Oral Testosterone-Triglyceride Conjugate in Rabbits: Single-Dose Pharmacokinetics and Comparison With Oral Testosterone Undecanoate

J. K. AMORY,* G. K. E. SCRIBA,† D. W. AMORY,‡ AND W. J. BREMNER*

*From the *Population Center for Research in Reproduction and the Department of Medicine, University of Washington, Seattle, Washington; †Department of Pharmaceutical Chemistry, University of Jena, Jena, Germany; and ‡Institute of Applied Physiology and Medicine, Seattle, Washington.*

ABSTRACT: Development of a safe and effective oral form of testosterone has been inhibited by the rapid hepatic metabolism of non-alkylated androgens. Since triglycerides are absorbed via lymphatics and bypass the liver, we hypothesized that a testosterone-triglyceride conjugate (TTC) might allow for safe and effective oral testosterone therapy. Therefore, we studied the single-dose pharmacokinetics of oral administration of TTC in rabbits. Female New Zealand rabbits were administered 2, 4, or 8 mg/kg of TTC in sesame oil by gastric lavage. Testosterone undecanoate (TU) by gastric lavage was used as a positive control. Blood was sampled from a catheter in the auricular artery at 0, 15, 30, 60, 90, 120, 180, 240, 360, 480, and 600 minutes after drug administration. Samples were assayed for testosterone by a fluoroimmunoassay. Mean serum testosterone, area under the curve (AUC), and terminal half-life were calculated. Oral TTC administration resulted in rapid and marked increases in serum testosterone. Oral TTC resulted in higher maximum serum testosterone concentrations than oral TU at 8 mg/kg (TTC: 28.6 ± 7.9 nmol/L vs TU: 11.9 ± 2.1 nmol/L; $P < .001$) and 4 mg/kg (TTC: 11.5 ± 4.2 nmol/L vs TU: 3.6 ± 1.0 nmol/L; $P < .001$). In addition, the AUC was 1.8 to 2.6 times greater for TTC than TU at both doses ($P < .05$). The terminal half-life for both TU and TTC was between 3 and 5 hours and was not significantly different. We conclude that oral TTC is rapidly absorbed from the rabbit intestine and results in elevated concentrations of serum testosterone. The absorption of TTC appears to be superior to that of TU; however, the in vivo persistence of the 2 compounds is similar. TTC may offer an alternative to the use of TU for oral testosterone therapy. Further testing of this compound is warranted.

Key words: Hypogonadism, androgen, lymphatics.

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Men with hypogonadism require testosterone replacement to maintain bone and muscle mass, strength, and sexual function (Katznelson et al, 1996; Wang et al, 1996; Behre et al, 1997; Bhasin and Bremner, 1997; Snyder et al, 2000; Wang et al, 2000). In the United States, testosterone is currently administered by intramuscular injections, transdermal patches, or gels. Each of these modes of testosterone delivery, however, has drawbacks. Injections, although inexpensive, must be given intramuscularly every 1 to 3 weeks to maintain normal serum testosterone levels and can be painful (Fossa et al, 1999). Patches can cause moderately severe skin reaction in more than half of subjects due to the vehicles that facilitate testosterone absorption across the skin (Amory and

Matsumoto, 1998). The newly available testosterone gel is safe and effective (Swerdloff et al, 2000), but it is expensive and must be applied to a fairly large area of skin and care must be taken to avoid inadvertent vicarious exposure to women and children.

