ORIGINAL ARTICLE

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An open-label, phase 2, single centre, randomized, crossover design bioequivalence study of AndroForte 5 testosterone cream and Testogel 1% testosterone gel in hypogonadal men: study LP101

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SUMMARY

We compared a novel 5% testosterone (T) cream (AndroForte 5, Lawley Pharmaceuticals, Australia) with a 1% T gel (Testogel, Besins Healthcare, Australia). Using an open-label crossover design, subjects were randomized to one of two treatment sequences using either the T gel or T cream first in a 1 : 1 ratio. Each treatment period was 30 days with a 7–14 days washout period between them. On Days 1 and 30 of each treatment period blood was sampled at −15, −5 min, 0, 2, 4, 5, 6, 7, 8, 9, 10, 12 and 16 h post study drug administration. Sixteen men with established androgen deficiency aged between 29 and 73 years, who had undertaken a washout from prior testosterone therapy participated in the study. One subject failed to complete both arms and another was excluded post-completion because of a major protocol violation. Bioequivalence was established based on key pharmacokinetic (PK) variables: AUC, C_{avg}, C_{max}, T_{max}, % fluctuation (with and without baseline correction) for the two formulations of testosterone on Day 1 and Day 30. The ratio and 90% CI of AUC 0.99 (0.86–1.14), C_{max} 1.02 (0.84–1.24) and C_{avg} 0.99 (0.86–1.14) for T cream/T gel were within the predetermined bio-equivalence criteria of 80% to 125% at Day 30. There were no statistically significant differences between secondary biochemical markers: serum dihydrotestosterone (DHT), oestradiol (E2), sex hormone-binding globulin (SHBG), luteinizing hormone (LH) and (FSH). The two testosterone formulations were shown to be bioequivalent.

INTRODUCTION

The varieties of testosterone products presently available in Australia include intermediate-acting mixed testosterone esters, long-acting testosterone undecanoate in oil-based injections (Schulte-Beerbuhl & Nieschlag, 1980; Snyder & Lawrence, 1980; Behre & Nieschlag, 1998), an oral testosterone undecanoate capsule (Nieschlag *et al.*, 1975), transdermal patches (Meikle *et al.*, 1992; Dobs *et al.*, 1999), gel (Wang *et al.*, 2000, 2004) or solution (Wang *et al.*, 2011).

The transdermal administration of testosterone has gained favour because it is non-invasive, avoids extensive first-pass hepatic metabolism and theoretically maintains not only physiological circulating testosterone concentrations, but also mimics the diurnal variation in testosterone level observed in eugonadal young men (Gooren & Bunck, 2003). Recently a 2% testosterone alcohol-based solution for application under the arm has become available, but transdermal androgen formulated as a 1% testosterone gel (T gel), (Testogel, Besins Healthcare, Sydney, Australia), is most commonly used worldwide.

AndroForte 5 (Lawley Pharmaceuticals, Perth, Australia) is a new 5% (50 mg/mL) alcohol-free topical testosterone cream (T cream) approved for use in men with confirmed androgen deficiency.

We evaluated the bioequivalence of the T cream and the T gel based on pharmacokinetic parameters in hypogonadal men over a 30-day treatment period in a randomized crossover design.

MATERIALS AND METHODS

Study population

Males aged between 29 and 73 years with confirmed androgen deficiency were recruited for the study (LP101) by advertisement. The Australian Pharmaceuticals Benefits Scheme guidelines were used for defining androgen deficiency which includes established pituitary or testicular disorder or testosterone level of less than 8 nmol per litre or testosterone levels between 8 and 15 nmol/L with high luteinizing hormone (LH)

(greater than 1.5 times the upper limit of the eugonadal reference range for young men, or greater than 14 IU/L, whichever is higher).

Subjects on established androgen replacement therapy observed the following washout period prior to entering the study: 7 days for transdermal testosterone or oral testosterone undecanoate, 6–12 weeks for intramuscular injections of testosterone esters and 6 months since last dose for subcutaneous testosterone pellets or testosterone undecanoate injections.

Inclusion/exclusion criteria

Inclusion criteria included BMI of 18–35 kg/m^2, normal digital rectal examination of the prostate within the last 6 months, prostate-specific antigen <4 ng/mL, liver function tests, serum lipids and haematological parameters within acceptable limits, calculated eGFR ≥30 mL/min, negative urine drug screen and the absence of any urinary tract infection. Participants were required to limit alcohol intake to no more than 21 units per week for the duration of the study and in addition to abstain from alcohol, caffeine, chocolate and vigorous physical activity 24 h prior to Day 1 and 30 of each study period.

