ORIGINAL ARTICLE

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Pharmacokinetics of Testosterone Enanthate After Intramuscular Injection for Transgender Men

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Abstract

Aim: To clarify the pharmacokinetics (PKs) of testosterone enanthate intramuscular injection in transgender men.

Materials and Methods: This is a single-center nonblind single-drug administration study. After screening, 10 Japanese transgender men were administered 250 mg of testosterone enanthate intramuscularly. If the subjects had already received testosterone treatment, a screening test was carried out after washout for at least 4 weeks. The total testosterone (TT) and free testosterone (FT) and estradiol (E2) levels were examined for 4 weeks. At days 0 and 28, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels were examined.

Results: Mean age was 31.1 ± 7.2 years, and four subjects received first-time testosterone administration. Mean TT level at baseline was under the cutoff value in hypogonadal men. Mean time to reach its maximum level was 1.7 days, and in all cases within 3 days. Then, the level decreased and reached <3.0 ng/mL at day 21. Mean time to maximum level of FT was 3.4 days, but in three cases it was at day 7. The coefficient of variation in apparent total clearance and distribution volume for TT was large. The PK parameters of E2 could not be calculated in four cases. In the remaining six, E2 gradually decreased and reached the nadir at day 21. LH and FSH were not different before and after injection. No specific adverse event associated with testosterone administration was observed.

Conclusions: PK profiles in transgender men after testosterone enanthate 250 mg single injection were clarified.

Keywords: gender dysphoria; gender incongruence; pharmacokinetics; testosterone; transgender men

Introduction

Gender identity is the internal sense of being male or female or identifying with both or neither. The terms transgender and gender incongruent are adjectives for persons with gender identities that are not aligned with the sex recorded at birth.¹ If gender incongruence is persisting and leads to distress, there can be gender dysphoria.² The treatments for those persons are classified into psychiatric (e.g., mental support) and physical. As physical treatments, medical (gender-affirming hormone treatment [GHT]) or surgical (gender-affirming surgery) interventions would be performed to align appearance with gender identity.

According to the clinical practice guidelines published by the endocrine society, the concept of GHT is to suppress endogenous sex hormone secretion determined by the person's genetic/gonadal sex, and maintain sex hormone levels within the normal range for the person's affirmed gender.³ For transgender men, an androgen is given to induce masculinization. Although several types of testosterone formulations are now available, GHT using testosterone enanthate (3-oxoandrost-4-en-17 β -yl heptanoate) intramuscular injection has been used worldwide for many years. Enarmon Depot[®] intramuscular injection is one of the parenteral androgen preparations (testosterone enanthate) launched in Japan. In our

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country, this preparation has been covered by insurance for male hypogonadism (eunuchoidism), male infertility due to loss of spermatogenesis, aplastic anemia, myelofibrosis, and renal anemia since 1993, but not yet for transgender men.

When testosterone enanthate is used as GHT, the guideline suggests that administration of 100 to 200 mg every 2 weeks is the ideal regimen.³ Then serum testosterone should be measured every 2 to 3 months until levels are in the normal physiological male range (3.2–10.0 ng/mL). In the case of testosterone enanthate injection, the testosterone level should be measured midway between injections (probably 1 week after injection). If the level is >7.0 or <3.5 ng/mL, the dose should be adjusted accordingly.³ However, there are few published data about the details in pharmacokinetic (PK) profiles of serum testosterone levels after GHT (specially testosterone enanthate) in transgender men.^{4–7} In addition, there has been no data in Asian subjects. Thus, the appropriate dosage and dose interval for this type of exogenous testosterone treatment have not been determined. Therefore, the aim of this study was to clarify the details in PKs of testosterone enanthate 250 mg after intramuscular administration to Japanese transgender men.

Materials and Methods

This was a single-center nonblind single-drug administration study. The study included transgender men without hysterectomy and oophorectomy who provided written informed consent from September 2015 to March 2018. All of them were 20 years old or older, with a body mass index (BMI) from 18.5 to 30.0 kg/m². The study protocol was approved by the institutional review board of the study site (approval no. 272-57) and was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki.

The exclusion criteria for the study were cardiovascular, renal, or hepatic impairments; drug hypersensitivity; suspected malignant disease; polycythemia; uncontrolled hypertension; sleep apnea; or considered unsuitable for the trial by doctors. In general, blood sampling, including the screening test, was done during the morning after at least 30 min of bed rest. If the candidate had already received GHT, the screening test was carried out after having discontinued testosterone administration for at least 4 weeks. We checked the following data as screening test: examination of the presence of subjective and objective symptoms, height, weight, blood pressure, pulse, urinal-

ysis, blood cell count, biochemistry, and hormonal data. The hormonal data examined were total testosterone (TT), free testosterone (FT), estradiol (E2), luteinizing hormone (LH), and follicle-stimulating hormone (FSH).

