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Pharmacokinetic Properties of Testosterone Propionate in Normal Men

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ABSTRACT. The pharmacokinetic characteristics of testosterone propionate were studied in normal men after a single im dose of 25 mg testosterone propionate-19,19,19-d_3. Plasma levels of testosterone propionate-19,19,19-d_3, its active metabolite testosterone-19,19,19-d_3, and endogenous testosterone were measured by gas chromatography-mass spectrometry. Testosterone propionate-19,19,19-d_3 was gradually transferred from the im injection site to the systemic circulation. The plasma levels of testosterone propionate-19,19,19-d_3 were maintained at 2–4 ng/ml between 3 and 36 h after administration. Plasma testosterone-19,19,19-d_3 levels were maintained above the physiological testosterone level for 48 h, while plasma levels of endogenous testosterone changed little. (*J Clin Endocrinol Metab* **63:** 1361, 1986)

TESTOSTERONE propionate is a short-acting parenteral form of testosterone used primarily in the treatment of hypogonadism, oligospermia, and impotence (1). Although the clinical use of testosterone propionate has been investigated, pharmacokinetic studies have been limited by the lack of a specific and sensitive assay. Testosterone propionate in pharmaceutical preparations has been measured by gas chromatography (GC) (2, 3), high performance liquid chromatography (4), high performance thin layer chromatography (5), infrared spectrophotometry (6), and colorimetry (7). However, none of these methods allowed measurement of testosterone propionate in biological fluids, because of low sensitivity and low selectivity. In attempts to examine the disposition of testosterone propionate, RIA (8–10) and competitive protein binding assay (11, 12) have been used to detect changes in unesterified testosterone concentrations in plasma after im administration of testosterone propionate. However, no direct evidence is available regarding the pharmacokinetics of im administered testosterone propionate. RIA and competitive protein binding assay cannot address this problem, since this type of assay cannot differentiate between endogenous testosterone and testosterone derived from administered testosterone propionate.

We have initiated studies designed to characterize the pharmacokinetic properties of im administered testosterone propionate and to determine the effect of testosterone propionate on endogenous testosterone levels. We reported previously the determination of plasma testosterone propionate levels by GC-mass spectrometry-selected ion monitoring (GC-MS-SIM) (13). The present paper describes the applications of GC-MS techniques for the pharmacokinetic study of deuterated testosterone propionate after the im administration of testosterone propionate-19,19,19-d_3 to normal men.

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Materials and Methods

Chemicals

Testosterone propionate-19,19,19-d_3 (testosterone propionate-19-d_3) and testosterone-19,19,19-d_3 (testosterone-19-d_3) were synthesized in our laboratory, as described previously (13, 14). Their isotopic compositions were 99.0% deuterium atoms (d_3, 97.8%; d_2, 2.2%; d_1, 0.0%). Unlabeled testosterone propionate (Tokyo Chemical Industry, Tokyo, Japan) and trifluoroacetic anhydride (Nakarai Chemicals Ltd., Kyoto, Japan) were obtained commercially. The testosterone propionate-19-d_3 preparation for im injection was prepared by dissolving 25 mg testosterone propionate-19-d_3 in 1 ml sesame oil containing 20% benzyl benzoate, followed by membrane filtration to insure sterility.

GC-MS-SIM

GC-MS-SIM measurements were made in the EI-mode with a Shimadzu LKB-9000B gas chromatograph-mass spectrometer equipped with Shimadzu high speed multiple ion detector-peak matcher 9060S. The GC column was a glass column (id,

2 m × 3 mm) packed with 1.5% OV-1 on Shimalate W (80–100 mesh). The injector, column, and ion source temperatures were 260, 230, and 270 C, respectively. Helium was used as the carrier gas at a flow rate of 30 ml/min. The electron energy was set at 20 eV, and the trap current at 60 μA. The multiple ion detector was focused at the molecular ions of the trifluoroacetate derivatives of testosterone propionate (d_0, m/z 440; d_3, m/z 443) and testosterone (d_0, m/z 480; d_3, m/z 483) to obtain peak height ratios.