Exclusion criteria included untreated obstructive sleep apnoea, clinically significant non-malignant disease including cardiovascular or cerebrovascular event within 12 months prior to randomization or major surgery within 3 months of randomization, prior history of prostate cancer or other malignancy, receipt of therapy with another investigational drug within 4 weeks of Day 1, current smoker or past smoker who has ceased smoking within the past year, elevated blood pressure (≥140/90 mmHg) at screening or a diagnosis of hypertension unless on stable therapy for at least 3 months, history of mental illness requiring ongoing psychotropic medications, active substance abuse, known human immunodeficiency virus or hepatitis B infection, planned elective surgery during the study, generalized skin disease on the abdomen that may be affected by or affect testosterone transdermal absorption, dementia or altered cognitive function that would interfere with subject safety or compliance to the study procedures, severe voiding symptoms as identified on the International Prostate Symptom Score questionnaire, the ongoing use of any medication, herbal remedy, or foodstuff (e.g. grapefruit juice) known to be strong inducers/inhibitors of CYP3A4, affect the production (e.g. opiates, GnRH agonists) or action (e.g. spironolactone) of androgens and/or affect the production of SHBG (e.g. thyroxine, insulin, growth hormone, anti-epileptics), unless the dose has been stable for at least 3 months.

It was anticipated that 16 participants be enrolled in the study and allow for a 25% drop out and a total of 12 evaluable participants.

This study was approved by institutional review boards/ethics committees of the participating institutions (University of Adelaide, Australia and New England Research Institutes, Inc, Watertown, MA, USA) and included on the Australia New Zealand Clinical Trial Register number ACTRN12610000834005.

The screening tests were performed within 28 days (within 14 days for blood tests) of Day 1 of treatment. Participants who met all inclusion and none of the exclusion criteria were randomized in a 1 : 1 ratio to receive either T cream (Treatment A) followed by T gel (Treatment B) or vice versa as outlined in Fig. 1.

Figure 1 LP101 study timeline.
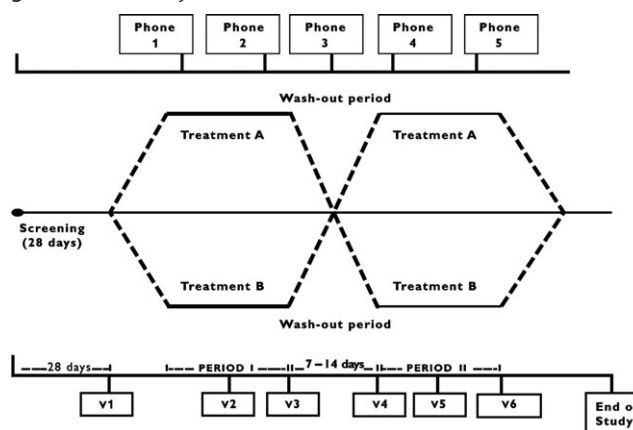
The T cream was supplied with a dose measuring applicator graduated in 0.5 mL increments. Each participant applied a 2 mL (100 mg T) dose to the torso which was massaged into the skin until not visible (approx. 30–60 sec). The T gel is a 1% preparation of testosterone dissolved in a hydro alcoholic solution. Each sachet contains 50 mg of testosterone (in 5 g gel). After opening the sachet, subjects immediately applied the full contents of the sachet onto the torso, gently smoothed to form a thin layer and left to dry.

Treatment compliance was monitored by the site pharmacy auditing of returned unused trial product and via review of patient study dairies.

Intensive pharmacokinetic sampling was conducted, following an overnight fast, on Days 1 and 30 of periods 1 and 2 at −15, −5 and 0 min pre-dose, then at 2, 4, 5, 6, 7, 8, 9, 10, 12, 16 and 24 h post administration. One PK sample was collected on Day 15 of periods I and II (visits 2 and 5). Following the application of transdermal product, participants were provided a standard breakfast, lunch and dinner. These were served at ~1, ~4 and 10 h post-dose respectively. During the pharmacokinetic sampling period, alcohol, caffeinated beverages, chocolate and vigorous physical activity were not allowed from the 24 h prior to admission, up to and including the time the participants was discharged from the unit.

