A Pharmacokinetic Study of Injectable Testosterone Undecanoate in Hypogonadal Men

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ABSTRACT: Testosterone undecanoate (TU) replacement for hypogonadal men when administered orally but requires multiple doses per day and produces widely variable serum T levels. We investigated the pharmacokinetics of a newly available TU preparation administered by intramuscular injection to hypogonadal men. Eight patients with Klinefelter’s syndrome received either 500 mg or 1,000 mg of TU by intramuscular injection; 3 months later, the other dose was given to each man (except to one, who did not receive the 1,000-mg dose). Serum levels of reproductive hormones were measured at regular intervals before and after the injections. Mean serum T levels increased significantly at the end of the first week, from less than 10 nmol/L to 47.8 ± 10.1 and 54.2 ± 4.8 nmol/L for the lower and higher doses, respectively. Thereafter, serum T levels decreased progressively and reached the lower-normal limit for adult men by day 50 to 60. Pharmacokinetic analysis showed a terminal elimination half-life of 18.3 ± 2.3 and 23.7 ± 2.7 days and showed a mean residence time of 21.7 ± 1.1 and 23.0 ± 0.8 days for the lower and higher doses, respectively. The area under the serum T concentration–time curve and the T-distribution value related to serum T concentration were significantly higher following the 1,000-mg dose than following the 500-mg dose. The 500-mg dose, when given as the second injection, yielded optimal pharmacokinetics (defined as mean peak T values not exceeding the normal range and persistence of normal levels for at least 7 weeks), suggesting that repeated injections of 500 mg at 6–8-week intervals may provide optimal T replacement. The mean serum levels of estradiol were normalized following the injections, and prolactin levels were normal throughout the study. Significant decrease of serum luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels was observed, with the decrease in LH levels being more pronounced. There were no significant differences in serum LH and FSH levels between the two doses. Sex hormone–binding globulin (SHBG) levels before any T therapy were near the upper limit of normal for adult men and were reduced by approximately 50% just prior to the second dose of TU. The decreased SHBG levels produced by the first TU injection could have led to lower peak total T levels and to a more rapid clearance of T following the second TU injection. We conclude that single-dose injections of TU to hypogonadal men can maintain serum T concentration within the normal range for at least 7 weeks without immediately apparent side effects. It is likely that this form of T would require injections only at 6–8-week or longer intervals, not at the 2-week intervals necessary with currently used T esters (enanthate and cypionate). This injectable TU preparation may provide improved substitution therapy for male hypogonadism and, in addition, may be developed as an androgen component of male contraceptives.

Key words: Testosterone, LH, FSH, estrogen, SHBG.

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Testosterone undecanoate (TU) is an unsaturated aliphatic fatty acid ester of testosterone (T) that is partially absorbed via the gut lymphatic system after oral administration (Horsl et al, 1976). The lymphatic absorption of TU and its metabolite prevents these compounds from being directly inactivated by the liver. After entering the peripheral circulation, these compounds are hydrolyzed and can exert their androgenic activity (Horsl et al, 1975; Nijs, 1987). TU-replacement therapy can provide adequate T levels in hypogonadal men if three doses per day are used (Nieschlag et al, 1975; Skakkebaek et al, 1981; Schirrmeier et al, 1983). The high cost and multiple dosing each day make oral TU problematic for treating testicular failure.

Testosterone enanthate (TE) or testosterone cypionate (TC), injected intramuscularly, are the most commonly used androgen preparations for the treatment of androgen deficiency (Bagatell and Bremner, 1996), and TE has been assessed for contraceptive efficacy in clinical trials (World Health Organization, 1990, 1996; Cummings and Bremner, 1994; Wu et al, 1996). Pharmacokinetic studies indicate that the major limitations of TE and TC include

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relatively high peak levels and limited duration of T levels within the normal range (approximately 2 weeks), following an injection. Disadvantages of TE and TC highlight the need for long-acting preparations of T with more stable delivery kinetics. Preclinical studies in long-term orchidectomized monkeys showed that injectable TU has more favorable pharmacokinetics and pharmacodynamics than TE (Partsch et al., 1995).

**Materials and Methods**

**Subjects**

Eight patients with Klinefelter’s syndrome were recruited. Age of the patients was 23 ± 3 years (range, 16–26 years), their height was 171 ± 7 cm (range, 158–182 cm), and their weight was 63 ± 14 kg (range 47–90 kg). They were healthy, other than their hypogonadism. All subjects who had received previous hormonal therapy for hypogonadism had stopped a minimum of 6 weeks before the baseline period started. All subjects gave written consent to participate in the study after an explanation of the study’s purpose, benefit, and possible risks.