After the screening test, the subjects judged eligible for study inclusion were injected with 250 mg of testosterone enanthate intramuscularly (day 0). Thereafter, hormonal data (TT, FT, and E2) and vital signs (blood pressure and pulse) were evaluated at days 1, 2, 3, 7, 14, 21, and 28 after injection. Blood cell count, biochemistry, LH, and FSH were examined at days 0 and 28. Each blood sample was left to stand at room temperature for 30 min, and then centrifuged at 1500 g for 10 min. For FT, 0.3 mL of serum was put in a polypropylene tube and cryopreserved at –20°C. For the other hormones, residual serum (>0.8 mL) was kept in a tube and preserved at 4°C until measurement. FT was measured by radioimmunoassay (Free Testosterone RIA kit®; SML, Tokyo, Japan) and the others by chemiluminescence (ARCHITECT®; Abbott, Chiba, Japan).

The primary outcome was the changes in plasma concentration of serum TT and FT for 28 days after testosterone enanthate administration. In addition, the following PK parameters were calculated: the maximum concentration ($C_{\max}$), area under the blood concentration–time curve (AUC), time-to-arrival to $C_{\max}$ ($T_{\max}$), and elimination half-life ($t_{1/2}$). The commercial software (Phoenix WinNonlin 7.0®; CERTARA, Tokyo, Japan) was used to calculate these PK parameters. In contrast, apparent total clearance (CL_{tot}/F), apparent distribution volume (V_d/F), and the absorption rate constant (k_a) were calculated for each patient by using solver add-in (nonlinear least squares method) in Microsoft Excel (Office 365®; Microsoft, Redmond, WA). A one-compartment model with first-order absorption was applied to the PKs of testosterone after intramuscular injection. These parameters were estimated by using Equation (1).

$$C = \frac{F \cdot D \cdot k_a}{V_d(k_a - CL_{\text{tot}}/V_d)} \left(e^{-\frac{CL_{\text{tot}}}{V_d}t} - e^{-k_a t} \right) + C_0, \quad (1)$$

where C is TT serum concentration, F is bioavailability, D is dosage as testosterone, k_a is absorption rate constant, V_d is distribution volume, CL_{tot} is total clearance, t is days after administration, and C_0 is TT serum concentration before testosterone administration.

CL_{tot}/F and V_d/F were calculated as proportional to body weight. If the estimated k_a exceeded 10 day^{–1}, we judged that it was difficult to accurately estimate the

Table 1. Clinical Characteristics of Study Participants

Case number ^a	Previous dose and duration of hormonal therapy	Age (years)	Body weight (kg)	Body mass index (kg/m ²)	Comorbidity
T-01	First time	26.4	64.6	24.9	Diabetes mellitus
T-02	250 mg/2–3 weeks, 1 year	27.1	63	24.5	
T-04	250 mg/2 weeks, 9 years	42.7	52.5	21.3	Chronic obstructive pulmonary disease, asthma
T-05	First time	43.1	70.5	27.0	Dyslipidemia, depression
T-07	First time	26.5	70	26.3	
T-08	250 mg/3–4 weeks, 6 years	26.5	75	28.4	Depression, insomnia, gastroenteritis
T-09	250 mg/2 weeks, 1 month	21.8	58	24.0	
T-10	250 mg/4 weeks, 4 years	33.1	72.5	27.1	Depression
T-11	250 mg/2 weeks, 4 years	26.4	46	19.8	
T-12	First time	37.5	52	19.3	Depression
Mean ± SD		31.1 ± 7.2	62.4 ± 9.4	24.3 ± 3.0	

^aT-03 and T-06 withdrew from the study.
SD, standard deviation.

absorption process of testosterone, and CL_{tot}/F and Vd/F were calculated by using a one-compartment model after intravenous injection [Eq. (2)].

$$C = \frac{F \cdot D}{Vd} e^{-\frac{CL_{tot}}{Vd}t} + C_0. \quad (2)$$

Secondary outcomes were the serum E2, LH, and FSH levels, and, in the case of E2, the PK parameters were also calculated. All adverse events were closely monitored, and details were reported throughout the study period for safety assessment.

