Evaluation of the Pharmacokinetic Profiles of the New Testosterone Topical Gel Formulation, Testim^{TM}, Compared to AndroGel$^{®}$

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ABSTRACT: A two-period, randomized, complete crossover study was performed to evaluate the pharmacokinetic profiles of Testim^{TM} (AA2500), a new 1% testosterone topical gel formulation, compared to AndroGel$^{®}$, an already available 1% testosterone topical gel. Twenty-nine hypogonadal subjects received a single dose (50 mg testosterone) of each formulation seven days apart. C_{max} estimates for total testosterone, dihydrotestosterone and free testosterone were greater (30, 19 and 38%, respectively) following the application of Testim^{TM} compared to AndroGel$^{®}$. Similarly, AUC_{0-24} estimates for total testosterone, dihydrotestosterone, and free testosterone were greater (30, 11 and 47%, respectively) following the application of Testim^{TM} compared to AndroGel$^{®}$. Confidence intervals for C_{max} and AUC_{0-24} were not wholly contained within the bioequivalence limits for testosterone, therefore Testim^{TM} and AndroGel$^{®}$ are not bioequivalent with Testim^{TM} providing higher serum levels and greater bioavailability than AndroGel$^{®}$. Copyright © 2003 John Wiley & Sons, Ltd.

Key words: pharmacokinetics; testosterone; AA2500; Testim^{TM}; AndroGel$^{®}$

Introduction

Insufficient secretion of testosterone due to aging and absence of, loss of, or injury to testosterone producing organs contributes to the main cause of low serum testosterone concentrations in the male [1]. Signs and symptoms associated with low testosterone include decreased sexual desire with or without impotence, fatigue and loss of energy, mood depression, osteoporosis, decreased muscle mass, and regression of secondary sexual characteristics [1]. The main reason for testosterone replacement therapy in hypogonadal men is to normalize serum concentrations of this hormone in order to improve these symptoms [2].

In order to provide sustained physiological serum testosterone concentrations, a variety of exogenous formulations have been developed [3]. Some forms alter the androgen molecule either to prevent rapid degradation after oral administration or to slow absorption of the injected intramuscular form into the circulation. Other products, such as transdermal preparations, pellets for subcutaneous placement, and sublingual preparations have been developed to overcome the first pass effect phenomena.

Studies with a topical gel formulation (AndroGel$^{®}$, Unimed) have shown that a single application to skin of the shoulders, upper arms, and abdomen was rapidly absorbed, with serum testosterone concentrations peaking 18-24 h after application [4]. With continued daily

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reapplications, steady state serum levels of testosterone were maintained [4,5]. In this report, we evaluate the pharmacokinetics of a single dose of the new topical gel formulation Testim™ compared to the commercially available Andro-Gel® in a two-period, complete crossover study.

Material and Methods

Materials

Testim™ was supplied as unit-dose foil tubes containing 50 mg of testosterone in 5.0 g of gel (Lot No. PLCF, Auxilium Pharmaceuticals, Inc., Norristown, PA). AndroGel® was supplied as marketed unit-dose aluminium foil packets containing 50 mg of testosterone in 5.0 g of gel (Batch Number 00152, Unimed Pharmaceuticals, Inc., Buffalo Grove, IL).

Subjects

A total of 180 subjects over the age of 45 were screened to find twenty-nine (29) hypogonadal male subjects to participate in the study, conducted at Orlando Clinical Research Center, Orlando, FL. The mean (S.D.) age of the subjects was 61.2 (8.9) years. Mean (S.D.) height was 70.2 (2.7) inches, and mean (S.D.) BMI was 27.1 (3.1). Of the 29 male subjects enrolled in the study, 25 were Caucasian, two were Asian, one was Black, and one was Hispanic. Nineteen subjects had an 0800 h (± 30 min) serum testosterone level below 250 ng/dl. Ten subjects had an 0800 h (± 30 min) serum testosterone level between 250 and 300 ng/dl. The study protocol was approved by an appropriate institutional review board and all subjects gave written informed consent before participation. Other than their hypogonadal condition, subjects were considered to be in otherwise good health based upon the results of medical history, physical examination, clinical laboratory evaluations, and electrocardiogram obtained within 3 weeks prior to initial study drug administration.