Main outcome measures

Primary outcome

Pharmacokinetic variables: AUC, C_{avg}, C_{max}, T_{max}, % Fluctuation (with and without baseline correction) on Day 1 and Day 30.

Secondary outcomes

- Proportion of samples within or outside target blood testosterone range (above [%AT], below [%BT])
- Serum profiles of other reproductive hormones including: DHT, E2, SHBG, LH and FSH
- Quality of Life using the Short Form (36) Health Survey (SF-36) Questionnaire (Ware & Sherbourne, 1992) and sexual function using the Sexual Desire Inventory (SDI-2) (Spector *et al.*, 1996) and International Index of Erectile Function Questionnaire (Rosen *et al.*, 1997).
- Safety and tolerability of T cream via the monitoring and evaluation of adverse events.

Hormone assays

A validated stable isotope dilution liquid chromatography–tandem mass spectrometry (LC–MS/MS; API-5000) (AB SCIEX, Concord, Canada) was used to measure serum total testosterone (TT) (LOQ:0.01 nmol/L; interassay CV:6% at 0.40 nmol/L; 5% at 1.49 nmol/L, 2% at 8.16 nmol/L), dihydrotestosterone (DHT) (LOQ: 0.20 ng/mL: interassay CV: 11% at 0.39 ng/mL; 9% at 1.55 ng/m/L, 9% at 7.81 ng/L) and oestradiol (E2) (LOQ: 5 pg/mL: interassay CV: 7% at 20 pmol/mL; 7% at 75p/mol/L, 4% at 416pmol/L)(ANZAC Research Institute, Sydney, NSW) (Harwood & Handelsman, 2009).

The SHBG samples were analysed Institute of Medical and Veterinary Science, Adelaide, Australia in accordance with the relevant standard operating procedures. Serum SHBG levels were determined by diluting serum to 1 : 21 by adding SHBG sample diluent (Siemens Medical Solutions, Tarrytown, NY), and then assayed using the Immulite 1000 (Siemens, Wales, UK) auto analyser- a solid-phase, two-site chemiluminescent, immunoassay; (interassay CV, 4.0% at 32.3 nmol/L).

Serum follicle-stimulating hormone (FSH) and LH levels were determined by automated enzyme immunoassay using the Centaur XP (Siemens, Dublin, Ireland) autoanalyser (interassay CV, 7.4% at 5.9 mIU/mL, 6.2% at 47.4 mIU/mL and 5.2% at 89.6 mIU/mL for FSH; and 4.8% at 4.1 mIU/mL, 4.8% for 30.8 mIU/mL and 5.0% at 57.0 mIU/mL for LH).

Statistical analyses

Standard pharmacokinetic parameters (AUC, C_{avg}, T_{max} and C_{max}) were obtained from profiles of intensively sampled blood testosterone concentrations. The pharmacokinetic profiles of each formulation were evaluated, and compared with and without correcting for baseline using fixed effect models in Proc GLM (SAS v9.3) (SAS Institute Inc. Cary, NC, USA). The natural log of each variable was analysed. The model covariates included patient ID, treatment and period with and without adjustments for baseline.

The Lsmeans statement in Proc GLM was used to create the estimates of treatment effect and 90% CI's these were then transformed back to the original scale to give a ratio. Adjustments for baseline were carried out by including the average baseline testosterone as covariate in the model using two methods. One method used the baseline values from Day 1 and Day 30. The other method adjusted only for the baseline testosterone on Day 1. It was thought that ongoing treatment may have confounded the baseline measurement on Day 30, and the baseline measurement on Day 1 was a better representation of a patient's baseline testosterone concentration. In practice both methods of baseline adjustment and unadjusted analyses gave similar results.

Statistical analyses were jointly conducted by New England Research Institute (NERI) and John Wlodarczyk Consulting Services.
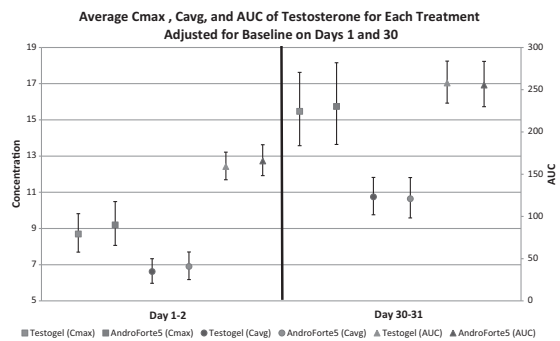
RESULTS

