Correcting endogenous concentrations of testosterone influences bioequivalence and shows the superiority of TDS®-testosterone versus Androgel®

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Abstract. Aim: Circulating concentrations of endogenous compounds such as testosterone, complicate the analysis of pharmacokinetic parameters when these compounds are administered exogenously. This study examines the influence of three correction methods of accounting for endogenous concentrations on the determination of bioequivalence between two testosterone formulations. Methods: 12 healthy males received 50 mg TDS®-testosterone, TDS®-placebo, and 50 mg Androgel® in a randomized placebo controlled study. Three correction methods (1,2 and 3) to remove the influence of endogenous testosterone from the exogenous blood concentrations data were carried out before the calculation of the AUC and C_{max}. The relative bioavailabilities between two treatments were then performed for the AUC and C_{max} for all the corrected and uncorrected data. Correction 4 was performed on the AUC and the C_{max} values and the average values were calculated for both active treatments. Results: The relative bioavailability comparison of the AUC and C_{max}, showed that the TDS®-testosterone and Androgel® was bioequivalent by using uncorrected data (CI: 93 – 120%; AUC_{0-12} and 88 – 117%; C_{max}). However, they were not bioequivalent when using all the corrections data ((Corr. 1; CI: 52 – 106%; AUC_{0-12} and 50 – 258%; C_{max}), (Corr. 2; CI: 71 – 655%; AUC_{0-12} and 87 – 286%; C_{max}), (Corr. 3; CI: 67 – 315%; AUC_{0-12} and 88 – 157%; C_{max})). TDS®-testosterone also showed the higher AUC_{0-12} and C_{max} compared to Androgel® for uncorrected and all the Corrections 1, 2, 3 and 4. Conclusions: Different results obtained in the relative bioavailability between TDS®-testosterone and Androgel® for uncorrected data and corrected data, suggests that correcting endogenous concentrations is important for the proper determination of bioequivalent for endogenous compounds such as testosterone.

Introduction

Testosterone, an endogenous hormone is the most important androgen secreted into the blood by the Leydig cells in the testes. In women, testosterone also is probably the principal androgen and is synthesized both in the corpus luteum and the adrenal cortex by similar pathways [Snyder 2001]. Testosterone deficiency in men (hypogonadal) is always associated with incomplete development of male sex characteristics. At this stage, testosterone replacement therapy may be indicated. There are a few treatments currently available to increase the amount of testosterone in the systemic circulation such as oral and sublingual preparations [Johnsen et al. 1974, Stuenkel et al. 1991], transdermal patches [Dobs et al. 1999, Korenman et al. 1987], subcutaneous implants [Handelsmam et al. 1990] and also the gel applications [Jockenhovel 2003, Swerdloff et al. 2003]. However, the fact that ingestion of testosterone is not an effective way to replace testosterone [Snyder 2001] due to first pass metabolism, topical applications of testosterone containing gels and patches avoid the hepatic catabolism and are among the more successful attempts to deliver testosterone systemically.

In the evaluation of new drug preparations, a bioequivalence (BE) study in healthy volunteers normally has to be conducted in order to show that the new preparation is as effective as current preparations. Guidelines for the determination of bioequivalence of pharmaceutically active formulations are fully documented by Food and Drug Administration (FDA) in the USA [US FDA 2006] and by the Committee for Proprietary Medicinal Products (CPMP) for the European Union [EMEA 2006]. Bioequivalence studies compare the rate and extent of absorption for a new treatment against that of the reference product using the parameters of maximum serum concentration (C_{max}) and area under the curve (AUC). For exogenously administered endogenous compounds, like testosterone, the comparison needs to allow for the normal circulating concentrations of the compound.

The calculations of AUC and C_{max} are normally performed by the measurement of blood concentrations time profiles from pre-dose (0 h) with zero value of drug concentrations up to several hours post-dose with the concentrations of drug at the last measuring point approaching zero. However, the circulating concentrations of testosterone, complicate the analysis of pharmacokinetic parameters when this compound is administered exogenously. Therefore, correction of the data to remove the influence of endogenous testosterone is necessary to obtain the concentration of testosterone that is attributable to the exogenous source.

Except levothyroxine sodium [US FDA 2006] and potassium chloride [US FDA 2006], the current BE guidelines offer no guidance on the correction for the endogenous concentrations. In this study, we have demonstrated four approaches to the correction for serum concentration data for the presence of endogenous concentrations and documented how these methods influenced the apparent bioequivalence of testosterone in healthy males.