Study design

This was a randomized, open-label, two-way, complete crossover study. Subjects were ran-

domly assigned in equal numbers to two sequences of treatment: treatment A-Testim™ and treatment B-AndroGel®. The two formulations were applied to different body sites with the first period application to the right shoulder and the second period application to the left shoulder. All subjects were housed and supervised during each dosing period from approximately 12 h prior to dosing until after the 24 h blood collection was completed. Subjects returned the following morning to the study center for their 48 h blood collection.

Blood collection

Approximately 10 ml of whole blood samples were collected at the following times: 0 h (predose), 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 15, 18, 24, and 48 h after dosing in each period. The samples were separated by centrifugation at $1500 \times g$ for 10 min at 18°C. The resulting sera were transferred into plastic tubes and immediately frozen and stored at –20°C (± 3°C) until assayed.

Analytical methods

Serum samples were analyzed for total testosterone (T), free testosterone (FT), and dihydrotestosterone (DHT) by specific radioimmunoassay techniques. The radioimmunoassay came in a kit complete with standard concentration steroid solutions and a diluent solution. The T and FT radioimmunoassays were produced by Diagnostics Products Corporation (Los Angeles, CA); the DHT radioimmunoassay was produced by Diagnostics Systems Laboratories (Webster, TX) [6–8]. All radioimmunoassays were performed by ICON Laboratories (Farmingdale, NY), who were accredited to perform the assays.

Data Analysis

Pharmacokinetic analysis

For all three analytes (T, DHT, and FT), baseline-adjusted concentrations were used in the analysis. These were calculated by subtracting the pre-dose concentration from all concentrations following application. As a result, several negative baseline-adjusted concentrations were generated;

these were imputed with zero unless observed at the last sampling time point, in which case they were ignored for pharmacokinetic purposes.

Individual profiles of the baseline-adjusted serum concentrations of each of the three analytes against actual sampling time after application of either Testim^{TM} or AndroGel$^{®}$ were generated for each subject and session. Pharmacokinetic parameter estimates for all three analytes were derived for each individual prior to the calculation of mean parameter estimates for each formulation and analyte. Pharmacokinetic parameters were estimated using WinNonlin pharmacokinetic software. Non-compartmental modeling was used to generate parameter estimates, using WinNonlin model 200 [9]. The terminal elimination phase was identified by regression analysis within WinNonlin, using at least 3 data points in each serum concentration vs time profile.

The following parameter estimates were calculated for each analyte where possible for each subject. C_{max} was the observed maximum concentration after dosing. t_{max} was the time at which C_{max} was apparent. Both parameters were determined by direct inspection of the serum concentration vs time data point values. AUC_{0-24} was the area under the serum concentration vs. time curve from zero to 24 h calculated using the linear trapezoidal method. $t_{1/2}$ (the terminal elimination half-life) was calculated by regression analysis of the terminal elimination slope.

Statistical analysis

With the following assumptions using log (AUC_{0-24}), 30 subjects were required to demonstrate average bioequivalence: 90% power, equivalence limits = (80, 125%), an estimated within subject standard deviation = 0.248 natural logarithm (nmol/l), and no difference between mean values.

Following a logarithmic transformation, AUC_{0-24} and C_{max} values for T, DHT, and FT were subjected to analysis of variance (ANOVA) techniques, including terms for subject, period, and formulation. A first order carryover effect was included in the model but was removed if found to be not significant at the 10% significance level.

Point estimates and 90% confidence intervals for the difference of the test formulation (Testim^{TM}) to the reference formulation (Andro-Gel$^{®}$) were constructed using the error variance obtained from the ANOVA. The point and interval estimates were then back transformed to give estimates of the ratio of the test formulation relative to the reference formulation. If the 90% confidence interval for the measure of relative bioavailability (i.e. AUC_{0-24} and C_{max} ratio) was within the acceptance range of 0.80–1.25 [10], then the two formulations were judged to be bioequivalent. The t_{max} was analyzed and point estimates and 90% confidence intervals for the difference between the two formulations were constructed in a similar manner without use of a log transformation.