Participants

The first subject was enrolled on 8 February 2011 and the last subject was enrolled on 6 October 2011. The final subject's last visit occurred on 22 December 2012. One hundred and eighty subjects were assessed for eligibility, of which sixteen (16) consented and enrolled in the study. They were randomized according to intervention AB (AndroForte 5 – Testogel) ($n = 8$) or BA (Testogel – AndroForte 5) ($n = 8$). Seven waivers were granted during the course of the study including four subjects receiving eligibility waivers regarding their body mass index (BMI). One subject withdrew early owing to personal reasons. Fifteen subjects completed the study. Upon review of the source data, an additional subject was identified as having a major protocol violation and excluded from the analysis.

All subjects were Caucasian. Ages ranged from 29 to 73 years with baseline mean and standard deviation BMI's of 30.7 (6.3) kg/m^2, height 177.1 (7.8) cm and weight 96.6 (23.2) kg.

Results adjusted for baseline on days 1 and 30

The estimated ratios of treatment effect and corresponding 90% confidence intervals are presented in Table 1. The estimates from models with baseline adjustment were very similar to the estimates from the unadjusted models. The 90% CI's for AUC and C_{avg} were well within the bioequivalence range. The CI for C_{max} was within the range at day 30–31 and just above the range at day 1–2.

Table 1 Ratio of treatment effect between AndroForte5 and Testogel after adjusting for baseline[a] with 90% confidence limits

Variable	Day	Ratio	Lower 90% CI	Upper 90% CI
AUC	1–2	1.04	0.89	1.22
AUC	30–31	0.99	0.86	1.14
C_{max}	1–2	1.06	0.88	1.28
C_{max}	30–31	1.02	0.84	1.24
C_{avg}	1–2	1.04	0.89	1.22
C_{avg}	30–31	0.99	0.86	1.14

[a]Baseline is defined as the average of the three readings prior to treatment on days 1 and 30.

Table 2 Adverse events by period, overall and product

Adverse event	AndroForte5			Testogel		
	Period 1	Period 2	Overall	Period 1	Period 2	Overall
Application site rash	0 (0%)	0 (0%)	0 (0%)	1 (13%)	0 (0%)	1 (6%)
Fatigue	1 (13%)	0 (0%)	1 (7%)	2 (25%)	2 (25%)	4 (25%)
Pain in extremity	1 (13%)	0 (0%)	1 (7%)	1 (13%)	0 (0%)	1 (6%)
Headache	5 (63%)	0 (0%)	5 (33%)	2 (25%)	2 (25%)	4 (25%)
Lethargy	2 (25%)	1 (14%)	3 (20%)	0 (0%)	1 (13%)	1 (6%)
Memory impairment	1 (13%)	0 (0%)	1 (7%)	0 (0%)	0 (0%)	0 (0%)
Libido decreased	1 (13%)	0 (0%)	1 (7%)	0 (0%)	0 (0%)	0 (0%)
Mood altered	1 (13%)	1 (14%)	2 (13%)	0 (0%)	0 (0%)	0 (0%)
Pruritus	0 (0%)	1 (14%)	1 (7%)	0 (0%)	0 (0%)	0 (0%)
Rash	0 (0%)	1 (14%)	1 (7%)	0 (0%)	1 (13%)	1 (6%)

Figure 2 Changes in baseline adjusted average AUC, C_{max} and C_{avg} at Days 1 and 30 for each treatment throughout the study.

Figure 3 Mean serum total testosterone levels over 24 h at Day 1 and Day 30 by product.
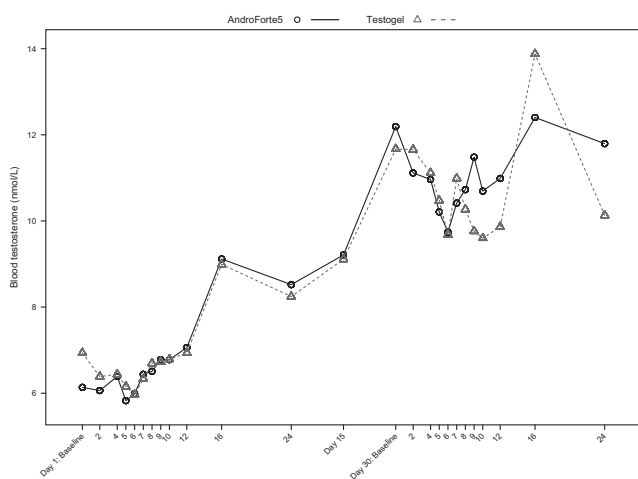
These results are graphically displayed in Fig. 2.

