was evaluated by a pump seal efficiency test. The filled containers were placed in the upright position at 30° for 3 days. The containers were weighed before and after the test period. The change in the weight of the container was recorded and the leakage rates were calculated. Average weight per metered dose was also measured. The initial weight of the container was recorded, and the container was weighed again after successive deliveries were sprayed from the MDTS. The difference between the initial and final weight of the container divided by the number of deliveries sprayed from the containers was used to represent the average weight per metered dose. The drug content per spray was determined by actuating ten sprays in a beaker containing methanol. This solution was shaken for 10 min and the drug content was analyzed by HPLC. Content uniformity was assessed by analyzing the drug content in 5th, 10th, 20th, 30th and 50th doses emitted from the pump.

2.5. In vitro skin permeation experiments

2.5.1. Skin isolation and preparation

Hairless mice weighing 22 ± 2 g provided by the SLAC Laboratory Animal Company Ltd. (Shanghai, China) were used. All animal studies were performed in accordance with Ethical

Table 1 Composition of investigated formulations.

Ingredient (% *w/v*)	F1	F2	F3	F4	F5	F6	F7	F8	F9[c]
Testosterone	5	5	5	5	5	5	5	5	5
PE[a]	–	–	–	–	5	5	5	5	0
FFP[b]	5	5	5	5	0	0	0	0	0
Absolute alcohol	Add to 10 mL								

[a]PEs used in F5–F8 were azone, IPM, NMP and PG, respectively.
[b]FFPs used in F1–F4 were Eudragit® E PO, Eudragit® RL PO, Plasdone® S630 and PVP K30, respectively.
[c]F9 was used as the control group.

Guidelines for Investigations in Laboratory Animals and was approved by National Pharmaceutical Engineering and Research Center. The dorsal skin was excised after the mice were killed by cervical dislocation. Fresh prepared skin was stored in the freezer at −20 °C without repeatable freeze and thaw recycles. Prior to permeation experiments, the skin was thawed and subcutaneous fat, tissue and capillaries of the skin were carefully removed. After cutting into pieces, the skin was mounted between the donor and receptor compartment of the Franz diffusion cells with the stratum corneum facing the donor compartment.

2.5.2. *Permeation studies*

The permeation area of Franz diffusion cells was 3.14 cm^2 and the receiver volume was 7.0 mL. 40% PEG 200 was used as the receiver medium. Assembled diffusion cells in triplicate were placed in a transdermal permeation diffusion instrument and maintained isothermally at 32 °C. The receptor compartment was stirred with a magnetic stirrer at 220 rpm. The air bubbles that remained in the receptor cell were carefully removed by gentle tilting of the diffusion cells. Since the drug administration area is about 12 cm^2 (in bioequivalence study) for each pump, we used a micropipette to delivery 50 μL drug solution precisely in order to mimic the true situation of drug administration and the uniformity of drug on the skin sample. In the experiments for PE screening we applied 100 μL drug solution in order to amplify the effect of PE for observation. The system was maintained at 32 °C for 2 h. Samples of 0.3 mL were withdrawn at 2, 4, 6, 8, 10 and 24 h for HPLC analysis and an equivalent volume was added to the system to supplement the volume loss. After centrifugation at 17,800 × *g* for 3 min, the supernatant was used for analysis.

2.6. *In vivo animal experiments*

Healthy female Sprague-Dawley rats weighing 220±20 g provided by the SLAC Laboratory Animal Company Ltd. (Shanghai, China) were used in this study. The animals were housed four per cage in laminar flow that were maintained at 22±2 °C and relative humidity of 50–60%. The animals were kept in these facilities for at least 1 week prior to the experiment and were fasted for at least 24 h before commencing the experiments. Before administration, the abdominal hair was shaved using an electric clipper carefully and allowed to heal for 24 h. The animals were divided into three groups with four animals in each group. The first and second groups were given the testosterone MDTS or the Testopatch®, respectively. The remaining group did not receive any treatment and served as the control group. Blood samples (250–300 μL) were collected *via* the retro-orbital plexus using a sterilized glass capillary tube. Before the experiments, blood samples of 300 μL were taken to measure the basal level of endogenous testosterone in each rat. After application of testosterone MDTS or the Testopatch®, blood samples were collected at the scheduled sampling times. The blood samples of rats in the control group were also collected to record the level of endogenous testosterone during the experiment. After centrifugation at 17,800 × *g* for 3 min, the separated serum of 100 μL was transferred into another neat tube and frozen at −20 °C until the determination of testosterone concentration by UPLC–MS/MS analysis.