**Androgen Preparation**

Injectable TU was obtained from Zhejiang Xian Ju Pharmaceutical Corporation, Zhejiang, People’s Republic of China. The preparation was available in ampules containing 250 mg of the ester in 2 ml of tea seed oil.

**Study Design**

Two pretreatment blood samples were taken for baseline estimation of serum hormones. Then, the eight patients were divided randomly into two groups. A prospective crossover clinical trial was performed with injectable TU at doses of 500 mg and 1,000 mg (equivalent to 315 mg and 630 mg pure T, respectively). For the first group (n = 5), 500 mg TU was used first; for the second group (n = 3), 1,000 mg TU was used first. The two treatment periods were separated by an intervening washout period of 3 months. All eight patients received both doses of TU, except for one patient, who received only a single dose of 500 mg TU. Blood samples were collected by venipuncture, allowed to clot for 24 hours, and processed by centrifugation thereafter. All serum samples were stored at −70°C until analysis.

**Hormone Assays**

Reagents for radioimmunoassay (RIA) of serum T, estradiol (E2), and prolactin (PRL) were supplied by the Diagnostic Products Corporation (Los Angeles, California). All samples from one subject were analyzed in a single assay. Sensitivity of the assay was 0.14 nmol/L, 3.7 pmol/L, and 20 mIU/L for T, E2, and PRL, respectively. The intra-assay coefficient of variation for serum T, E2, and PRL was 7.6%, 5.8%, and 6.2%, respectively. The mean interassay coefficient of variation was less than 10% for all three hormones. Levels of serum luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were measured by time-resolved fluorometry (Delfia, Wallac Oy, Finland). Sensitivity was 0.018 IU/L and 0.016 IU/L for LH and FSH, respectively. All samples from one individual were analyzed in the same assay. The mean intra- and interassay coefficients of variation for serum LH were 4.4% and 9.3%; the mean intra- and interassay coefficients of variation for serum FSH were 5.9% and 8.7%. Sex hormone-binding globulin (SHBG) levels were measured by the Nichols Institute (San Juan Capistrano, California) with double-antibody RIA kits supplied by Diagnostic System Laboratories (Webster, Texas). The lower detection limit was 5.0 nmol/L. The mean intra-assay and interassay coefficients of variation for serum SHBG were 4.0% and 8.8%, respectively.

**Statistics and Pharmacokinetic Analysis**

Pharmacokinetic data were analyzed by software designed jointly for pharmacokinetics by the Chinese Pharmacology Association and by the Research Institute for Computation of Mathematics and Scientific Data, Academy of China. A two-sided t-test was used to assess statistical significance between the two doses. Results are expressed as mean ± SEM. P < 0.05 was considered significant.

**Results**

**Testosterone Pharmacokinetics**

A comparison of the pharmacokinetic parameters between the two doses, merged from the crossover injections, is given in Table 1. There were no significant differences in mean residence time (MRT), terminal elimination half-life (T½β), maximal T concentrations (Cmax), time to reach maximum concentration (Tmax), or clearance rate of T at steady status of serum T concentration (CL/t(s)) between the two doses. In contrast to those parameters, injection of 1,000 mg TU resulted in a greater area under the serum T concentration–time curve (AUC) and in a larger T-distribution value related to serum T concentration (V[t(c)]) at steady state. Mean serum levels of T at both doses, merged from the crossover injections, increased promptly after injection of TU (Fig. 1). Mean serum T levels increased significantly by the end of the first week from less than 10 nmol/L to 47.8 ± 10.1 and 54.2 ± 4.8 nmol/L for the lower and higher doses, respectively. Thereafter, elevated serum T levels decreased progressively and reached the lower-nor-
FIG. 1. Serum T levels (mean ± SEM) merged from crossover injections at two doses of TU to the hypogonadal men (--- normal range). Asterisks indicate a significant difference between the two doses (P < 0.05).

The normal limit for adult men by day 50 to 60. There was a clear dose-response relationship between injection dose and serum T levels.

Mean T data from the two groups of patients are compared in Figure 2. Comparison of the serum T levels obtained when 500 mg was given first, versus when it was given 3 months after the 1,000-mg dose, showed quite different pharmacokinetics. The serum T concentrations were remarkably higher when the 500-mg dose was given as the first injection than when it was given as the second.