Distributional assumptions underlying the statistical analyses were assessed by visual inspection of residual plots. Normality was examined by normal probability plots, while homogeneity of variance was assessed by plotting the studentized residuals against the predicted values for the model. If the distributional assumptions of a lognormal (AUC_{0-24} and C_{max}) distribution or normal (t_{max} and $t_{1/2}$) distribution in the parametric approach were doubtful, a corresponding non-parametric approach was followed [11]. Geometric means, within subject's coefficients of variation (CV_w) and between subject coefficients of variation (CV_b) were calculated for the log transformed parameters, using the following formula: $CV_w(\%)=[exp(mse)-1]^{1/2*}$ 100, where mse = mean square error from the analysis of variance model. $CV_b(\%) = [exp(SD^2)-1]^{1/2*}$ 100, where S.D. = standard deviation of the natural logarithmically transformed data (Table 1).

All estimations of the terminal elimination phase slope (lambda-z) were considered to be utilizing non-elimination phase data; consequently, no $t_{1/2}$ estimates were included in the

Table 1. Geometric mean ($CV_b\%$) C_{max} and AUC_{0-24} for testosterone

	C_{max} (ng/dl)	AUC_{0-24} (ng*h/dl)
Testim^{TM}	480 (70.3)	5864.5 (77.9)
AndroGel$^{®}$	368 (60.9)	4499.1 (77.9)

summary statistics or statistical analysis for all three analytes.

As a result of a significant sequence effect in the analysis of variance models for C_{max}, the following additional supplementary analysis using Period 1 values only was performed for C_{max} and AUC_{0-24} for both T and FT analytes. Following a logarithmic transformation, Period 1 AUC_{0-24} and C_{max} values for T and FT were subjected to ANOVA techniques, including a term for formulation. Point estimates and 90% confidence intervals for the difference of the test formulation to the reference formulation were constructed using the error variance obtained from the ANOVA. The point and interval estimates were then back transformed to give estimates of the ratio of the test formulation relative to the reference formulation.

For t_{max} for all three analytes and for DHT (AUC_{0-24} and C_{max}), the assumptions of normality and homogeneity of variance were not satisfied. Therefore t_{max} and DHT (AUC_{0-24} and C_{max}) were analyzed and point estimates and 90% confidence intervals for the difference between the two formulations were constructed using the methods outlined in Hauschke *et al.* [11].

Results

Serum concentrations of all three analytes (T, DHT, and FT) increased rapidly following application of either Testim™ or AndroGel®. Three concentration maxima were observed for all analytes in the majority of profiles; these were apparent at approximately 3–4 h, 8–10 h, and 18–24 h post application, respectively (see Figure 1). Consequently, individual t_{max} varied considerably, ranging from 1.00 to 24.02 h for T, 2.98 to 48.00 h for DHT, and 1.00 to 24.05 h for FT. In the majority of profiles, concentrations started to decline by 24 h post application.

The unreliability of the lambda-z determinations resulted in a large variability in apparent $t_{1/2}$ values. Therefore the $t_{1/2}$ estimates for T, DHT, and FT were not considered valid and are not presented.

Testosterone

Systemic exposure to T was highly variable following topical application of either Testim™ or AndroGel®. Mean estimates of both AUC_{0-24} and C_{max} were consistently higher following
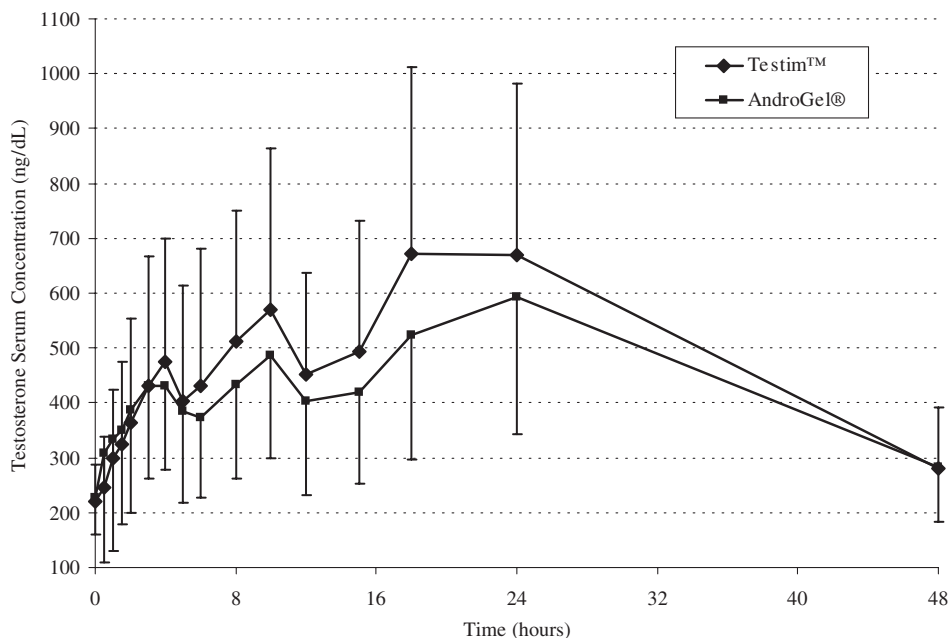