Table 2 Skin irritation score scale.

Grading	Description of irritant response
0	No reaction
+	Weakly positive reaction (usually characterized by mild erythema across most of the treatment site)
+ +	Moderate positive reaction (usually distinct erythema possibly spreading beyond the treatment site)
+++	Strongly positive reaction (strong, often spreading erythema with odema)

2.7. *Skin irritation study*

The Draize patch test was carried out using rats as the animal model[13]. The optimized formulation was sprayed on the patch of shaven skin and occluded with adhesive tapes and the resulting reactions such as erythema and edema were scored after 24 h. The skin irritation score scale is shown in Table 2.

2.8. *Analytical method*

2.8.1. *HPLC analysis*

The samples of testosterone in the *in vitro* experiments were analyzed using a HPLC system consisting of a system controller (SCL-10 ATVP; Shimadzu, Japan), a binary pump (LC-10 ATVP, Shimadzu), a UV−Vis detector (SPD-10 AVP, Shimadzu), a column oven and an auto injector (SIL-10A, Shimadzu). The separation was obtained through a C18 reversed phase analytical column (150 mm × 4.6 mm, 5 μm) (Shimadzu, Japan) using the mobile phase consisting of methanol/water (60/40, *v/v*). The peak area correlated linearly with testosterone concentration in the range 1–100 μg/mL. The obtained linear equation is $A=73{,}624C-56{,}654$ ($R^2=0.999$). The coefficient of variation (CV) was below 2% at three levels of 5, 25 and 100 μg/mL.

2.8.2. *In vivo UPLC–MS/MS analysis of testosterone*

The analyte was recovered from the plasma samples by liquid–liquid extraction (LLE) after thawing thoroughly at room temperature.[14]

Briefly, 10 μL of testosterone propionate solution was added to the serum sample as an internal standard and mixed well by vortexing, and then LLE was performed by the addition of *tert*-butyl methyl ether followed by vortex extraction for 3 min. After centrifugation at 17,800 × *g* for 5 min, the upper organic layer was transferred into a clean tube and evaporated to dryness at 40 °C with a vacuum centrifugal concentrator (miVac DUO, Genevac). The residue was reconstituted with 200 μL of mobile phase and mixed by vortexing for 30 s. Analysis of testosterone was performed with UPLC–MS/MS system equipped with a system controller (SCL-10 ATVP; Shimadzu), a binary pump (LC-10 ATVP; Shimadzu), a UV–Vis detector (SPD-10 AVP, Shimadzu), a column oven and an auto injector (SIL-10A; Shimadzu) with an electrospray ionization (ESI) interface. The UPLC separation was obtained through a C18 reversed phase analytical column (Shim-pack XR-ODS III) (2.0 mm × 75 mm, 1.6 μm) using a mobile phase of methanol and 10 mM ammonium acetate buffer at a flow rate of 0.3 mL/min. The column temperature was set at 40 °C, detection wavelength at 241 nm and an injection volume of 10 μL. A gradient elution was carried out as shown in Table 3.

The MS/MS conditions were set as follows: The ionization method was ESI, which was operated in positive single ion monitoring (SIM+) mode. Nitrogen was used as the nebulizer and desolvation gas with a flow rate of 3 and 15 L/min. The capillary temperature and voltage were set at 400 °C and 3.0 kV. Desolvation temperature was 400 °C. Quantification was performed using the multiple reaction monitoring mode with a transition of *m/z* 289.25→109.20 for testosterone and *m/z* 345.30→109.20 for the internal standard. The data were acquired and analyzed by Shimadzu Labsolutions software. Retention times were 2.8±0.1 and 3.2±0.1 min for the internal standard and testosterone, respectively. The analytical column and mobile phase used for the assay provided a clear separation between testosterone and the internal standard. There was no interference from any endogenous material. The limit of detection by this method was 1 ng/mL. The method recovery rate was 100.80±1.87%, and the extraction recovery rate was 80.45±3.21%. The intra-day and inter-day assay coefficients of variation were 1.89% and 3.68%, demonstrating good reproducibility.