Older forms of oral testosterone are alkylated at the 17-carbon position, greatly reducing their hepatic metabolism and improving their oral bioavailability. Unfortunately, these compounds are associated with an unacceptably high rate of liver toxic effects, including cholestatic jaundice, peliosis hepatis, and even liver tumors in one third to half of long-term users (Westaby et al, 1977; Turani et al, 1983; Lowdell et al, 1985). As a result, such 17-alkylated forms of testosterone are not considered safe for long-term use by most experts in the field (Snyder, 2001). Therefore, oral administration of testosterone is currently only safely accomplished by the use of testosterone undecanoate (TU), which is commercially available in many countries but not the United States. When administered orally, a portion of TU is absorbed via lymphatics and thereby bypasses hepatic ''first-pass'' metabolism (Coert et al, 1975; Nieschlag et al, 1975; Horst et al, 1976). TU is lymphatically absorbed due to its long lipophilic side chain in a fashion similar to triglycerides. The absolute
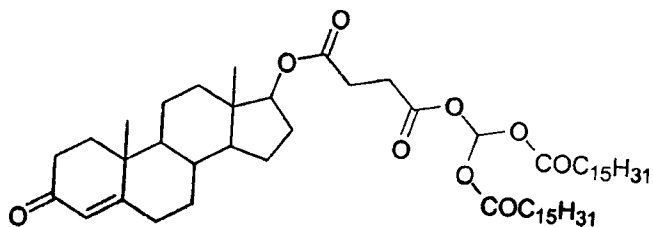
Figure 1. The molecular structure of the testosterone-triglyceride conjugate ($C_{58}H_{98}O_6$); molecular weight = 939.
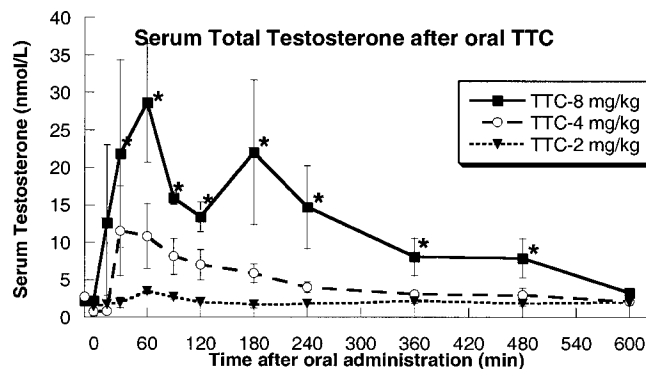

Figure 2. Serum total testosterone after oral administration of 2, 4, and 8 mg/kg of testosterone-triglyceride conjugate to female rabbits. Data are mean ± SEM. *$P < .05$ between groups. Conversion for testosterone: 1 nmol/L = 288 pg/mL.

bioavailability of testosterone after oral TU administration, however, is only approximately 6% (Täuber et al, 1986), implying that most of an oral TU dose is absorbed via the portal circulation and metabolized by the liver.

We hypothesized that a testosterone-triglyceride conjugate (TTC) would show superior absorption via lymphatics than TU and would therefore result in higher maximal serum levels of testosterone and improved bioavailability. One of us (G.K.E.S.) has synthesized such a compound and reported on its in vitro characteristics (Scriba, 1995a). Moreover, this type of drug-triglyceride conjugate has been demonstrated to improve the oral delivery of other poorly water-soluble drugs, such as phenytoin (Scriba et al, 1995b). In this report, we present our initial studies of the single-dose pharmacokinetics of a TTC after oral administration in a rabbit model.

Materials and Methods

Testosterone Compounds

TTC (Figure 1) was synthesized by the N,N-dicyclohexylcarbodimide–mediated esterification of 2-(1,3-dipalmitoylglyceryl)succinic acid mono ester with testosterone (Scriba, 1995a). Purity was assessed by high-performance liquid chromatography and found to be greater than 99%. Lyophilized TTC was suspended in sterile sesame oil (Prima Pharm Inc, San Diego, Calif) at a concentration of 40 mg/mL by overnight stirring before administration. TU in 40-mg capsules (Andriol) was obtained from Organon Pharmaceuticals (Oss, Netherlands). Capsules were incised and the TU oil was aspirated and suspended in sesame oil before administration.

Experimental Animals

Juvenile female New Zealand white rabbits (Western Oregon Rabbit Center, Philomath, Ore) weighing 2 to 4 kg were obtained and allowed to eat ad libitum before dosing. Animals were sedated with 2 mg/kg of acepromazine maleate (Boehringer Ingelheim Vetmedica Inc, St Joseph, Mo), positioned in a restraining device, and the dorsal auricular artery cannulated to facilitate frequent blood sampling. After baseline blood samples were obtained, animals underwent insertion of an orogastric tube according to published techniques (Burr et al, 1987), TTC, TU, or sesame oil placebo was instilled, and the tube removed. Doses of TTC and TU were normalized for testosterone content (ie,

60% [wt/vol] for TU [molecular weight, 480] and 30% [wt/vol] for TTC [molecular weight, 939]). Arterial blood samples were obtained 15, 30, 60, 90, 120, 180, 240, 360, 480, and 600 minutes after drug administration. Heparin (50 U in 0.5 mL) was instilled in the catheter after each blood draw to ensure catheter patency. Each dose of TTC or TU was tested in triplicate. Animals were restudied after a 2-week rest interval to allow their blood volume to return to normal. The Animal Use Committee of the University of Washington approved this protocol.

Testosterone Measurements

Rabbit serum was obtained from whole blood by centrifugation (15 minutes at $2000 \times g$) and frozen immediately at $-70°C$ until assayed. Serum levels of testosterone were determined using a highly specific fluoroimmunoassay (Diagnostic Systems Laboratories, Webster, Tex). The assay sensitivity was 0.8 nmol/L, and the midrange intra-assay and interassay coefficients of variation were 7.5% and 9.3%, respectively. Neither TTC nor TU was found to cross-react in the assay up to a concentration of 100 nmol/L.

