Intramuscular injection of testosterone undecanoate for the treatment of male hypogonadism: phase I studies

H M Behre, K Abshagen, M Oettel1, D Hübner1 and E Nieschlag

Institute of Reproductive Medicine of the University, Domagkstrasse 11, D-48129 Münster, Germany and 1Jenapharm GmbH & Co. KG, Jena, Germany

(Correspondence should be addressed to E Nieschlag)

Abstract

Objective: In the search for long-acting testosterone preparations suited for substitution therapy of hypogonadal men, testosterone undecanoate (TU) dissolved in either tea seed oil or castor oil was investigated.

Design: In study I, 1000 mg TU in tea seed oil (125 mg/ml) were injected in equal parts into the gluteal muscles of seven hypogonadal men. In study II, 1000 mg TU in castor oil (250 mg/ml) were injected into one gluteal muscle of 14 patients.

Results: In comparison with published data on testosterone enanthate, most widely used for i.m. injections, the kinetic profiles of both TU preparations showed extended half-lives and serum levels not exceeding the upper limit of normal. The castor oil preparation had a longer half-life than TU in tea seed oil (33.9 ± 4.9 vs 20.9 ± 6.0 days (mean ± S.E.M.)).

Conclusion: The longer half-life and the smaller injection volume make TU in castor oil a strong candidate for further applications in substitution therapy and in trials for male contraception.

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Introduction

Testosterone has been used for substitution therapy for almost six decades. Since the number of patients suffering from hypogonadism and requiring such therapy is relatively small there has not been much drive to develop new testosterone preparations beyond subdermal implants developed in the 1940’s, enanthate and cypionate esters for i.m. injections developed in the 1950’s and oral testosterone undecanoate (TU) developed in the 1970’s. Although still in use, these preparations are not ideal because of their kinetics, resulting in either supraphysiological or fluctuating serum testosterone levels, and because of the inconvenience of frequent application (for review see reference 1). Only the possibility of new and more widespread indications stimulated a search for alternative application modalities. One result was transdermal systems well suited for long-term substitution because of almost physiological serum testosterone levels (2–4) and because of the possibility for immediate interruption of the treatment if required (e.g. when substituting hypogonadism in senescence) (5). For younger patients and for hormonal male contraception, however, long-acting testosterone preparations continue to be required.

Under the auspices of the WHO, testosterone bucinate was synthesized and tested as an i.m. injection, and in phase I studies showed an extended half-life of 29.5 days (compared with 4.5 days for conventional testosterone enanthate (TE)) (6). However, further development of this ester was hampered by the lack of an industrial partner. We therefore turned to TU prepared for i.m. injection in China and exhibiting an extended half-life in hypogonadal men (7). After confirmation of the long half-life in monkeys (8) we tested the Chinese preparation in hypogonadal men. Since this preparation is based on tea seed oil, uncommon in the Western pharmacopoeia, TU was manufactured for clinical use in castor oil, granting a higher solubility than tea seed oil. Here we present results from phase I studies in hypogonadal men using both the Chinese and the new TU preparation.

Subjects and methods

Testosterone preparations

The TU preparation (3-oxoandrost-4-ene-17β-y1-undecanoate) used in study I was provided and manufactured by Zhejiang Xian Ju Pharmaceutical Corp. (Zhejiang, People’s Republic of China). The steroid was dissolved in tea seed oil at a concentration of 125 mg/ml. TU used in study II was prepared by Jenapharm GmbH & Co. KG (Jena, Germany). The batch used for all i.m. injections had a concentration of 250 mg TU dissolved in 1 ml castor oil.
Study design and patients

Study I was performed as a therapeutic trial in agreement with German Drug Law. The protocol of study II was approved by the Ethics Committee of the University of Münster and the State Medical Board. Both studies were conducted at the Institute of Reproductive Medicine in Münster, in agreement with the Declaration of Helsinki and in accordance with Good Clinical Practice. All subjects gave written informed consent.

Seven Caucasian patients (25–51 years) with primary (n = 4) or secondary (n = 3) hypogonadism without additional diseases were enrolled in study I (Table 1). In study II, 14 Caucasian patients (19–45 years) with primary (n = 9) or secondary (n = 5) hypogonadism without additional diseases participated (Table 1). Three of seven (study I) and 10 of 14 patients (study II) had been treated in the past with i.m. TE and one patient in each study with oral TU before recruitment for the study. One patient did not reappear on day 28 of study II or later; his data are only included up to day 21 after TU injection.