2.9. Data analysis

The cumulative amount Q (μg/cm^2) of testosterone that permeated through skin was calculated by

$$Q_n = \frac{C_n \times V_0 + \sum_{i=1}^{n-1} (C_i \times V_i)}{A} \quad (1)$$

Table 3 Gradient conditions for UPLC.

Time (min)	A (%)[a]	B (%)[b]
0	40	60
0.5	40	60
0.8	70	30
1.5	70	30
1.8	95	5
3.0	95	5
3.2	40	60
4.5	40	60

The flow rate was 0.3 mL/min.
[a]Methanol.
[b]10 mM ammonium acetate buffer.

where A is the effective area, V is the volume of receptor cell, C_n is the drug concentration at time point "n", V_0 is the volume of receptor cell, and C_i is the testosterone concentration at time point "i". The cumulative amount of testosterone that permeated through the skin was plotted *versus* time (h). Each data point was expressed as the mean±SD of three determinations. The flux data were subjected to student's *t*-test and one-way analysis of variance (ANOVA) to determine the level of significance. The data was considered to be significant if $P<0.05$.

The enhancement ratio (ER) was determined using the following equation:

$$ER = Flux_{with\ enhancer}/Flux_{without\ enhancer} \quad (2)$$

Peak plasma concentration during the dosing period (C_{max}) and the time of peak plasma concentration (T_{max}) was directly determined from the pharmacokinetic data. The pharmacokinetic parameters such as area under curve (AUC_{0-t}), half-life of elimination from plasma ($t_{1/2}$) and mean residence time (MRT) were calculated by noncompartment analysis following transdermal application using DAS 2.0 software. Relative bioavailability F (%) was calculated from

$$F(\%) = (AUC_{optimal\ formulation} \cdot dose_{Testopatch})/(AUC_{Testopatch} \cdot dose_{optimal\ formulation}) \times 100\% \quad (3)$$

The drug administration area of each pump was calculated by

$$A_x = W_0 \times A_0/W_x \quad (4)$$

where W_0 and A_0 is the known weight and area of the piece that was clipped from the paper; W_x is the weight of the paper after certain MDTS actuation; and A_x is the area of the pump. Taking paper with area of 10 cm × 10 cm and weighted 0.8166 g as a sample, W_0 is 0.8166 g, A_0 is 100 cm^2.

3. Results and discussion

3.1. Solubility

To ensure stable collection conditions, a 40% (*v/v*) PEG water solution was used as the receptor fluid. Based on the hypothesis that PE acts as a "transporter vehicle" for the drug, the solubility of testosterone in different PE can be one of the criterions for the PE screening. The more drug solubilized in the vehicle, the higher was the transdermal flux[15,16]. From the results shown in Table 4, the solubility of testosterone in different PEs is NMP>PG>azone>IPM. NMP showed the greatest solubility for testosterone, and NMP subsequently was used as a solubilizer in most cases. PG and azone showed similar ability to solubilize testosterone.

3.2. Preparation of MDTS

Based on the practical application of MDTS, we took 2 min as the critical point for the drying time of the film formed. For the measurement of stickiness, there should not be fiber left on the film when pressing some cotton on the film for 1 min. The film formed by the formulation incorporating FFP was transparent and cohesive (Table 5). By varying the ratio of the FFP, based on the visualization of the film formed, we chose 5% as the content of FFP. All formulations containing PE or both FFP and PE were clear in appearance.

3.3. *In vitro* evaluation

The effects of FFP, PE, testosterone concentration and the PE content on skin permeation were investigated to optimize the testosterone MDTS formulation.