FIG. 2. Serum T levels (mean ± SEM) in two groups of hypogonadal men receiving 500 mg and 1,000 mg of TU intramuscularly in varying order (--- normal range).
injection. Following 500 mg given as the second injection, the pattern of mean T levels obtained was within the normal adult male range from week 1 through at least week 7. Mean peak T levels did not exceed the normal male range at the end of week 1. This was true despite the fact that basal levels were higher when 500 mg was given as the second injection (the higher basal levels were presumably a continued manifestation of the 1,000-mg dose given 3 months earlier). The tendency for the 500-mg dose given as the second injection to result in lower peak T levels is also suggested by the data from the first group; despite a higher basal level (at week 22, compared with week 0), the peak level obtained following the 1,000-mg dose did not exceed the peak level obtained following the preceding 500-mg dose (Fig. 2).

Estradiol
Mean serum E2 levels rose following T injections and stayed above baseline for 5 to 6 weeks (Fig. 3). Mean serum E2 levels showed the same pattern as those of serum T following 500 mg TU given as the second injection.

Gonadotropins and Prolactin
Mean serum LH and FSH levels were decreased by TU injection at both doses (Figs. 4 and 5). The two doses of TU did not show dose-response effects on suppression of mean serum LH and FSH levels. Mean serum LH and FSH levels were lower following the second dose, but this difference did not achieve statistical significance. Serum PRL levels remained unchanged in both doses throughout the study.

Serum SHBG Levels
SHBG levels before any T therapy were 40.0 ± 8.2 and 46.5 ± 2.5 nmol/L for the subjects in the first and second dose groups, respectively, which was near or above the upper limit of normal in adult men (6–44 nmol/L). SHBG levels were reduced to 18.5 ± 7.5 and 19.5 ± 0.5 nmol/L at 3–4 months following the 500-mg and 1,000-mg doses, respectively. Furthermore, the SHBG level was reduced from 19.5 ± 0.5 nmol/L to 11.5 ± 6.5 nmol/L, following 500 mg TU given as the second injection when serum T achieved peak level. However, the SHBG level remained at 20.5 ± 10.5 nmol/L following 1,000 mg TU given as the second injection when serum T achieved peak level.

Clinical Features
Improved secondary sexual characteristics were noted after one to two injections of TU. All subjects reported increased libido. The common report from subjects was that TU injections stimulated their appetite. Weight gain (2 kg, on average) was found. The parameters of hematology and clinical chemistry, including liver and kidney functions, were within normal reference ranges during the follow-up periods. No local reaction was seen at the injection site. No fluctuation in mood was found. Gyneco-
mastia was found in two subjects before TU injection. No
changes in breast size were found in these two subjects
or in the remaining subjects after TU injections.

**Discussion**

This study is the first detailed pharmacokinetic investiga-
tion of the injectable preparation of TU with two doses in
hypogonadal men. The pharmacokinetic profile of the in-
jectable TU preparation is advantageous compared with the
T esters currently available for androgen therapy. TC shows
pharmacokinetics similar to TE, which has a MRT of
8.5 days and a T½ (β) of 4.5 days (Schulte-Beerbühl and
Nieschlag, 1980; Snyder and Lawrence, 1980; Sokol et al.,
1982; Bøthe et al., 1990, 1992a). Therefore, TE and TC
must be injected approximately every 2 weeks. Also, TE
and TC lead to early increases in serum T levels and may
be associated with subnormal values shortly before the
next injection. To attain physiological mean serum T lev-
els, it is necessary to accept some supraphysiological T
levels and some fluctuations in circulating T levels be-
tween injections, which may be experienced as unpleasant
by patients and can cause unwanted side effects. The in-
jectable TU preparation shows a more favorable pharma-
cokinetic profile, with a MRT of 21.73 ± 1.1 and 22.98
± 0.81 days and a T1/2 (β) of 18.3 ± 2.32 and 23.68 ±
2.65 days for the two doses. Thus, the long duration ob-
served in the monkey (Partsch et al., 1995) was also seen
in humans. To computerize these pharmacokinetic data, a
one-compartment model with first-order absorption was
chosen according to the criteria of good fit describing ki-
netics for the batch data. The pharmacokinetic parameters
were nonlinearly distributed between lower and higher doses.
Most parameters, such as MRT, T½ (β), and Cmax were
very similar for the two doses, although the AUC and
the V(f) were greater by 50% at the higher dose. Our
study has demonstrated substantial patient-to-patient vari-
ation in the serum concentration achieved following similar
doses. This can be attributed to differences in completeness
of absorption, volume of distribution, and elimination.
There are several factors affecting pharmacokinetics of T
esters. The kinetics of T entry into the circulation is dic-
tated by the rate-liming release of the esters from the oily-
vehicle injection site (World Health Organization Special
Programme, 1992). The rate of T-ester release is influenced
by the volume and type of oil, the site of injection and its
blood flow, and by the nature of the side-chain–ester moi-
ety, especially its hydrophobicity (Belmann et al., 1976).
In addition, the longer chains do produce longer duration
effects, which exploit more fully the side-chain–ester moi-
egies (World Health Organization Special Programme,
1992). The injectable TU preparation (longer carbon-chain
ester than TE) enhances the capability to develop a depot-
release preparation while maintaining more stable serum T
levels.
When comparing the pharmacokinetics of TU with those of testosterone buciclate (TB), a novel testosterone ester (Weinbauer et al., 1986; Rajalakshmi and Ramakrishna, 1989; Rajalakshmi et al., 1991), major pharmacokinetic differences between TU and TB exist in the following parameters: MRT (21.73 vs. 65 days), $T_{\beta} (\beta)$ (18.3 vs. 29.5), and Cmax (40.5 vs. 13.1) after a single injection of 600 mg TB (equivalent to 380 mg unesterified T) in hypogonadal men (Behre and Niesschlag, 1992b). However, TB is delayed in development and may not be available for further clinical work in the near future.