Testosterone propionate-19-d_3 administration studies

The study subjects were two normal men, 27 and 24 yr old, weighing 72 and 61 kg, respectively. To determine the secretory dynamics of endogenous testosterone, heparinized blood samples (10 ml) were obtained at 0800, 1100, 1400, 1700, and 2000 h for 4 days. The men then were given 25 mg testosterone propionate-19-d_3, im, at 0800 h. Ten-milliliter heparinized blood samples were obtained 5 min before and 3, 6, 9, 12, 24, 27, 30, 33, 36, 48, 54, 60, 72, 78, and 84 h after dosing. Plasma was separated by centrifugation and stored at −20 C until analysis.

Sample preparation for GC-MS-SIM

To measure the secretory dynamics of endogenous testosterone, plasma samples were subjected to GC-MS-SIM analysis, as described previously (15). Briefly, 10 ng testosterone-19-d_3 were added as an internal standard to 1.0 ml plasma, and the plasma sample was extracted with ether. The ether extract was cooled to −15 C and centrifuged to remove plasma lipids. After purification of the ether extract by thin layer chromatography and derivatization with trifluoroacetic anhydride, 1–3 μl sample dissolved in 20 μl *n*-hexane were analyzed by GC-MS-SIM. This assay has a sensitivity of 0.1 ng/ml plasma and an interassay coefficient of variation of 3.6% (5.9 ng/ml plasma) based on measurement of three samples in triplicate.

To measure testosterone propionate-19-d_3 in plasma, plasma samples were subjected to the GC-MS-SIM analytic method described previously (13). Briefly, 20 ng unlabeled testosterone propionate were added as an internal standard to 1.0 ml plasma. To avoid hydrolysis of testosterone propionate during the extraction procedure and the presence of numerous interfering peaks at m/z 440 and 443, the plasma sample was cooled to 4 C and extracted with *n*-hexane. The *n*-hexane extract was cooled to −15 C and centrifuged to remove plasma lipids. After derivatization with trifluoroacetic anhydride, 1–3 μl sample dissolved in 20 μl *n*-hexane were analyzed by GC-MS-SIM. This assay has a sensitivity of 0.2 ng/ml plasma and an interassay coefficient of variation of 3.5% (2.6 ng/ml plasma) based on measurement of three samples in triplicate.

The double isotope dilution method was employed to measure testosterone-19-d_3 and endogenous testosterone in plasma simultaneously. Plasma samples (1.0 ml each) were divided into two aliquots each. To one aliquot of each sample was added testosterone-19-d_3 (10 ng) as an internal standard. The two aliquots of plasma were cooled to 4 C and then subjected to GC-MS-SIM analysis, as described previously (15). The calculations used to determine testosterone-19-d_3 and endogenous testosterone levels, as assayed by the double isotope dilution method, were described previously (16).

Data analysis

Independent modeling techniques, described by Yamaoka *et al.* (17) and Riegelman and Collier (18), were used to calculate the pharmacokinetic parameters of testosterone propionate-19-d_3 and testosterone-19-d_3. The estimation of the elimination half-life ($t_{1/2}$) was obtained from at least five points of the terminal log-linear portion of the curve. The area under the plasma concentration time curve (AUC) was calculated by the trapezoidal method. The area under the first moment of the curve (AUMC) was computed similarly by multiplying each individual plasma concentration by its time. Extrapolations through infinity were calculated from the last detectable plasma concentration and the terminal elimination half-life. The systemic plasma clearance (CL_s), the mean residence time (MRT), and the steady state volume of distribution (V_{ss}) were determined with Eq I–III (19).

$$CL_s = \text{dose/AUC} \quad \text{(I)}$$

$$MRT = AUMC/AUC \quad \text{(II)}$$

$$V_{ss} = CL_s\ MRT \quad \text{(III)}$$

Results

Endogenous plasma testosterone profiles

Before studying the disposition of testosterone propionate, we determined the levels of endogenous plasma testosterone for 4 days in the two normal men (Fig. 1). The mean plasma testosterone concentration in subject 1 was 6.6 ng/ml, with a range of 4.0–8.9 ng/ml. Subject 2 had a mean value of 6.9 ng/ml and a range of 4.7–9.3 ng/ml. Testosterone concentrations had a circadian rhythm, with the highest levels between 0800 and 1100