In study I, 1000 mg TU (2 × 4 ml on each side) were injected into each of the patient’s musculus glutaeus medius on day 0 between 08.00 and 09.00 h. In study II, 1000 mg TU (4 ml volume on one injection side) were injected into one of the patient’s musculus glutaeus medius at the same time of the day. The blood sampling scheme thereafter was identical in both studies. Blood samples for hormone determinations were drawn between 08.00 and 10.00 h at two control examinations (days −14 and −7), shortly before and 4 h (only study II) and 1, 2, 3, 5 and 7 days after TU injection, and then weekly up to day 56 (week 8). Blood samples for hormone determinations were separated at 800 g and stored at −20 °C until assayed.

Serum testosterone and estradiol were measured in all samples of both studies, while dihydrotestosterone (DHT), sex hormone-binding globulin (SHBG), luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were determined only in study II.

In study II, clinical chemistry (total protein, albumin, cholesterol, low density lipoprotein (LDL) cholesterol, high density lipoprotein (HDL) cholesterol, triglycerides, glucose, alkaline phosphatase, creatinine, aspartate aminotransferase, alanine aminotransferase, gamma-glutamyltransferase, total bilirubin, uric acid, urea nitrogen, sodium, potassium, calcium, chloride and prostate-specific antigen (PSA)) and hematology (red cell count, hemoglobin, hematocrit, white cell count, platelet count, prothrombin time (Quick test) and partial thromboplastin time) were performed at the control examinations, shortly before injection of the testosterone ester, and then weekly up to the end of the study. Urinalysis was performed before TU injection and at the final examination of study II. Blood and urine samples were taken after a 10 h fast.

Table 1 Anthropomorphic and clinical data of patients.

<table>
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<tr>
<th>Study</th>
<th>Age (years)</th>
<th>Height (cm)</th>
<th>Weight (kg)</th>
<th>BMI* (kg/m²)</th>
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*BMI = body mass index.
**Immunoassays**

Testosterone and DHT were separated in extracted serum samples by HPLC before measurement by RIAs. The detection limits for testosterone and DHT were 0.28 and 0.14 nmol/l respectively. The intra- and interassay coefficients of variation for testosterone were 6.6 and 9.8% respectively, and for DHT 7.2 and 12.3% respectively. In our laboratory the normal range for testosterone after separation by HPLC is 10–35 nmol/l and the upper normal limit for DHT is 2.9 nmol/l. Estradiol was measured by RIA (Sorin Biomedica, Saluggia, Italy). The detection limit for estradiol was 37 pmol/l. The intra- and interassay coefficients of variation for estradiol were 6.8 and 8.2% respectively. The upper normal limit for estradiol is 250 pmol/l.

Serum LH, FSH, SHBG and PSA were determined by specific fluorimmunoassays (Pharmacia GmbH, Freiburg, Germany). The lower detection limits for FSH, LH and SHBG were 0.25 IU/l, 0.12 IU/l and 6.3 nmol/l respectively. The intra- and interassay coefficients of variation for LH were 4.5 and 6.4% respectively, for FSH 3.7 and 5.4% respectively, and for SHBG 4.8 and 7.4% respectively. In our laboratory the normal range for LH is 2–10 IU/l, for FSH 1–7 IU/l and for SHBG 11–71 nmol/l. The lower detection limit for PSA is 0.5 µg/l and the upper reference limit is 4 µg/l. Not detected PSA levels were defined as 0.25 µg/l. The intra- and interassay coefficients of variation for PSA were 5.8 and 11.7% respectively. All analytical methods were executed and documented in accordance with the principles of Good Laboratory Practice.

**Pharmacokinetic evaluations and statistics**

For evaluation of TU pharmacokinetics and for removal of between-subject variations in basal endogenous testosterone the increments from the subject’s own baseline testosterone values were analyzed. Analysis included AUC (area under the concentration versus time curve, calculated by the trapezoidal method), C_{max} (concentration maximum, calculated from the individual data), t_{max} (time of reaching C_{max}, calculated from the individual data), and t_{1/2} (terminal elimination half-life) (9).

Significant variations over time of any variable were evaluated by ANOVA for repeated measures. In case of a general effect over time, values at single time points were analyzed in more detail by comparison with the baseline value before injection using the Duncan multiple comparison test for repeated measures. When necessary, analysis was performed on logarithmically transformed data. P values <0.05 were considered significant. Unless otherwise stated, results are given as mean ± S.E.M.

**Results**

The i.m. injections of 1000 mg TU into either one or both gluteal regions were well tolerated by all 21 hypogonadal patients included in the studies. No serious adverse effects were observed. In study II, detailed weekly diaries of 4 out of 14 patients revealed some discomfort at the injection site persisting not longer than 1 week after injection; one patient reported some pain at the injection site on day 14. No patient reported the injections to be more painful or inconvenient than former i.m. injections. One patient reported transient testicular pain at day 28. Clinical examinations revealed no new occurrence of gynecomastia nor any enlargement or soreness of the liver; one patient showed sporadic signs of acne 2 and 5 weeks after TU injection. No patient discontinued treatment because of side-effects.