3.3.1. *Effect of FFP on skin permeation*

The transdermal permeation profiles of formulations containing different FFPs showed that their transdermal flux is higher than that of the control group, the ER for each formulation containing FFP was Plasdone® S630 > Eudragit® E PO > PVP K30 > Eudragit®RL (Fig. 1). This might be attributed to the fact that FFP delayed the crystallization of testosterone after its administration and lead to greater transdermal flux. These results supported the finding that these materials had the ability to inhibit the drug crystallization[17,18].

3.3.2. *Effect of PE on skin permeation*

Among the PEs studied in this experiment, azone showed the greatest transdermal flux (Fig. 2). The ER for each formulation containing PE was azone > IPM > PG > NMP. Although NMP showed the greatest solubilizing ability for testosterone, NMP can also be functioned as solubilizer. The solubility of testosterone in NMP was 518.9 mg/mL, much higher than the others, which can be attributed to this reason. Trottet et al.[19] had shown that PG would permeate though the skin and might carry the drug with it, as shown by correlations *in vitro* between the permeation of both PG and drug. The investigation of the influence of PE on drug permeation from volatile formulations reconfirmed the conclusion stated by Trottet et al. Hadgraft et al.[20] reported that IPM remained in the skin after administration to form a "patchless drug reservoir" instead of permeating through the skin as with PG. Azone is non-irritant to human skin, even in undiluted form, reversible in its action and hardly absorbed through human skin[21]. The permeation enhancement ability of azone was higher than IPM. Since we found that IPM showed the greatest permeation ability during the development of a pressure sensitive adhesive (PSA) patch for testosterone, it seems that the contradictory results suggest different mechanisms between the two dosage forms we developed. This indicates that the solubility of testosterone in PE is a critical factor in determination of transdermal flux.

Table 4 Solubility of testosterone in different solvents ($n=3$).

Solvent	Solubility (mg/mL)
Water	0.024 ± 0.007
Normal saline	0.020 ± 0.003
PBS (pH 7.4)	0.020 ± 0.005
40% PEG 200	0.321 ± 0.021
Azone	92.380 ± 2.233
NMP	518.9 ± 10.367
PG	103.0 ± 4.269
IPM	6.830 ± 0.758

3.3.3. *Effect of testosterone and azone with various concentrations*

Plasdone® S630 showed the greatest transdermal drug flux in the investigation of the effect of FFP on skin permeation, as shown in Fig. 1. It almost stopped drug release during 10–24 h. While the formulation containing azone showed continuous release during 0–24 h. This difference might be attributed to the different mechanism which needed further investigation. So we selected formulation containing testosterone and azone without Plasdone® S630 for further optimization. By changing the concentrations of testosterone and

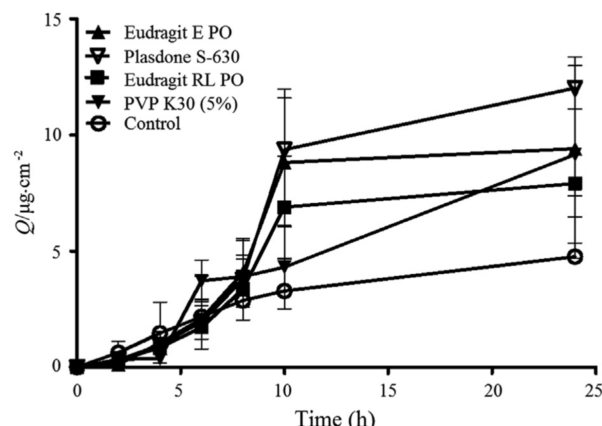

Figure 1 Percutaneous permeation profiles of testosterone MTDS containing different film-forming polymers (Mean ± SD; $n=3$).

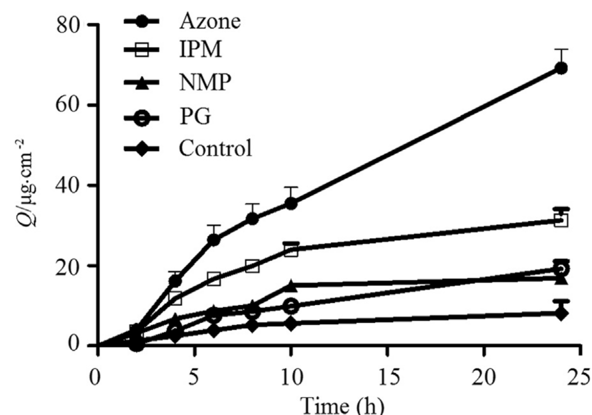

Figure 2 Percutaneous permeation profiles of testosterone MTDS containing different penetration enhancers (Mean ± SD; $n=3$).