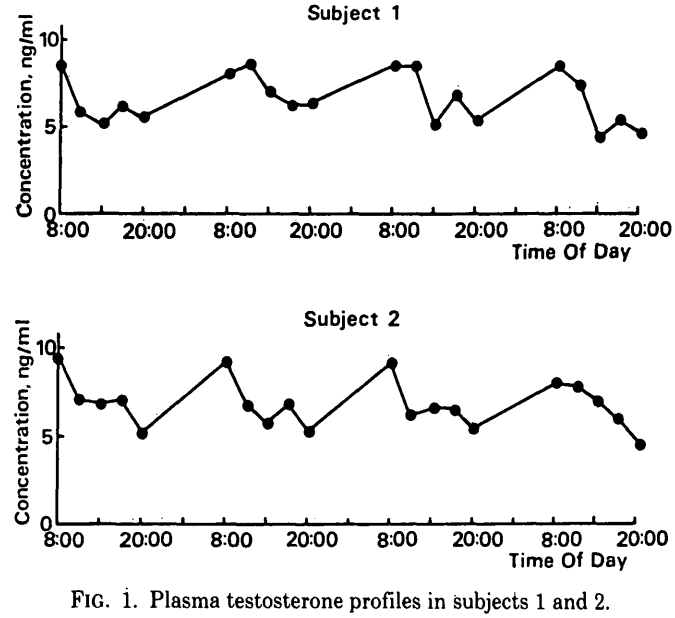

FIG. 1. Plasma testosterone profiles in subjects 1 and 2.

h and the lowest levels between 1700 and 2000 h in both men.

Plasma testosterone propionate-19-d_3 and testosterone-19-d_3 concentrations

Plasma testosterone propionate-19-d_3 levels were maintained at 2–4 ng/ml between 3 and 36 h after im administration of a single 25-mg dose of testosterone propionate-19-d_3 (Fig. 2) and thereafter decreased very slowly. Testosterone-19-d_3 was detected in the first blood sample taken 3 h after the injection. The maximum plasma testosterone-19-d_3 levels (15.0 ng/ml for subject 1 and 11.5 ng/ml for subject 2) occurred between 24 and 27 h. Thereafter, the decline of testosterone-19-d_3 was parallel to that of testosterone propionate-19-d_3 in both subjects. The pharmacokinetic parameters characterizing the disposition of testosterone propionate-19-d_3 and testosterone-19-d_3 are summarized in Table 1.

Effect of testosterone propionate-19-d_3 on plasma endogenous testosterone

Endogenous plasma testosterone levels were measured at various times for 84 h after the im testosterone propionate-19-d_3 injection. The results are shown in Fig. 2. Endogenous testosterone levels just before the administration of testosterone propionate-19-d_3 were 7.2 ng/ml in subject 1 and 7.5 ng/ml in subject 2. After the administration of testosterone propionate-19-d_3, endogenous testosterone levels varied between 3.8 and 7.8 ng/ml and 4.4 and 7.5 ng/ml in subjects 1 and 2, respectively.