Statistical Analysis

Testosterone measurements were averaged from each time point to obtain the mean and SEM. Differences between means were compared by a t test. Area under the curve (AUC) was calculated using the trapezoid rule from $t = 0$ to the last measured level without smoothing or curve fitting (PK Solutions, Summit Research, Montrose, Colo). The terminal half-life (T½) was calculated from $t = 90$ to the last measured level. For all comparisons, $\alpha < .05$ was considered statistically significant.

Results

Testosterone-Triglyceride Conjugate

Immediately before administration of TTC, serum total testosterone levels were less than 1.0 nmol/L. Oral administration of TTC resulted in dose-dependent increase in serum testosterone that persisted for up to 10 hours after administration (Figure 2). The maximum concentra-
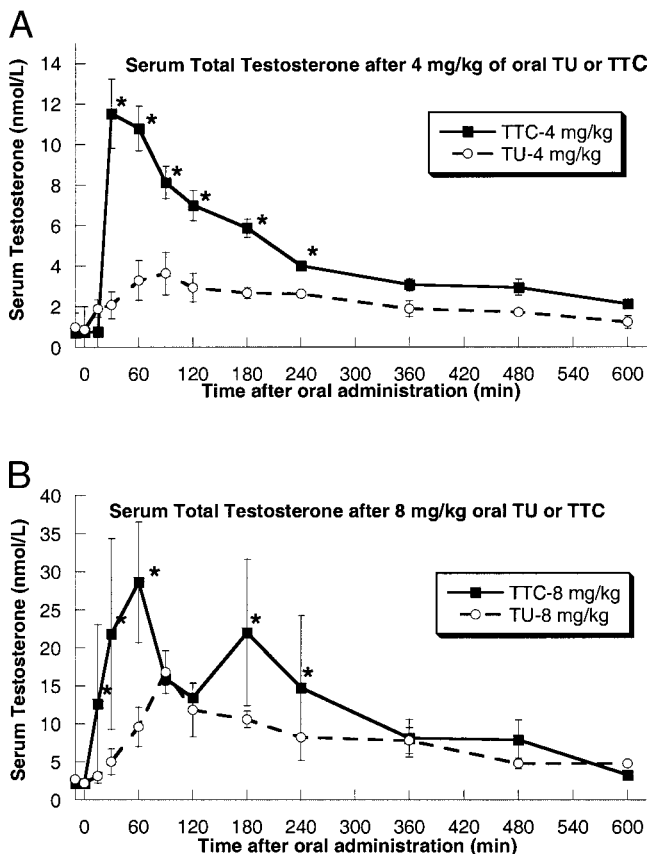
A

B

Figure 3. Serum total testosterone after oral administration of 4 mg/kg (A) or 8 mg/kg (B) of testosterone undecanoate to female rabbits. Data from the testosterone-triglyceride conjugate are included for comparison. Data are mean ± SEM. *$P < .05$ between testosterone-triglyceride conjugate and testosterone undecanoate. Conversion for testosterone: 1 nmol/L = 288 pg/mL.

tions of serum testosterone observed with each dose of TTC were as follows: 2 mg/kg: 4.1 ± 1.5 nmol/L; 4 mg/kg: 11.5 ± 4.2 nmol/L; and 8 mg/kg: 28.6 ± 7.9 nmol/L ($P < .01$ for trend). The time to reach the maximum concentration at each dose was between 30 and 60 minutes in all cases. Serum total testosterone levels decreased rapidly after peaking, slowly returning to baseline throughout the 10-hour observation period.

Testosterone Undecanoate

Immediately before administration of TU, serum total testosterone levels were less than 1.0 nmol/L. Oral administration of TU resulted in dose-dependent increase in serum testosterone that persisted for up to 10 hours after administration (Figure 3A and B). The maximum concentrations of serum testosterone observed after oral administration of TU were 3.6 ± 1.0 nmol/L for 4 mg/kg and 11.9 nmol/L ± 3.4 nmol/L for 8 mg/kg ($P < .01$). The time to reach the maximum concentration at each dose was between 90 and 120 minutes. After peaking, serum total testosterone levels returned to baseline slowly throughout the 10-hour observation period.