Methods

Study design and treatments

This was a single-dose, randomized, three-way crossover study (with 3 treatments, 3 periods and 6 sequences) with a minimum of 1 week washout period between each treatment. Twelve healthy males successfully completed the study. The three treatments were TDS®-testosterone 50 mg/ml (metered pump spray), TDS®-placebo (metered pump spray), and Androgel® 1% (50 mg/5 g gel). The dose was applied to the left arm and gently rubbed into the skin. Approximately 4 ml of blood was collected at –0.5 and 0 h to establish a baseline measurement of serum testosterone concentration. Subsequently, serial blood samples were collected at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 10, 12 and 24 h post-dose. The serum concentrations of testosterone were analyzed using ELISA method. Full details of the study are described elsewhere [Chik et al. 2006].

Correction methods

Three methods of data correction were used to subtract the influence of endogenous concentrations of testosterone from the total concentration measurements. The area under the curve (AUC) and the maximum concentration C_{max} were then calculated from the data generated in Corrections 1, 2 and 3.

Correction Method 1

The mean pre-dose testosterone concentration (–0.5 and 0 h) was subtracted from each testosterone concentration after dosing for each subject and treatment.

Correction Method 2

The endogenous data were modelled from the placebo data using a polynomial equation and subtracted from the measured treatment values. The endogenous concentration obtained for each time point was then subtracted from the analogous time point for active treatments.

Correction Method 3

The concentrations on the placebo day were subtracted from the active treatment

concentrations at the analogous time point for each subject.

Correction Method 4

The testosterone C_{max} and AUC values calculated on the placebo day were subtracted from the parameters calculated from the two active treatment days.

Some of the negative values generated after subtraction in Corrections 1, 2 and 3 were considered 0. The serum testosterone concentrations (ng/ml) were then plotted versus times (h) for uncorrected and Corrections 1, 2 and 3.
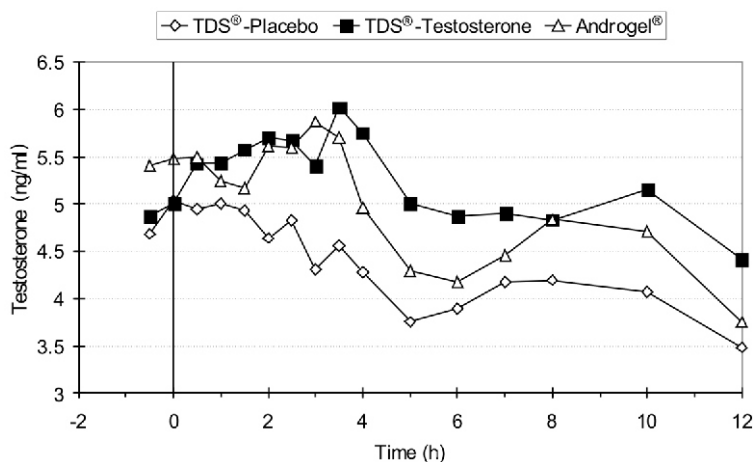

Figure 1. Plots of mean serum testosterone concentration (ng/ml) vs. time (h) for each treatment based on uncorrected data.
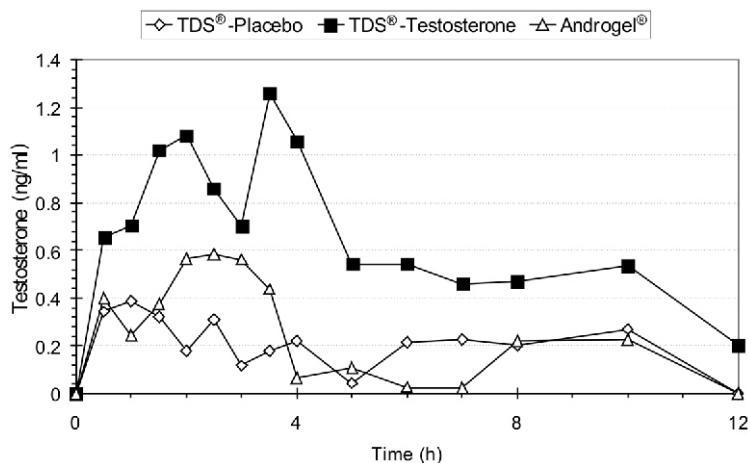

Figure 2. Plot of mean serum testosterone concentration (ng/ml) vs. time (h) for each treatment based on Correction 1.