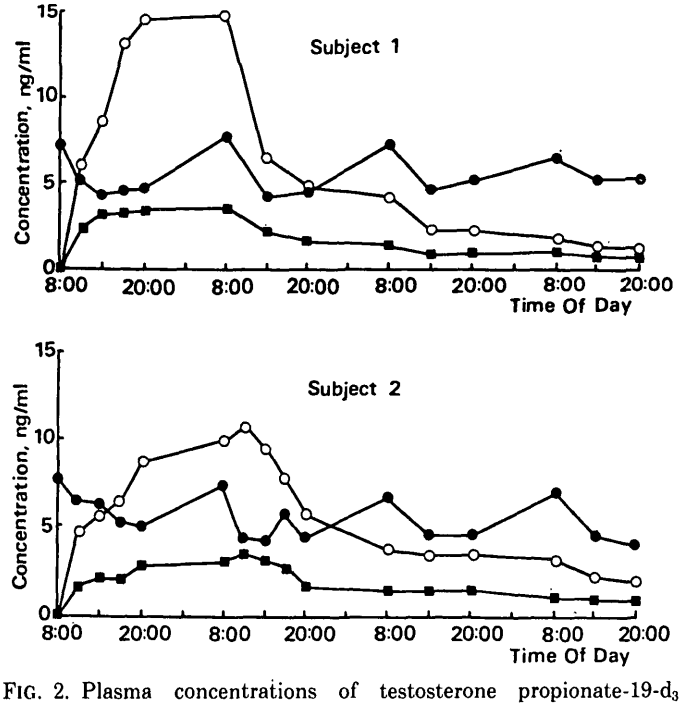

FIG. 2. Plasma concentrations of testosterone propionate-19-d_3 (■——■), testosterone-19-d_3 (○——○), and endogenous testosterone (●——●) *vs.* time in subjects 1 and 2. An im dose of 25 mg testosterone propionate-19-d_3 was given to the two normal men at 0800 h.

TABLE 1. Parameters characterizing the disposition of testosterone propionate

Parameter	Subject 1	Subject 2
$t_{1/2}$ (h)	26.7	23.1
CL_s (ml/min)	2317.4	1958.0
V_{ss} (liters/kg)	74.9	122.5
MRT_{TP} (h)	38.8	63.6
MRT_T (h)	30.5	54.4
AUC_{TP} (ng·h/ml)	179.8	212.8
AUC_T (ng·h/ml)	535.0	571.0

MRT_{TP}, The mean residence time of testosterone propionate; MRT_T, the mean residence time of testosterone; AUC_{TP}, the area under the plasma concentration time curve of testosterone propionate; AUC_T, the area under the plasma concentration time curve of testosterone.

Discussion

The use of GC-MS and stable isotopically labeled drugs as tracers makes it possible to provide a precise analytical method for both the parent compound and metabolites with high sensitivity and selectivity (20–22). The application of this method allowed us to follow the plasma levels of testosterone propionate-19-d_3 and its active metabolite testosterone-19-d_3 for 84 h after the im administration of testosterone propionate-19-d_3.

Intramuscularly administered testosterone propionate-19-d_3 was readily detected in the circulation, demonstrating clearly that the testosterone propionate-19-d_3 was transferred from the injection site in the muscle to the systemic circulation.

Nieschlag *et al.* (8) measured the total plasma exogenous and endogenous testosterone levels by RIA after the im administration of 25 mg testosterone propionate to normal men. Total plasma testosterone levels were maintained above the physiological levels for 24 h after drug administration. However, it was not possible to determine exactly the contribution of administered testosterone propionate to the total concentration of circulating testosterone. With the aid of the double isotope dilution assay employed in this study, endogenous testosterone and testosterone derived from administered testosterone propionate could be differentiated easily. Conversion of testosterone propionate-19-d_3 to testosterone-19-d_3 was fast in the systemic circulation, and testosterone propionate-19-d_3 injection resulted in exogenous testosterone levels above the normal testosterone level (>4 ng/ml) for 48 h. Moreover, exogenous testosterone continued to contribute to the total plasma levels for up to 84 h.

Significant decreases in plasma LH occur after the im administration of testosterone preparations to normal men (8, 12, 23–25). This decrease is caused by the negative feedback effect of exogenous testosterone on LH

secretion, which, in turn, should result in suppression of testicular secretion of testosterone. However, no direct evidence concerning the effect of exogenous testosterone on endogenous testosterone levels is yet available. In our study, administration of testosterone propionate-19-d_3 was followed by a gradual decline in plasma endogenous testosterone levels, which then returned to pretreatment levels 24 h after the administration of testosterone propionate-19-d_3. However, the circadian testosterone rhythm, as documented in these men before testosterone propionate administration, could have resulted in the curves obtained after testosterone propionate administration. Thus, we cannot conclude that endogenous testosterone production was suppressed by the single dose of testosterone propionate administered.