Comparative Pharmacokinetics

Oral administration of TTC resulted in significantly higher maximum concentrations and greater AUC values when compared with oral TU (Table). At 8 mg/kg, the maximum serum testosterone concentration achieved by oral administration of TTC was 2.4 times higher than the maximum concentration in the TU group, whereas at 4 mg/kg, the maximum serum testosterone concentration was 3.2 times that achieved in the TU group ($P < .01$). The AUC for TTC was 1.8 times that of TU in the 8-mg/kg group and 2.6 times that of TU in the 4-mg/kg group. The T½ was 3 to 5 hours for both compounds at both

Pharmacokinetic parameters after oral administration of testosterone-triglyceride conjugate and testosterone undecanoate*

	Dose of Compound, mg/kg		
Parameter	2	4	8
Testosterone-triglyceride conjugate			
Cmax (nmol/L)	4.1 ± 1.5	11.5 ± 4.2†‡	28.6 ± 7.9†‡
Tmax (h)	1.0 ± 0.3	0.5 ± 0.4	1.0 ± 0.6
AUC (nmol-h/L)	30 ± 12	77 ± 23†‡	149 ± 34†‡
Terminal half-life (h)	3.4 ± 0.4	3.8 ± 0.8	3.1 ± 0.7
Testosterone undecanoate			
Cmax (nmol/L)	ND	3.6 ± 1.0‡	11.9 ± 2.1‡
Tmax (h)	ND	1.5 ± 0.5	2.0 ± 0.9
AUC (nmol-h/L)	ND	29 ± 6.7‡	81 ± 25‡
Terminal half-life (h)	ND	4.0 ± 1.1	4.7 ± 1.3

* Data are mean ± SEM. Cmax indicates maximum concentration; Tmax, time to maximum concentration; AUC, area under the curve; and ND, not determined.
† $P < .05$ for the comparisons between testosterone triglyceride conjugate and testosterone undecanoate.
‡ $P < .01$ for comparisons between indicated concentration and lower dose of a given compound.

doses tested and did not differ significantly between groups.

Discussion

In this report, we have demonstrated that a TTC is rapidly absorbed from the rabbit intestine and results in markedly elevated concentrations of serum testosterone for several hours. These levels of serum testosterone exceed those achieved by the oral administration of an equivalent dose of TU. These increased serum concentrations of testosterone imply that the oral bioavailability of TTC is superior to that of TU, which is known to be approximately 6% (Täuber et al, 1986). In addition, the AUC after oral administration of TTC is greater than that of TU. Much of the increase is attributable to the high initial concentration achieved by TTC, since both compounds exhibited similar T½ in this rabbit model. This is likely due to the fact that once absorbed, both compounds are rapidly hydrolyzed to testosterone and then metabolized in a similar fashion.

Since TU is thought to gain access to the systemic circulation via absorption by the intestinal lymphatics (Coert et al, 1975; Nieschlag et al, 1975; Horst et al, 1976), our initial hypothesis was that enhanced lymphatic absorption of testosterone could be accomplished by conjugating testosterone to a triglyceride molecule. Although our study does not directly demonstrate lymphatic absorption of TTC, given its extremely lipophilic molecular structure, it seems likely that TTC is absorbed primarily via the lymphatics. It is interesting to note, however, that the time required to reach maximum concentration was longer with the oral TU than with TTC. The reason for this difference requires further study and may reflect differential rates of in vivo hydrolysis between the 2 compounds.

Could TTC be useful for testosterone therapy in men? The metabolism of testosterone in rabbits has been extensively studied (Mahoudeau et al, 1973; Bourget et al, 1984), and it has been shown that rabbits metabolize testosterone at a rate twice that of humans (Wang et al, 1967). Therefore, the pharmacokinetics of TTC in humans may be superior to those demonstrated in this report. It is possible that alterations in the chemistry of compounds like TTC may allow for twice or possibly even once-daily oral administration in humans. Oral TU is widely used in Europe and Canada for testosterone replacement and is usually given 2 to 3 times daily, but the resulting testosterone levels can be highly variable (Davidson et al, 1987; Conway et al, 1988). Importantly, unlike 17-alkylated forms of testosterone, oral TU is thought to be safe for long-term use in humans (Gooren, 1994).

Novel means of testosterone delivery are needed given the large number of patients who require testosterone therapy. In addition to the treatment of male hypogonadism, more convenient modes of testosterone administration may be useful in bringing the promise of male hormonal contraception to fruition (Amory and Bremner, 2000). The ease of administration of an oral form of testosterone makes it an attractive alternative for patients; however, the effects of long-term testosterone-triglyceride administrations are unknown. Long-term use of such a compound will require additional studies of its safety, efficacy, pharmacokinetics, and pharmacodynamics.

Acknowledgment

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