Data are shown as the mean ± standard deviation. Using the measured values and calculated data, plasma concentration–time profiles were made. Missing, non-applicable, and abnormal data were not supplemented in this study. Moreover, all the data were monitored, and analysis was independently done by a trusted third party to ensure data transparency (RPM Co., Ltd. Tokyo, Japan).

Results

Clinical characteristics of the subjects

A total of 12 transgender men provided informed consent and underwent the screening test, but 2 withdrew

their consent to participate in the study (T-03 and 06). Finally, 10 subjects were included and received testosterone injection (Table 1). Of these, four were new cases for testosterone administration (T-01, 05, 07, and 12). The mean age of the subjects was 31.1 ± 7.2 years old and BMI was 24.3 ± 3.0 kg/m². Six subjects had comorbidity (there was some overlap), including diabetes (1), chronic obstructive pulmonary disease/asthma (1), dyslipidemia (1), insomnia (1), gastroenteritis (1), and depression (4). The blood pressure and pulse before the drug administration were $113.8 \pm 7.7/68.6 \pm 7.7$ mmHg and 75.8 ± 16.6 times/min, respectively. There were no significant changes in these values during the study period.

Primary outcome

The mean serum TT and FT levels after testosterone enanthate administration for each sampling day are given in Table 2 and Figure 1. C_{max} , AUC, T_{max} , and $t_{1/2}$ are given in Table 3. The transition of both TT and FT levels was almost parallel for 28 days. The TT of each subject at baseline was under the cut-off value of hypogonadal men, which is defined as 3.0 ng/mL.⁸ The mean T_{max} of TT was 1.7 days, and

Table 2. Mean Serum Levels of Total Testosterone, Free Testosterone, and Estradiol at Each Evaluation Point

Day of measurement	Day 0	Day 1	Day 2	Day 3	Day 7	Day 14	Day 21	Day 28
Total testosterone, ng/mL (N=10)								
Mean ± SD	0.75 ± 0.5	27.99 ± 17.5	21.69 ± 9.6	23.33 ± 15.8	14.48 ± 4.7	5.81 ± 2.4	2.72 ± 1.6	1.33 ± 0.8
Free testosterone, ^a pg/mL (N=10)								
Mean ± SD	2.78 ± 1.3	53.22 ± 28.5	51.96 ± 25.3	48.52 ± 23.5	42.9 ± 22.4	13.87 ± 4.7	7.26 ± 4.7	3.84 ± 2.5
Estradiol, ^b pg/mL (N=10)								
Mean ± SD	93.2 ± 84.1	91.5 ± 61.9	91.4 ± 66.4	92 ± 57.6	71.2 ± 52.0	42 ± 39.4	45.1 ± 37.9	132 ± 127.8

^aUpper limit value: 100 pg/mL.

^bLower limit value: 10 pg/mL.

FIG. 1. Plasma concentration–time profiles of mean TT and FT levels. The black and white dots (●), (○) indicate the mean values of TT and FT, respectively, at each study point in transgender men injected intramuscularly with testosterone enanthate 250 mg. Each error bar indicates a standard deviation. FT, free testosterone; TT, total testosterone.

all cases reached C_{max} within 3 days. The C_{max} of TT was 29.4 ± 18.1 ng/mL, and mean TT decreased gradually to <3.0 ng/mL again at day 21. The mean T_{max} of FT was 3.4 days, and three cases reached C_{max} at day 7. Estimated PK parameters of TT for each patient are given in Table 4.

Secondary outcomes

Mean E2 levels are given in Table 2, and the calculated PK parameters are given in Table 3. Calculation of the parameters was possible in six cases. In these cases, the mean E2 level decreased and reached its

Table 3. Calculated Pharmacokinetic Parameters for Total Testosterone, Free Testosterone, and Estradiol

Pharmacokinetic parameters					
Total testosterone (N = 10)					
	C_{max} (ng/mL)	AUC_{0-t} (ng · d/mL)	$AUC_{0-\infty}$ (ng · d/mL)	t_{max} (day)	$t_{1/2}$ (day)
Mean ± SD	29.4 ± 17.1	$252.3 \pm 95.6.8$	265.9 ± 98.2	1.7 ± 0.9	6.6 ± 1.9
Free testosterone (N = 10)					
	C_{max} (pg/mL)	AUC_{0-t} (pg · d/mL)	$AUC_{0-\infty}$ (pg · d/mL)	t_{max} (day)	$t_{1/2}$ (day)
Mean ± SD	59.1 ± 24.7	625.1 ± 246.4	673.5 ± 263.8	3.4 ± 2.4	7.39 ± 2.9
Estradiol ^a					
Number	10	10	6	10	6
Mean ± SD	190.3 ± 119.1	1922.8 ± 958.5	4821.3 ± 4098	9.9 ± 11.9	25.7 ± 8.6

^aCalculation was impossible for four subjects because the E2 level did not approach a standard decay curve.