Pharmacokinetic and statistical analysis

C_{max} was determined by observation and AUC was calculated using the linear trapezoidal method. The AUC and C_{max} for the uncorrected and corrected data were determined from 0 – 12 h data for each treatment. Analysis of variance (ANOVA) were carried out on the log transformed AUC and C_{max} to determine the bioequivalence between TDS®-testosterone and Androgel®. Formulations were considered bioequivalent if the 90% confidence interval (CI) of the ratio, test to reference, was contained within 80 to 125% [US FDA 2006, EMEA 2006, Pabst et al. 1990]. The 0 – 24 h data were only used in Correction 2, where the two models (0 – 12 and 0 – 24 h) of endogenous testosterone were plotted. All the statistical analyses were carried out using Win Nonlin Professional ver. 5.2.1.

Results

Serum testosterone profile

Uncorrected serum testosterone data

The mean testosterone serum concentration-time profile for TDS®-testosterone, TDS®-placebo and Androgel® are shown in Figure 1. Higher testosterone concentration profiles were observed for both the active treatments compared to placebo. Serum testosterone profiles for TDS®-testosterone and Androgel® were lower at the pre-dose, and increased after the treatments application. Both the active treatments achieved the highest concentration after 3 – 4 h post-dose and declined following the 12 h period. However, TDS®-placebo showed the decreased in serum testosterone profile from pre-dose until the 12 h post-dose.

Correction Method 1

Following the 12 hours sampling period, TDS®-testosterone showed the higher profile of testosterone concentrations compared to Androgel® and TDS®-placebo (Figure 2). Androgel® also showed the small increased from 0 up to 2.5 hours and decrease through
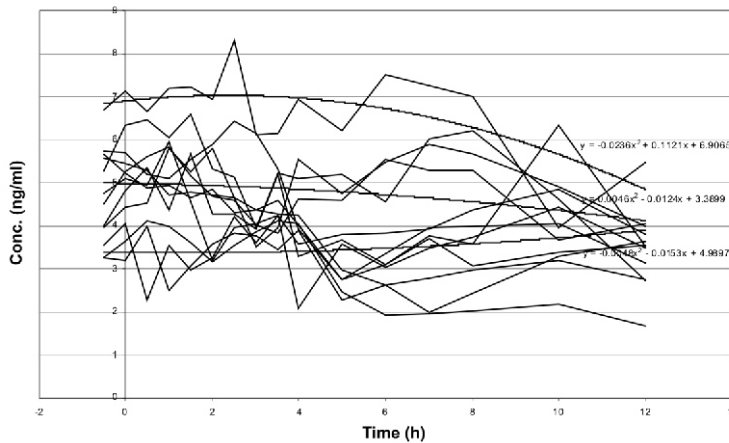
Figure 3. Plots of concentration vs. time for TDS®-placebo in 12 subjects with examples of polynomial regression on selected lines.
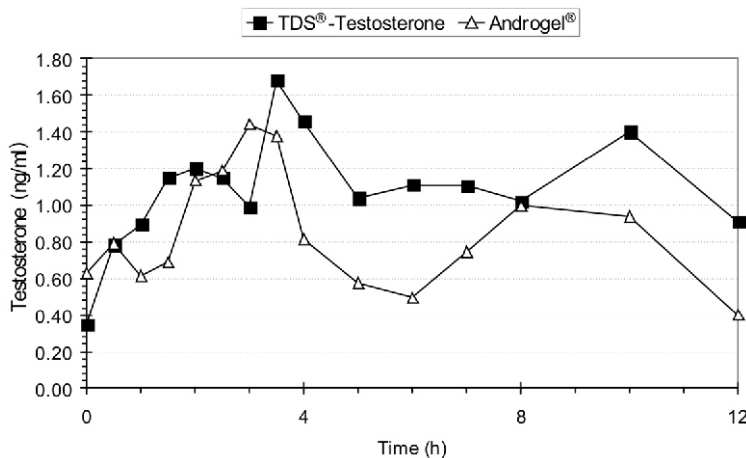