Figure 1. Mean (S.D.) testosterone levels following a single dose of Testim™ or AndroGel® testosterone gel 50 mg TD in 29 hypogonadal men

application of Testim™ compared to Andro-Gel®. The adjusted geometric means ($CV_b\%$) from the analysis of variance models for C_{max} and AUC_{0-24} are shown in Table 1.

The ratio of the treatment comparison for both C_{max} and AUC_{0-24} was 1.30, indicating that values for Testim™ were 30% greater than for AndroGel®. The 90% confidence interval for C_{max} ratio was (1.10, 1.55), and for AUC_{0-24} it was (1.08, 1.57). Since neither of these confidence intervals was wholly contained within the bioequivalence limits of 0.80 to 1.25, Testim™ and AndroGel® are not bioequivalent.

Statistical analysis revealed a sequence effect whereby the magnitude of the difference between the two formulations was less when AndroGel® was applied in Period 1 compared to Period 2. Therefore, the ANOVA model for C_{max} included a term for sequence in the model since this was statistically significant at the 10% significant level. The results using Period 1 values only are consistent with the results obtained from the analysis including both treatment periods. In addition, as the two formulations were applied to different body sites (right/left shoulder), it was considered unlikely that AndroGel® could inhibit the absorption of Testim™ via a topical effect on dermal or muscular tissue.

Median t_{max} values were similar between the two formulations. Median values for Testim™ and AndroGel® were both 18.00 h, with an estimated treatment difference of 0 h and a 90% confidence interval of (−3.00, 3.00 h), indicating that there were no statistically significant differences between the two formulations with respect to t_{max}.

Dihydrotestosterone

As observed for T, systemic exposure to DHT was also highly variable following topical application of either Testim™ or AndroGel®. Similarly, DHT C_{max} and AUC_{0-24} estimates were consistently higher following application of Testim™ compared to AndroGel®. The assumptions of normality were not satisfied for the analysis of variance models for C_{max} and AUC_{0-24}; therefore, non-parametric analyses were performed. For both C_{max} and AUC_{0-24}, median values for

Table 2. Median (range) C_{max} and AUC_{0-24} for dihydrotestosterone[a]

	C_{max} (pg/ml)	AUC_{0-24} (pg∗h/ml)
Testim™	321 (23–964)	4891.0 (257.5–15259.1)
AndroGel®	313 (16–1038)	4091.7 (225.0–16034.5)

[a] Excluding one patient for AUC_{0-24} because there was insufficient sample volume for analysis in Period 2.

Testim™ were greater than for AndroGel® (see Table 2). For C_{max}, the estimated treatment ratio was 1.19, indicating that values for Testim™ were greater than for AndroGel®. Similarly for AUC_{0-24}, the estimated treatment ratio was 1.11. The 90% confidence interval for C_{max} ratio was (0.91, 1.36), and (0.95, 1.32) for AUC_{0-24}. As neither of these confidence intervals was wholly contained within the bioequivalence limits of 0.80 to 1.25, Testim™ and AndroGel® are not bioequivalent.

Median DHT t_{max} values for Testim™ and AndroGel® were both 18.00 h, with an estimated treatment difference of −0.01 h and a 90% confidence interval of (−3.99, 3.00 h), indicating that there were no statistically significant differences between the two formulations with respect to t_{max}.

Free testosterone

As for T and DHT, mean estimates were consistently higher following application of Testim™ compared to AndroGel®. For both C_{max} and AUC_{0-24}, the adjusted geometric mean for Testim™ was greater than for AndroGel® (See Table 3). For C_{max}, the ratio of the treatment comparison was 1.38, suggesting values for Testim™ were on average 38% greater than for AndroGel®. Similarly, for AUC_{0-24}, the ratio of the treatment comparison was 1.47, suggesting values for Testim™ were on average 47% greater than for AndroGel®. The 90% confidence interval

Table 3. Geometric mean ($CV_b\%$) C_{max} and AUC_{0-24} for free testosterone

	C_{max} (pg/ml)	AUC_{0-24} (pg*h/ml)
Testim™	20.08 (77.8)	240.7 (75.4)
AndroGel®	14.55 (69.8)	164.2 (90.0)

for C_{max} ratio was (1.14, 1.67) and (1.20, 1.79) for AUC_{0-24}. Since neither of these confidence intervals was wholly contained within the bioequivalence limits of 0.80 to 1.25, Testim™ and AndroGel® are not bioequivalent.