Of considerable interest is the observation in our work that the pharmacokinetic profiles of T were different when 500 mg TU was given as the first injection and when it was given as the second one (Fig. 2). Similarly, serum E2 levels showed the same pattern as serum T following 500 mg TU given as the second injection. The peak T values obtained were lower following the case of the 500-mg dose given as the second injection, compared with when the 500-mg dose was given first. This was true despite the higher basal T levels before the second injection. One explanation for this pharmacokinetic profile is that these long-term hypogonadal men may have induced faster clearance mechanisms for T by the time of the second injection. This is supported by the finding that SHBG concentration was reduced to 11.5 ± 6.5 nmol/L when 500 mg TU was given as the second injection, compared with 26.5 ± 8.2 nmol/L after 500 mg TU was given as the first injection when serum T achieved peak levels. The relationship between serum peak T value and SHBG levels is summarized as follows: 1) SHBG is a plasma glycoprotein, synthesized by the liver, that reversibly binds circulating androgens with high affinity (Anderson, 1974). 2) SHBG levels are higher in men with hypogonadism (Vermeulen et al., 1969; Anderson, 1974); our results confirm that administration of androgens causes a reduction in SHBG levels (Pearlman et al., 1967; Vermeulen et al., 1969). 3) It has been found that the main fraction of serum T is bound to SHBG. T is also bound partly to albumin, which has a low binding affinity. SHBG binding sites are nearly saturated in men, since their molar concentration is only marginally greater than the molar concentration of serum T (Anderson, 1974). Hence, there was a change in the binding of T from SHBG to albumin, due to reduction in SHBG level. This change in volume of distribution might lead to an increase in free T that could distribute deeper into the extravascular compartment or be metabolized quickly. 4) It has been proven that there is an inverse relationship between SHBG levels and the metabolic clearance rate of T (Petra et al., 1985). Thus, total serum T concentration measured by RIA decreased as a result of change in the volume of distribution and the increased MCR of T. In other words, decreased serum T at peak value of the 500 mg TU given as the second injection was induced by a change in the volume of distribution and by faster elimination of T.
Moreover, the decreased SHBG levels produced by the 1,000-mg TU injection given first could have led to a more rapid clearance of T following the 500-mg TU injection given second than that which occurred when 500 mg TU was given first and 1,000 mg TU was given second. We propose that the pattern of serum T following the 500-mg injections given second may give a more accurate estimate of eventual clinical results. The T levels obtained with the second–given 500-mg injections suggest that this formulation will be very successful in long-term studies in which repeated injections are involved.

Serum levels of both LH and FSH were decreased by TU injections. Serum LH and FSH at both doses tended to be lower after the second injections, compared with those after the first injections, a phenomenon partly due to the fact that serum LH and FSH levels had not returned to the baseline levels after the first injections. The lower basal gonadotropin levels before the second injections again confirm the long duration of action of this T preparation.

We conclude that the present study demonstrates that single-dose injections of 500 mg or 1,000 mg TU preparation to hypogonadal men can maintain serum T concentration within the normal range for over 50 to 60 days without notable side effects. The data demonstrated the most favorable serum T profile after the second injections of 500 mg TU, suggesting that repeated injections of 500 mg every 6–8 weeks are likely to produce satisfactory T replacement. Therefore, since injections could be relatively infrequent, this injectable TU preparation may provide an improved step for substitution therapy of male hypogonadism. In addition, TU may be developed as a male contraceptive, used alone or with additional gonadotropin suppressive agents, such as progestins or GnRH antagonists (Bremner et al., 1991; Behre et al., 1992a; Bagatell et al., 1993; Cummings and Bremner, 1994; Mergioglia et al., 1996).

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