Pharmacokinetics of serum T concentrations

Serum T concentrations were elevated from hypogonadal levels to eugonadal levels in all subjects at Day 30. The Day 30 testosterone C_{max} was 16.3 ± 6.5 and 19.4 ± 12.8 nmol/L for 5% cream and 1% gel respectively. Similarly C_{avg} for the same period was 11.4 ± 5.2 and 11.3 ± 3.7. Figure 3 provides a graphical representation of the similarity between the two products absorption profile over the 30 days treatment period.

There were no significant differences between treatments on serum DHT, E2, SHBG, LH or FSH and the expected pharmacodynamic effect (i.e. suppression) on LH in hypergonadotropic patients occurred with both treatments.

There was a low frequency of adverse events with no significant difference in treatment-related adverse events between the two testosterone formulations (Table 2).

DISCUSSION

Two medicinal products containing the same active substance are considered bioequivalent (BE) if their bio availabilities (rate and extent – AUC, C_{max} and C_{avg}) after administration in the same molar dose lie within acceptable predefined limits (80–125%). These limits are set to ensure comparable in vivo performance, that is similar in terms of safety and efficacy (EMEA Guidelines, 2013). Bioequivalence is generally confined to comparing generic preparations of a similar formulation and molar dose. The T cream and T gel used in this study fell within these predefined limits despite the significant differences between the respective strengths (5% vs. 1%), formulations (cream vs. gel) and the administered dose of testosterone (100 mg vs. 50 mg).

The T gel has a well-established pharmacokinetic, safety and clinical efficacy profile.

Other testosterone products have used the T gel as a benchmark comparator for clinical efficacy (Mazer *et al.*, 2005).

The product information for the comparator T gel (Testogel TGA approved PI, 2014), states that in the pivotal clinical trial of T gel vs. a transdermal T patch some individuals achieved suboptimal bioavailability (Swerdloff *et al.*, 2000). In that study 27.4% of subjects using a 50 mg daily dose of T gel were titrated upwards to 75 mg daily because their serum T levels were not elevated beyond the study inclusion criteria (10.4 nmol/L) after 60 days of treatment. The authors noted that despite increasing the dose by 50% this group had average serum T levels lower than those that remained on a 50 mg daily dose.

Our results for the T gel C_{max} and C_{avg} values at 30 days are significantly less than those quoted in the T gel PI (19.4 ± 12.8 and 11.3 ± 3.7 nmol/L compared with 30.4 ± 2.0 and 19.6 ± 1.1 nmol/L respectively). Previous pharmacokinetic data with the T cream (Lawley, 2001, 2004) applied to the lower abdomen showed serum T levels comparable to that in the pivotal T gel study (Swerdloff *et al.*, 2000). There can be significant variation in absorption between the transdermal testosterone options depending upon site of application, product type, product formulation and individual skin variation. During the development of the T patch it was established that testosterone absorption from the back > thigh > upper arm > abdomen > chest > shin (Meikle *et al.*, 1996). It is likely that application to the torso may not be as receptive to the absorption of testosterone compared with the shoulders, upper arms and abdomen. A review of patient diaries showed that participants were compliant during both treatment arms.

Our study period was not of sufficiently long duration nor was sufficiently powered to adequately determine QOL or symptoms of sexual function. Because of the short duration of the study our results do not adequately address all safety issues that may arise with longer term testosterone usage.

The strength of our study design was that owing to the crossover individuals acted as their own controls thus eliminating variations such as intersubject absorption variances. Both products restored serum testosterone levels from a hypogonadal to a eugonadal state which is the cornerstone for the effective management of androgen deficiency. From a bioequivalence standpoint the two products are bioequivalent and the subject numbers complied with EMEA guidelines.

Testosterone cream provides patients and clinicians with an additional option for the management of androgen deficiency in hypogonadal males.

ACKNOWLEDGEMENTS

DISCLOSURES

AUTHORS' CONTRIBUTIONS

G.W. and M.B. did the conception and study design; G.W. was the principal investigator and researcher; J.W. and R.H. conducted the data and statistical analysis and interpretation; M.B. drafted the manuscript; R.H., G.W. and J.W. edited and revised the manuscript; all authors approved the final version of the manuscript.

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