**Testosterone and DHT**

In study I, injections of TU in tea seed oil increased testosterone serum levels in a time-dependent pattern (Fig. 1, upper panel) \( (P < 0.001) \). One day after injection serum levels of testosterone rose from basal levels of 4.8 ± 0.9 to levels of 14.9 ± 1.4 nmol/l in the normal
range. C_{max} were seen between day 7 (30.5 ± 6.4 nmol/l) and 14 (29.9 ± 6.0 nmol/l). In three of seven patients, individual values exceeded the normal range with C_{max} of 52.6 nmol/l in one patient 14 days after injection. Mean testosterone levels remained in the normal range up to week 7 and were back in the hypogonadal range 8 weeks after injection (9.1 ± 1.4 nmol/l). Pharmacokinetic analysis revealed a t_{1/2} of 20.9 ± 6.0 days (Table 2).

In study II, after injection of 1000 mg TU in castor oil, serum levels of testosterone rose from basal levels of 5.0 ± 0.8 to normal levels of 12.3 ± 1.7 nmol/l 2 days after injection (Fig. 1, upper panel) (P < 0.001). C_{max} were seen between day 7 (22.0 ± 2.0 nmol/l) and 14 (21.5 ± 1.4 nmol/l). Individual values exceeded the normal range at days 3, 5 and 7 with C_{max} of 40.8 nmol/l 3 days after injection in only 1 of 14 patients. Mean levels in the lower normal range and significantly higher compared with baseline values were maintained up to week 8 after injection (11.5 ± 1.5 nmol/l). Pharmacokinetic analysis revealed a t_{1/2} of 33.9 ± 4.9 days (Table 2).

In study II, mean serum concentrations of DHT increased significantly after TU injection (P < 0.001) (Fig. 2, upper panel). C_{max} were seen between day 7 (1.4 ± 0.4 nmol/l) and 14 (1.4 ± 0.2 nmol/l). In 2 of the 14 patients, individual values of DHT exceeded the normal range: at day 7 in one patient (3.09 nmol/l) and from day 3 to 14 in the other (C_{max} 4.6 nmol/l). Serum concentrations of DHT remained significantly elevated compared with baseline up to day 35.

**Estrogens and SHBG**

In study I, TU administration in tea seed oil increased serum estradiol significantly (P < 0.001) from 47.8 ± 7.0 pmol/l to a C_{max} of 116.4 ± 13.9 pmol/l 14 days after injection (Fig. 1, lower panel). All individual values remained within the normal range.

In study II, administration of TU in castor oil increased serum concentrations of estradiol significantly (P < 0.001) from 55.3 ± 6.4 pmol/l to a C_{max} of 99.0 ± 9.0 pmol/l 14 days after injection (Fig. 1, lower panel). All individual values remained in the normal range throughout study II.

**Gonadotropins**

In the nine patients of study II with primary hypogonadism and elevated gonadotropins, LH was significantly suppressed from a baseline of 21.5 ± 2.5 to 14.3 ± 3.5 IU/l at day 35 (P < 0.005) (Fig. 3, upper panel). FSH serum concentrations were only marginally, but significantly, lowered by TU administration.

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**Table 2** Pharmacokinetic data (mean ± S.E.M.) of the two TU preparations after i.m. injection of 1000 mg TU in comparison with previously published kinetic data from TE and testosterone buciclate (TB).

<table>
<thead>
<tr>
<th>Preparation/concentration</th>
<th>Total dose injected (mg)</th>
<th>AUC (nmol×days/l)</th>
<th>C_{max} (nmol/l)</th>
<th>t_{max} (days)</th>
<th>t_{1/2} (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TU 125 mg/ml (tea seed oil)</td>
<td>1000</td>
<td>AUC_{(0–8 weeks)} 825 ± 93</td>
<td>30.1 ± 5.5</td>
<td>13.0 ± 3.7</td>
<td>20.9 ± 6.0</td>
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<tr>
<td>TU 250 mg/ml (castor oil)</td>
<td>1000</td>
<td>AUC_{(0–8 weeks)} 534 ± 49</td>
<td>19.3 ± 2.1</td>
<td>11.4 ± 1.5</td>
<td>33.9 ± 4.9</td>
</tr>
<tr>
<td>TB 200 mg/ml (aqueous suspension)*</td>
<td>600</td>
<td>AUC_{(0–16 weeks)} 377 ± 68</td>
<td>6.7 ± 1.2</td>
<td>25.8 ± 8.2</td>
<td>29.5 ± 3.9</td>
</tr>
<tr>
<td>TE 250 mg/ml (castor oil)†</td>
<td>250</td>
<td>AUC_{(0–3 weeks)} 376</td>
<td>39.4</td>
<td>10</td>
<td>4.5</td>
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</table>

* Data from reference (6).
† Data from reference (11) as re-analysed from reference (18).
from 39.7 ± 6.2 to a nadir of 35.3 ± 5.4 U/l at study day 21 (P = 0.017) (Fig. 3, lower panel).