Table 5 Evaluation of the film formed by MTDS incorporating FFP.

FFP	Appearance	Drying time	Outward stickiness
Eudragit® E PO	Transparent	Less than 2 min	No fiber left
Eudragit®RL	Transparent	Less than 2 min	No fiber left
Plasdone® S630	Transparent	Less than 2 min	No fiber left
PVP K30	Transparent	Less than 2 min	No fiber left

azone, the results indicated that the transdermal flux of testosterone responded positively with the ratio of azone when the drug is at a relatively low concentration of 5% or 10% (Fig. 3). This might be attributed to that the solubilized drug increased with the use of azone, and as a result the transdermal flux increased. When the drug concentration reached a relative high level of 15%, the transdermal flux was greatest when the azone ratio was 5%, but the percutaneous permeation profiles of testosterone were similar to those where the azone ratio was 7% and 9%[22,23]. It may be that the azone was not able to carry the drug into the skin fast enough after the volatile solvent evaporated, and the crystallization of the drug outside the skin warrants investigation[24].

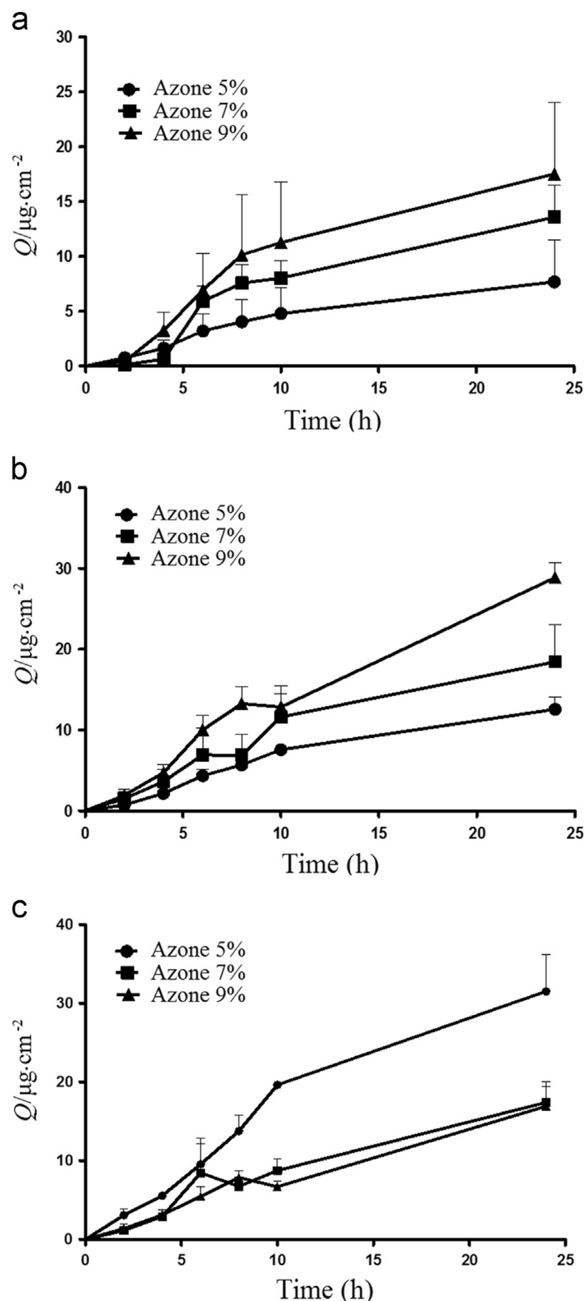

Figure 3 Percutaneous permeation profiles of testosterone (a) 5% *w/v*, (b) 10% *w/v* and (c) 15% *w/v* with azone content of 5%, 7% and 9% *v/v* (Mean±SD; n=3).