Figure 4. Plots of mean serum testosterone concentration (ng/ml) vs. time (h) for TDS®-testosterone and Androgel® based on Correction 2.
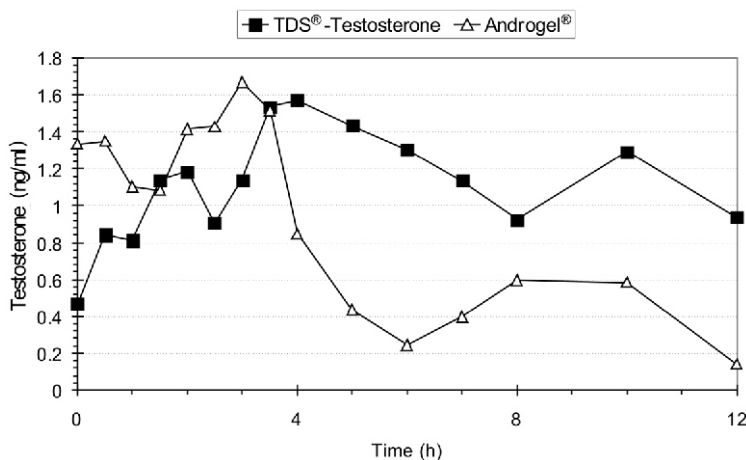

Figure 5. Plots of mean serum testosterone concentration (ng/ml) vs. time (h) for TDS®-testosterone and Androgel® based on Correction 3.

out the day to the same level as placebo. TDS®-placebo treatment remains constant and slightly fluctuated for the whole sampling period.

Correction Method 2

Figure 3 shows the testosterone profile for the placebo treatments for 12 subjects with the polynomial regression and equation on selected subjects. The plots for TDS®-testosterone and Androgel® quite a similar with the peaks at 3.5 h and 3 h, respectively. However, the testosterone concentrations were higher for TDS®-testosterone compared to Androgel® for most of the sampling times (Figure 4).

Correction Method 3

The mean serum testosterone concentrations for Androgel® were higher than TDS®-testosterone after application up to 3 h, but decreased dramatically to lower than pre-dose value, especially at 12 h post-dose (Figure 5). The serum testosterone concentrations profile for TDS®-testosterone were increased after application and peaked at 4 h, before decreased constantly throughout the day.

All the plots for serum testosterone concentrations (ng/ml) versus time (h) based on uncorrected and Correction Methods 1, 2 and 3 showed the higher testosterone profile for TDS®-testosterone than Androgel®.

Bioequivalence and Statistic

The relative bioavailability comparison of the AUC and C_{max} for TDS®-testosterone and Androgel® are summarized in Table 1 for all uncorrected, Correction Methods 1, 2 and 3 data. The AUC and C_{max} for TDS®-testosterone and Androgel® were not bioequivalence with TDS®-placebo using the uncorrected and Correction Method 1 data. However, the 90% CI of the AUC and C_{max} for the comparison between TDS®-testosterone and Androgel® gave a different result between uncorrected and Corrections 1, 2 and 3. TDS®-testosterone and Androgel® were found to be bioequivalent based on uncorrected data, but

they were not bioequivalent with Corrections 1, 2 and 3.

Table 2 summarizes the percentage differences of AUC and C_{max} between TDS®-testosterone and Androgel®. The differences were lower for uncorrected data compared to corrected data. Within the corrections data, the largest difference was seen in Correction Method 1. Similarly, the other correction methods showed higher AUC and C_{max} values for TDS®-testosterone compared to Androgel®. Thus, data corrected for endogenous serum testosterone concentrations showed increased testosterone profiles for TDS®-testosterone compared to Androgel®, with correction AUC by 30 to 190% and correction C_{max} by 12 to 55%, depending on the method of correction. ANOVA could not be performed on Correction Method 4 due to the negative values generated. Therefore, only the average of AUC and C_{max} were reported.

Discussion

In this study, we have shown the different outcomes in following further analysis of testosterone data from the study conducted in 12 healthy subjects [Chik et al. 2006]. These findings are important for the future development of TDS®-testosterone or any other delivery system for endogenous compounds. There are potential advantages and disadvantages which may influence the accuracy of each correction method.

The first correction method (Correction Method 1) was based on the assumption that the endogenous testosterone level remains constant at all times. Therefore, the mean pre-dose (–0.5 and 0 h) concentration value

Table 1. Relative bioavailability (90% CI) for uncorrected and all corrections data.