Table 4. Calculated CL_{tot}/F , Vd/F , and k_a for Total Testosterone

Case no.	CL_{tot}/F (L/day/kg)	Vd/F (L/kg)	k_a (day ⁻¹)
T-01	25.4	286.8	—
T-02	10.4	113.0	2.82
T-04	16.4	148.7	—
T-05	5.3	35.7	—
T-07	12.1	108.8	3.16
T-08	24.6	125.3	—
T-09	14.7	98.4	—
T-10	8.3	83.9	0.49
T-11	14.0	55.2	—
T-12	11.0	130.5	1.38
Mean $\pm$ SD	14.3 $\pm$ 6.5	118.7 $\pm$ 68.3	—
Coefficient of variation	0.45	0.58	—

CL_{tot}/F , apparent total clearance; k_a , absorption rate constant; Vd/F , apparent distribution volume.

nadir at day 21. The E2 level did not approach a standard decay curve in four cases (T-01, 05, 07, and 11). Therefore, we could not calculate the parameters in these cases. LH and FSH levels at baseline were 7.86 ± 8.5 and 5.20 ± 2.1 mIU/mL, respectively, and there were no differences in these parameters compared with those at day 28 (LH: 6.39 ± 6.1 , FSH: 4.52 ± 1.6 mIU/mL).

Safety assessment

Adverse events were observed in two subjects during follow-up. One subject (T-01) reported abdominal pain on day 10, and common cold-like symptoms on day 25. These symptoms were mild and relieved spontaneously within several days. The other (T-12) took an overdosing of sleeping pills on day 10. Although this event was thought to be a serious adverse event, it was regarded as unrelated to the study drug because this subject suffered from depression and said that similar events had occurred several times before study participation. Finally, this subject came to the hospital without any physical problem on day 14 and completed examinations until the end of the study period.

To investigate the effect of testosterone enanthate administration on blood cell count, we checked hemoglobin and hematocrit levels at screening and on day 28. The mean hemoglobin value changed from 14.2 ± 1.7 to 14.6 ± 1.9 g/dL. The mean hematocrit value changed from $43.9\% \pm 3.5\%$ to $45.3\% \pm 3.2\%$. None of them were statistically significant changes (hemoglobin: $p=0.243$, hematocrit: $p=0.214$, paired t -test).

Discussion

Medical expenses of GHT for transgender persons in Japan are not covered by insurance at present.

Although several testosterone formulations (oral, transdermal, and parenteral) are available as GHT for transgender men around the world, intramuscular testosterone enanthate injection is mainly used in our country. So far, there are few published data on the PKs when an exogenous androgen, especially for intramuscular injection of testosterone enanthate, is given to transgender men.^{4–7} In addition, there has been no published data on Asian subjects. Moreover, there are also little data about the changes of TT and FT in humans after exogenous testosterone administration using current assay methods.⁹

In this study, all subjects were given 250 mg of testosterone enanthate intramuscularly and examined for 28 days. A mean TT level of >3.0 ng/mL was maintained until day 14, but it was <3.0 ng/mL on day 21. The C_{max} reaches three to five times the upper-limit value of TT (6.0 or 10.0 ng/mL) in physiological males,^{3,5} and TTs of all subjects reached T_{max} within 3 days. It is said that super physiological concentration of testosterone has roller coaster effect.¹⁰ Therefore, we verified the effects when the serum TT concentration was much higher than the normal physiological range in males. We checked TT levels and all adverse reactions for consecutive 3 days after drug administration, then checked them every week until for 1 month. Although the number of subjects participated in the study was relatively small, no specific severe adverse reaction related to high testosterone level was observed.