References

1. Murad F, Haynes Jr RC 1980 Androgens and anabolic steroids. In: Gilman AG, Goodman LS, Gilman A (eds) The Pharmacological Basis of Therapeutics. Macmillan, New York, p 1448
2. Gazdag M, Szepesi G, Nyiredy S 1977 Gas chromatographic determination of a mixture of testosterone and estradiol esters in an oily injection. Z Anal Chem 288:281
3. Pawelczyk E, Opielewicz M 1978 Gas chromatography in drug analysis. IV. Determination of some steroid drugs. Farm Pol 34:531
4. Carignan G, Lodge BA, Skakum WJ 1980 High-performance liquid chromatographic analysis of testosterone esters in oily solution. J Pharm Sci 69:1214
5. Amin M 1980 Quantitative analysis of steroid hormones in pharmaceutical preparations by high performance thin-layer chromatography. In: Bertsch W, Hara S, Kaiser RE (eds) Instrumental HPTLC. Proceedings of the 1st International Symposium on Instrumental HPTLC. Bad Dürkhein, Huthig, Heidelberg, p 9
6. Mynka AF, Lutsevich DD 1975 Infrared-spectroscopy of synthetic analogs of androgenic hormones. Farm Zh (Kiev) 30:31
7. Fahmy E, Yassa DA, Wahba N 1974 Specific color reaction for the determination of testosterone. Analyst (Lond) 99:759
8. Nieschlag E, Cüppers HJ, Wiegelmann W, Wickings EJ 1976 Bioavailability and LH-suppressing effect of different testosterone preparations in normal and hypogonadal men. Horm Res 7:138
9. Herbert DC, Parker Jr CR, Bartke A 1978 Serum estradiol, testosterone and dihydrotestosterone in male monkeys treated with testosterone propionate. Endocr Res Commun 5:249
10. Keating RJ, Tcholakian RK 1983 *In vivo* patterns of circulating testosterone following castration and intramuscular testosterone propionate injections of adult male rats. Steroids 42:63
11. Capell PT, Paulsen CA, Derleth D, Skoglund R, Plymate S 1973 The effect of short-term testosterone administration on serum FSH, LH and testosterone levels: evidence for selective abnormality in LH control in patients with Klinefelter's syndrome. J Clin Endocrinol Metab 37:752
12. Morse HC, Horike N, Rowley MJ, Heller CG 1973 Testosterone concentration in testes of normal men: effects of testosterone propionate administration. J Clin Endocrinol Metab 37:882
13. Baba S, Fujioka M, Shinohara Y, Furuta T 1985 Determination of testosterone propionate in human plasma by gas chromatography-mass spectrometry. J Chromatogr 337:205
14. Deleted in proof.
15. Baba S, Shinohara Y, Kasuya Y 1979 Determination of plasma testosterone by mass fragmentography using testosterone-19-d_3 as an internal standard: comparison with radioimmunoassay. J Chromatogr 162:529
16. Baba S, Shinohara Y, Kasuya Y 1980 Differentiation between endogenous and exogenous testosterone in human plasma and urine after oral administration of deuterium-labeled testosterone by mass fragmentography. J Clin Endocrinol Metab 50:889
17. Yamaoka K, Nakagawa T, Uno T 1978 Statistical moments in pharmacokinetics. J Pharmacokinet Biopharm 6:547
18. Riegelman S, Collier P 1980 The application of statistical moment theory to the evaluation of *in vivo* dissolution time and absorption time. J Pharmacokinet Biopharm 8:509
19. Lassen NA, Perl W 1979 Tracer Kinetic Methods in Medical Physiology. Raven Press, New York, p 76
20. Baillie TA 1981 The use of stable isotope in pharmacological reseach. Pharmacol Rev 33:81
21. Garland WA, Powell ML 1981 Quantitative selected ion monitoring (QSIM) of drugs and/or drug metabolites in biological matrices. J Chromatogr Sci 19:392
22. Shinohara Y, Baba S, Kasuya Y, Knapp G, Pelsor FR, Shah VP, Honigberg IL 1986 Stable-isotope methodology in the bioavailability study of 17α-methyltestosterone using gas chromatography-mass spectrometry. J Pharm Sci 75:161
23. Muta K, Kato K, Akamine Y, Ibayashi H 1981 Age-related changes in the feedback regulation of gonadotrophin secretion by sex steroids in men. Acta Endocrinol (Copenh) 96:154
24. Sokol RZ, Palacios A, Campfield LA, Saul C, Swedloff RS 1982 Comparison of the kinetics of injectable testosterone in eugonadal and hypogonadal men. Fertil Steril 37:425
25. Schürmeyer Th, Nieschlag E 1984 Comparative pharmacokinetics of testosterone enanthate and testosterone cyclohexanecarboxylate as assessed by serum and salivary testosterone levels in normal men. Int J Androl 7:181