3.4. *In vivo evaluation*

Based on the above results, a formulation containing 10% *w/v* testosterone, 9% *v/v* azone and 91% *v/v* ethanol was selected as optimal. To compare the newly developed testosterone MDTS to the commercial product Testopatch® in pharmacokinetic profile, a bioavailability study was carried out in female rats. Fig. 4 depicts the profiles of mean plasma concentration of testosterone *versus* time following the transdermal application of experimental MDTS (100 μL, 12 cm^2, 10 mg) and Testopatch® (0.5 mg/cm^2, 12 cm^2, 6 mg). We chose 100 μL as the dosage of experimental MDTS to ensure the integrity of each actuation. Since the administration area of each actuation is 12 cm^2, we also delivered 12 cm^2 drug administration area for Testopatch®. The profile of blank serum sample of rats without testosterone administration was also determined. The results indicate that plasma testosterone concentrations increased promptly after administration and reached the peak level; thereafter the plasma concentrations gradually declined. The results with the control group showed that the effect of endogenous testosterone was limited. The pharmacokinetic parameters were calculated using a non-compartmental model and presented in Table 6. Compared with the testosterone MDTS group, the Testopatch® group showed a more steady plasma concentration profile. This difference might be attributed to the different transdermal mechanisms of these two dosage forms. In the testosterone MDTS group, the drug reservoir was formed in the skin after the drug administration. Once the reservoir formed, there was no extra drug to supply for the reservoir. For the Testopatch® group, the reservoir remained until the patch was removed. After the peak level was reached, the testosterone in MDTS group

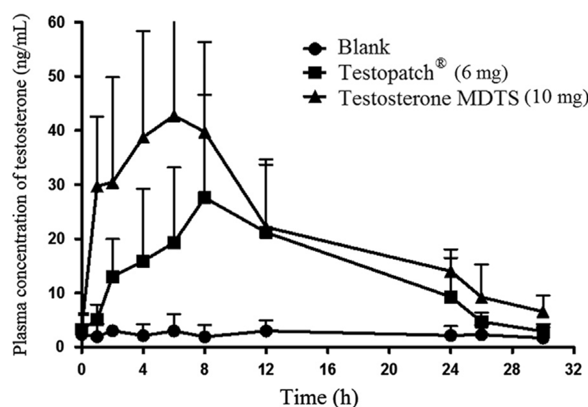

Figure 4 *In vivo* absorption profiles of testosterone after Testopatch®, testosterone MDTS and blank (mean±SD, n=4).

Table 6 Pharmacokinetic parameters after application of the experimental patch and Testopatch®.

Parameters (unit)	Experimental patch	Testopatch®
AUC_{0-t} (ng·h/mL)	687.37±98.90	517±80.72
MRT_{0-t} (h)	10.33±0.72	13.74±1.1
$t_{1/2}$ (h)	8.83±1.59	14.9±3.46
T_{max} (h)	5.32±1.31	9.00±2.00
C_{max} (ng/mL)	42.74±14.62	27.58±8.54

Data are expressed as Mean±SD, n=4.

Table 7 Evaluation of MDTS administration area.

No.	Weight (g)	Area (cm^2)	Mean area (cm^2)[a]
1	0.0986	12.07	11.89 ± 0.66
2	0.0898	11.00	
3	0.0978	11.98	
4	0.1045	12.80	
5	0.0948	11.61	

The statistical results of student't *t*-test and one-way ANOVA showed *P* levels >0.05.
[a]Data are expressed as Mean ± SD, $n = 5$.

Table 8 Evaluation of per-actuation content for testosterone MDTS.

Actuation times	Weight per pump (g)	Testosterone for per pump (mg)
1–5th	0.096 ± 0.003	9.59 ± 0.11
5–10th	0.098 ± 0.002	9.79 ± 0.13
10–20th	0.102 ± 0.001	9.97 ± 0.09
20–30th	0.102 ± 0.002	9.98 ± 0.14
30–50th	0.100 ± 0.001	9.95 ± 0.12

Data are expressed as Mean ± SD, $n = 6$.
The statistical results of a Student's *t*-test and one-way ANOVA showed *P* levels >0.05.

declined more quickly in the absence of a patch resevoir. The relative bioavailability (*F*) was 79.78% for the testosterone MDTS.