In this study, the coefficient of variation (CV%) of CL_{tot}/F and Vd/F was large (0.45 and 0.58, respectively), and especially interindividual differences in Vd/F was about eightfold. These large CV% might be due to high interindividual variation of bioavailability (F) of testosterone after intramuscular injection.¹¹ Therefore, if we use testosterone enanthate 250 mg as GHT, the masculinization effects would be visible more quickly than 100 or 200 mg at the start of GHT. In contrast, after the desired virilization has been obtained, maintenance administration at 100 or 200 mg may be appropriate in consideration of safety. A previous report showed the results of dose–response analysis of testosterone treatment and supports this concept.¹²

In vivo, FT is one of the activated testosterone forms, and the other is part of an albumin-binding type (if testosterone dissociates from albumin). These forms are called bioavailable testosterone. In contrast, the type binding with sex hormone-binding globulin does not

have bioavailability, and this assay is not covered by insurance in our country. For these reasons, we usually use FT to estimate bioavailable testosterone in the body. The target FT range of androgen supplementation in hypogonadal Japanese men is suggested to be from 8.5 to 11.8 pg/mL.¹³ This study showed that transitions in FT levels were parallel to TT, and the mean FT level at each study point was within the target range until day 21. Therefore, from this point of view, an interval of every 2 weeks may be appropriate for testosterone enanthate 250 mg injection.

The major effects of GHT for transgender men are masculinization and cessation of menses. Masculinization caused by testosterone in women is irreversible, but menses comes again with an extended interval or cessation of testosterone administration. One of the reasons is that the testosterone is thought mainly to suppress the hypothalamo-hypophyseal system and have little direct effect on ovarian function. However, a previous study showed that continuous exposure of transgender men to a high androgen dose caused pathognomonic changes in the ovarian cortex and stroma.¹⁴ Thus, high-dose androgen administration might affect ovarian hormonal function.

In this study, we also evaluated LH, FSH, and E2 levels as secondary outcomes. Although LH and FSH levels were evaluated only at two time points, there was no difference between before and after testosterone injection. The mean E2 level at each point decreased gradually toward the nadir on day 21, and then recovered on day 28. However, when the subjects were distributed based on whether the PKs of E2 could be calculated, there was a difference in transition of E2 levels among them. Four of 10 subjects with relatively lower baseline E2 levels could not calculate E2 PKs. Their serum E2 levels rapidly reached the nadir (at day 14), and thereafter mean E2 levels rebounded far from the baseline at day 28. Interestingly, three of these subjects received their initial testosterone treatment. Therefore, we hypothesized that previous hormonal therapy might affect plasma concentration–time profiles of E2. We checked each data of them, but small sample size would not allow us to evaluate the influence of previous hormonal treatment. There is a possibility that the ovulation cycle might have been affected in these subjects, but we did not investigate it before study inclusion.

Finally, limitations of this study should be addressed. First, we tested only testosterone enanthate 250 mg single administration. If we want to find out a truly appro-

priate method of GHT, several testosterone enanthate doses and repetitive administration should be tried. Second, the number of time points studied was limited. It would have been interesting to see hormone levels at days 4, 5, 6, 10, etc. Third, the number of subjects was relatively small. In this respect, we could not conclude the reason why CV% of CL_{tot}/F and Vd/F were large. Furthermore, the effects of testosterone on ovarian function are still unclear. Further study using large sample size may be helpful to clarify these questions. In any case, the necessary sample size for estimating PKs is generally set ~6 to 10 cases. Therefore, sample size for the primary outcome is adequate.

Conclusions

We clarified the PK profiles in Japanese transgender men after intramuscular injection of 250 mg of testosterone enanthate. If we use 250 mg for GHT, an injection interval of every 2 weeks may be appropriate.

Authors' Contributions

N.M. conceived and designed the study; N.M. and K.I. acquired data; K.I., S.F., and T.T. analyzed and interpreted data; K.I. and S.F. drafted the article; N.M. and T.T. revised the article for intellectual content; and N.M. gave final approval of the completed article. All authors have read and approved the final version of the article and agree with the order of presentation of the authors.

Data Statement

The research data are confidential.

Acknowledgments

We thank Hiroshi Morifuji and Katsunori Takahashi for data monitoring and analysis.

Author Disclosure Statement

No competing financial interests exist.

Funding Information

This study is partially supported by Aska Pharmaceutical Co., Ltd.

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Cite this article as: Ichihara K, Masumori N, Fujii S, Toda T (2020) Pharmacokinetics of testosterone enanthate after intramuscular injection for transgender men. *Androgens: Clinical Research and Therapeutics* 1.1, 15–21, DOI: 10.1089/andro.2020.0002.

Abbreviations Used

AUC = area under the blood concentration–time curve
 BMI = body mass index
 CV% = coefficient of variation
 E2 = estradiol
 FSH = follicle-stimulating hormone
 FT = free testosterone
 GHT = gender-affirming hormone treatment
 LH = luteinizing hormone
 PK = pharmacokinetic
 SD = standard deviation
 TT = total testosterone

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