Consistent with T, the statistical analysis revealed a sequence effect whereby the magnitude of the difference between the two formulations was less when AndroGel® was applied in Period 1, compared to Period 2. Therefore the ANOVA model for C_{max} included a term for sequence in the model since this was statistically significant at the 10% significant level. The results using Period 1 values only are consistent with the results obtained from the analysis including both treatment periods.

Although median FT t_{max} values for Testim™ and AndroGel® were 18.02 and 24.00 h, respectively, due to variability in t_{max}, the estimated difference in t_{max} was -1.50 h and a 90% confidence interval of (-4.00, 3.00 h). This indicated that there were no statistically significant differences between the two formulations with respect to t_{max}.

Discussion

Serum concentrations of the three analytes (testosterone (T), dihydrotestosterone (DHT), and free testosterone (FT)) increased rapidly following topical application of either Testim™ or AndroGel®. For all analytes, three concentration maxima were generally observed at 3–4 h, 8–10 h, and 18–24 h post application. In the majority of profiles, concentrations started to decline by 24 h post application. Systemic exposure to T, DHT, and FT was variable, but was consistently greater following application of Testim™ compared to AndroGel®. For all three analytes, the 90% confidence intervals for AUC_{0-24} and C_{max} were not wholly contained within the bioequivalence limits and, therefore, the two formulations are not bioequivalent with Testim™ providing significantly greater T and FT levels compared to AndroGel®.

The enhanced absorption of T with Testim™ may be attributed to the increased emollient qualities of this formulation due to inclusion of a pentadecalactone. The AndroGel® formulation, which is also hydroalcoholic in base, does not have the same emollient levels and may account for greater drying of the skin resulting in lower T absorption.

Conclusion

Application of 5.0 g of the new topical gel formulation, Testim™, to hypogonadal males was shown to produce significantly higher levels of testosterone and free testosterone than 5.0 g of AndroGel®.

Acknowledgements

References

1. Griffin JE, Wilson JD. In: *Harrison's Principles of Internal Medicine*, Fauci AS *et al*. (eds). (14th edn). McGraw Hill: New York, NY; 1998: 2087–2097.
2. Howell S, Shalet S. Testosterone deficiency and replacement. *Horm Res* 2001; **56** (Suppl) (1): 86–92.
3. McGriff NJ, Csako G, Kabbani M, *et al*. Treatment options for a patient experiencing pruritic rash associated with transdermal testosterone: a review of the literature. *Pharmacotherapy* 2001; **21**(11): 1425–35.
4. Wang C, Berman N, Longstreth JA, *et al*. Pharmacokinetics of transdermal testosterone gel in hypogonadal men: application of gel at one site versus four sites: A general clinical research center study. *J Clin Endocrinol Metab* 2000; **85**(3): 964–969.
5. Swerdloff RS, Wang C, Cunningham G, *et al*. Long-term pharmacokinetics of transdermal testosterone gel in hypogonadal men. *J Clin Endocrinol Metab* 2000; **85**(12): 4500–4510.
6. Diagnostics Products Corporation, Coat-A-Count®, Total Testosterone Assay Kit, product insert.
7. Diagnostics Products Corporation, Coat-A-Count®, Free Testosterone Assay Kit, product insert.
8. Diagnostics Systems Laboratories, Active™ Dihydrotestosterone Assay Kit, product insert.
9. WinNonlin v1.1. User Guide, Pharsight Corp., Mountain View, California, USA
10. Pabst G and Jaeger H. Review of methods and criteria for the evaluation of bioequivalence studies. *Eur J Clin Pharmacol* 1990; **38**(1): 5–10.
11. Hauschke D, Steinijans VW and Diletti E. A distribution-free procedure for the statistical analysis of bioequivalence studies. *Int J Clin Pharmacol Ther Toxicol* 1990; **28**(2): 72–78.