Traditional TDDS products relied mainly on their occlusive nature to increase the permeability of the drug candidates. Occlusion often causes an increased possibility of skin irritation at the application site. Occlusive systems can also provide an environment for microbial proliferation[25]. Besides, manufacturing and scale-up of multi-component patches has caused some distinct challenges to the formulator. Additionally, issues on formulation stability and drug crystallization on longer-term storage are still to be solved. MDTS represents an evaporative system which provides passive and non-occlusive delivery[26]. As a result of these characteristics, this system demonstrates very low skin irritation rates. This formula also enables the formulation a uniform distribution on the skin over a defined area after application. Comparing with the patch, the manufacturing and scale-up of MDTS is much easier. As the MDTS is easy to use, well tolerated and can avoid the skin irritation caused by the occlusive environment. The advantage of this dosage form would make up its relative low bioavailability.

3.5. Characterization of the MDTS formulation

In this study, we evaluated the drug administration of each pump. The results indicated that this MDTS formulation showed a uniform spray pattern (Table 7).

No leakage was observed from the MDTS containers when placed in the upright position at 30° for 3 d. Content uniformity was assessed for the 5th, 10th, 20th, 30th and 50th doses and the results indicated that the MDTS provides uniform content per actuation. Average weight per metered dose is an important quantitative parameter and the testosterone content per spray was also determined. The results indicated that the testosterone MDTS showed reproducible amounts of the formulation per actuation (Table 8).

3.6. Skin irritation study

No erythema or edema was found in the primary skin irritation studies with the optimized formulations (10% testosterone, 9% azone and 91% ethanol) on rat skin.

4. Conclusions

A novel transdermal drug delivery system was designed and evaluated *in vitro* and *in vivo*. The effects of FFPs, PEs, testosterone concentration and the content of enhancer azone on skin permeation were investigated to identify an optimized formulation. The final formulation provided satisfactory skin permeation with an appropriate combination of testosterone and azone content. The pharmacokinetic parameters of the optimal formulation showed a different plasma profile from that of a commercial product, indicating a likely different transdermal mechanism, which needs further investigation. Characterization of the testosterone MDTS indicated that it could deliver reproducible amounts of the formulation per actuation. No erythema or edema was found in the primary skin irritation studies with the optimized formulations in rats, and hence this application system was found to be safe and non-irritating for transdermal application. From the results obtained in this work it can be concluded that the MDTS has potential as an alternative therapeutic system for the transdermal delivery of testosterone.

Acknowledgments

This study was supported financially by the Major Projects for Drug Innovation and Development from National Science and Technology (2012ZX09304004).

References

1. Leichtnam ML, Rolland H, Wüthrich P, Guy RH. Identification of penetration enhancers for testosterone transdermal delivery from spray formulations. *J Control Release* 2006;**113**:57–62.
2. Leichtnam ML, Rolland H, Wüthrich P, Guy RH. Testosterone hormone replacement therapy: state-of-the-art and emerging technologies. *Pharm Res* 2006;**23**:1117–32.
3. Okimoto K, Rajewski RA, Stella VJ. Release of testosterone from an osmotic pump tablet utilizing (SBE)7m-[beta]-cyclodextrin as both a solubilizing and an osmotic pump agent. *J Control Release* 1999;**58**:29–38.
4. Guy RH, Hadgraft J. Transdermal drug delivery: a simplified pharmacokinetic approach. *Int J Pharm* 1985;**24**:267–74.
5. Gooren L, Bunck M. Transdermal testosterone delivery: testosterone patch and gel. *World J Urol* 2003;**21**:316–9.
6. Lopez RF, Seto JE, Blankschtein D, Langer R. Enhancing the transdermal delivery of rigid nanoparticles using the simultaneous application of ultrasound and sodium lauryl sulfate. *Biomaterials* 2011;**32**:933–41.
7. Thomas B, Finnin B. The transdermal revolution. *Drug Discov Today* 2004;**16**:697–703.
8. Leichtnam ML, Rolland H, Wüthrich P, Guy RH. Formulation and evaluation of testosterone transdermal spray. *J Pharm Sci* 2006;**95**:1693–702.