Body weight and blood pressure

Body weight as well as systolic and diastolic blood pressure did not change significantly throughout study II.

Clinical chemistry and hematology

No significant changes in clinical chemistry, including cholesterol, HDL cholesterol, LDL cholesterol and liver function tests were observed throughout study II. Hemoglobin, erythrocytes and hematocrit were not different from baseline. No significant change was seen in leukocyte or platelet counts throughout the study. No significant change was seen in blood coagulation as assessed by partial thromboplastin time and prothrombin time.

Serum levels of PSA increased significantly (P < 0.001) from baseline of 0.33 ± 0.06 to 0.56 ± 0.07 μg/l at day 28. Levels at day 56 of 0.48 ± 0.08 μg/l were no longer statistically different from baseline. All individual PSA values remained well within the normal range (Cmax 1.6 μg/l in one patient at day 35).

Discussion

Intramuscularly injected TE is the most widely used testosterone preparation when depot effects are required, e.g. for substitution of hypogonadism (1) or in trials for hormonal male contraception (10). However, after injection of the commonly administered dose of 200 or 250 mg, TE has the disadvantage that it produces supraphysiological serum testosterone levels during the days immediately following administration with a slow decline to the lower limit of normal following within 10–14 days (11). Patients dislike these swings in serum testosterone levels, which they experience as ups and downs in vigour, mood and sexual activity. Other testosterone esters in clinical use such as testosterone cypionate or cyclohexanecarboxylate show pharmacokinetic profiles almost identical to that of TE (12, 13), so that these preparations offer no therapeutic advantage.

Although the current study deals with a much higher dose of testosterone than administered in previous studies, TU does not result in supranormal serum testosterone levels, but in much prolonged action. Extrapolating from single-dose kinetics it appears that upon repeated injections of 1000 mg, injection intervals of 6–10 weeks will be possible. The prolonged intervals and the normal serum testosterone levels throughout the injection-free period would be welcomed by the hypogonadal patient requiring substitution as well as by the eugonadal male seeking contraceptive protection.

Although different routes of administration may yield different toxicological profiles for the same drug, the lack of serious side-effects from TU administered orally at doses of 80–160 mg/day over many years (14) gives reason to assume that TU applied i.m. might also be well tolerated. Indeed, no untoward side-effects have been reported from i.m. use in China (7, 15). The reason for the prolonged half-life of TU in comparison with TE is the longer aliphatic, and thus more hydrophobic, side-chain, comprising 11 instead of 7 carbon atoms. Similarly, testosterone buciclate has a prolonged duration of action, which is caused by the hydrophobic benzol ring incorporated into the side-chain.

Recently, it was shown in Chinese men that i.m. injection of 1000 mg TU dissolved in tea seed oil at a concentration of 125 mg/ml has a similar pharmacokinetic profile with a t½ β of 23.7 ± 2.7 days compared with our study with the tea seed oil preparation in Caucasian men (15). The longer duration of action of TU in castor oil compared with TU in tea seed oil could be due to the properties of the oils, the different concentrations (125 vs 250 mg/ml) and injection volumes (4 vs 8 ml), as well as unilateral vs bilateral gluteal application. It is conceivable that the larger surface of the depot produced by 2 × 4 ml injections leads to a slightly faster release of the testosterone

Figure 3 Serum concentrations (mean ± S.E.M.) of LH (upper panel) and FSH (lower panel) after single dose i.m. injections of 1000 mg TU in castor oil in nine men with primary hypogonadism (study II). Broken lines indicate normal ranges of LH and FSH.
ester, resulting in higher $C_{\text{max}}$ values and a slightly shorter half-life than the single 4 ml depot with more concentrated TU. It has been shown that different physico-chemical properties of the oil used as vehicle (16), as well as different injection volumes (17), may influence the kinetics of administered androgens.

In summary, i.m. TU in castor oil has a considerably longer half-life than conventional TE, producing serum levels in the normal range over 6 weeks. These properties make it an attractive candidate for substitution therapy as well as for use in male contraception.

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**References**


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