Correction Methods	Bioequivalence (90% CI)	
	AUC_{0-12}	C_{max}
Uncorrected	93 – 120	88 – 117
Correction Method 1	52 – 106	50 – 258
Correction Method 2	71 – 655	87 – 286
Correction Method 3	67 – 315	88 – 157

Reference: Androgel®.

Table 2. Percentage differences (%) in mean AUC and C_{max} between TDS®-testosterone and Androgel®.

	Mean value		Percentage differences (TDS-Androgel) (%)	
	AUC (ng/ml × h)	C_{max} (ng/ml)	AUC (ng/ml.h)	C_{max} (ng/ml)
Uncorrected				
TDS®-testosterone	61.92	6.63	7.18	1.47
Androgel®	57.77	6.54		
Correction Method 1				
TDS®-testosterone	7.47	1.69	190.15	54.90
Androgel®	2.57	1.09		
Correction Method 2				
TDS®-testosterone	13.61	2.42	36.37	29.41
Androgel®	9.98	1.87		
Correction Method 3				
TDS®-testosterone	13.86	2.88	30.07	16.45
Androgel®	10.65	2.48		
Correction Method 4				
TDS®-testosterone	11.15	0.95	59.29	11.76
Androgel®	7.00	0.85		

can be used to subtract the post-dose values to obtain the correct amount from exogenous administrations. The advantage of this method is that the pre-dose values were obtained exactly before the administration of testosterone, which can minimize the period error. However, this method also offers some disadvantages, as it is known that testosterone secretion is likewise pulsatile and diurnal. In healthy males, recorded plasma testosterone concentration is normally higher in the morning, at about 8 a.m. and lowest in the evening (~8 p.m.) [Snyder 2001], and also slightly fluctuated over the day and night. This condition can be seen in the pattern of mean testosterone concentrations from placebo treatment in Figure 1, which was higher at the beginning and lower throughout the day.

The second correction (Correction Method 2) was based on the inhibition of the gonad regulating cycle, which can cause the body to reduce the production of testosterone when its level is too high. During exogenous administration of testosterone, endogenous testosterone release may be inhibited through feedback inhibition of pituitary luteinizing hormone (LH), resulting in declining levels of testosterone. Correction Method 2 may offer a slight advantage over Correction Method 1, as the average level of testosterone production can be estimated through the best fit of polynomial regression the placebo modelled data. These data can, therefore be accepted as an average endogenous level of testosterone on that particular subject. However, the inhibition of endogenous testosterone productions may only happen with large doses of exogenously administered testosterone. No data were available to date to suggest the exact amount of testosterone that can inhibit the production of testosterone. Assuming the diurnal variation of testosterone as explained previously, Correction 1 and 2 have a similar disadvantage.

The third method of correction (Correction Method 3), by assuming the analogous placebo data as endogenous level of testosterone can offer more advantages over the first two methods. Subtraction of the treatment values at the analogous time points of the placebo can overcome the diurnal variation as the above two methods did not. However, this method may has disadvantages as the analogous placebo values, which obtained in different period may not represented the endogenous testosterone level on the day of the active treatments administered. This can be explained by the high variability in the pre-dose testosterone concentration values (0 h). As manifest by the within subject coefficient of variation (CV) of the C0 values which ranged from 1.4 to 28.2 %. The study by Andersson and co-workers [Andersson et al. 2003] also suggested that there was variability in the testosterone levels from month to month. To minimize this error, placebo treatment may need to be conducted a day before the active treatment is given. Considering all the outcomes and the results obtained in this analysis, different perspectives of studies need to be conducted in order to find the most accurate method that can be accepted for the bioequivalent assessment of endogenous compounds such as testosterone. These findings can give an insight into bioequivalence assessment on the future development of exogenously administered endogenous compounds. The authors of this paper also hope to have given valuable guidelines in the conduct of the bioequivalence study of endogenous compound.

Conclusion

Different results obtained in the relative bioavailability between TDS®-testosterone and Androgel® for uncorrected data and corrected data, suggest that correcting endogenous concentrations is important for the proper determination of bioequivalent for endogenous compound such as testosterone. Without endogenous data corrections, an incorrect conclusion about bioequivalence may result with products being declared bioequivalent when they were actually not bioequivalent or vice versa. TDS®-testosterone also showed superiority compared to Androgel® with data corrections.

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