9. Morgan TM. Metered dose transdermal spray (MDTS). In: Hagraft J, editor. *Modified release drug delivery technology*. New York: Marcel Dekker; 2003, p. 523–31.
10. Raghavan SL, Trividic A, Davis AF, Hadqraft J. Effect of cellulose polymers on supersaturation and *in vitro* membrane transport of hydrocortisone acetate. *Int J Pharm* 2000;**193**:231–7.
11. Zurdo Schroeder I, Franke P, Schaefer UF, Lehr CM. Development and characterization of film forming polymeric solutions for skin drug delivery. *Eur J Pharm Biopharm* 2007;**65**:111–23.
12. Bakshi A, Bajaj A, Malhotra G, Madan M, Amrutiya NA. Novel metered dose transdermal spray formulation for oxybutynin. *Indian J Pharm Sci* 2008;**70**:733–9.
13. Agner Tove, Serup Jørgen. Sodium lauryl sulphate for irritant patch testing-a dose-response study using bioengineering methods for determination of skin irritation. *J Derma* 1990;**95**:543–7.
14. Ma J, Wang C, Luo H, Zhu Z, Wu Y, Wang H. Design and evaluation of a monolithic drug-in-adhesive patch for testosterone based on styrene–isoprene–styrene block copolymer. *J Pharm Sci* 2013;**107**:2221–34.
15. Williams AC, Barry BW. Penetration enhancers. *Adv Drug Delivery Rev* 2012;**64**:128–37.
16. Morgan TM, Reed BL, Finnin BC. Enhanced skin permeation of sex hormones with novel topical spray vehicles. *J Pharm Sci* 1998;**87**:1213–8.
17. Pellett MA, Davis AF, Hadgraft J. Supersaturated solutions evaluated with an *in vitro* stratum corneum tape stripping technique. *Int J Pharm* 1997;**151**:91–8.
18. Raghavan SL, Trividic A, Davis AF, Hadgraft J. Effect of cellulose polymers on supersaturation and *in vitro* membrane transport of hydrocortisone acetate. *Int J Pharm* 2000;**193**:231–7.
19. Trottet L, Merly C, Mirza M, Hradgraft J, Davis AF. Effect of finite doses of propylene glycol on enhancement of *in vitro* percutaneous permeation of loperamide hydrochloride. *Int J Pharm* 2004;**30**:213–9.
20. Santos P, Watkinson AC, Hadgraft J, Lane ME. Influence of penetration enhancer on drug permeation from volatile formulations. *Int J Pharm* 2012;**439**:260–8.
21. Adachi Y, Hosaya K, Sugibayashi K, Morimoto Y. Duration and reversibility of the penetration-enhancing effect of Azone. *Chem Pharm Bull* 1988;**36**:3702–5.
22. Santos P, Machado SR, Watkinson AC, Hadcraft J, Lane ME. The effect of drug concentration on solvent activity in silicone membranes. *Int J Pharm* 2009;**377**:70–5.
23. Miselnicky SR, Lichtin JL, Lichtin L, Sakr A, Bronaugh RL. Influence of solubility, protein binding, and percutaneous absorption on reservoir formation in skin. *J Soc Cosmet Chem* 1988;**39**:169–77.
24. Leveque N, Raghavan SL, Lane ME, Hadgraft J. Use of a molecular form technique for the penetration of supersaturated solutions of salicylic acid across silicone membranes and human skin *in vitro*. *Int J Pharm* 2006;**318**:49–54.
25. Taylor LJ. Effect of occlusion on the percutaneous penetration of linoleic acid and glycerol. *Int J Pharm* 2002;**249**:157–64.
26. Morgan TM. Enhanced skin permeation of sex hormones with novel topical spray vehicles. *J Pharm Sci* 1998;**87**:1213–8.