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ORIGINAL ARTICLE

Testosterone undecanoate in the functional compartments of the male reproductive tract

Undécanoate de testostérone dans l'appareil reproducteur de l'homme

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Abstract *Background*: Assessment of testosterone undecanoate's (TU) presence in the functional compartments of the male reproductive tract has never been performed despite the evidence that its documented beneficial effect in male infertility might be mediated through an epididymal action and this study was set to examine this possibility.
Materials and methods: In 18 normozoospermic volunteers TU has been administered (40 mg t.i.d.) for 6 days with serum measurements of TU, total testosterone (T), DHT, E2, SHBG, FSH, LH, and PRL before and at the end of medication. Steroid hormones (T, E2, and TU) were also assayed in seminal plasma. In a selected group of 7 men with previously diagnosed non-obstructive azoospermia TU, T, and E2 were assayed in the extracts of testicular biopsy material taken before ICSI and at the end of the same medication.
Results: A marked rise of serum DHT (average 148%, $P < 0.001$) has been found after treatment, whereas T, E2, FSH, LH, SHBG, and PRL did not significantly change. Measurable amounts of TU were found in the serum of all men but only in 6 cases in seminal plasma (11.1 ± 8.0 ng/mL) and all of them in semen delivered 7-8 h after the last TU capsule was taken. In dilution fluid from testicular tissue extracts, no detectable amounts of TU were found whereas mean values of 92.5 ± 54.3 pg/mL and 43.8 ± 16.3 ng/mL for E2 and T were observed. Positive correlations among TU and E2, T or DHT concentrations were found in serum samples ($P < 0.01$, 0.02, and 0.002) as well as between E2 and T ($P < 0.01$), E2 and DHT ($P < 0.001$), or T and DHT ($P < 0.001$).
Conclusion: It is concluded that TU was identified and measured for the first time in seminal plasma of a fair percentage (33%) of men on this medication and was associated in all men with a marked rise of DHT concentration, a known epididymal function promoter, in the absence of an effect on pituitary and gonadal activity. On this evidence, it appears that a beneficial effect of TU on epididymal function may be a distinct possibility.

Keywords Testosterone undecanoate · Dihydrotestosterone · Seminal plasma · Peripheral blood

Résumé L'évaluation de la présence d'undécanoate de testostérone (TU) dans les compartiments fonctionnels de l'appareil reproducteur masculin n'a jamais été réalisée malgré le fait que son effet bénéfique documenté dans l'infertilité masculine pourrait être médié par une action épididymaire; cette étude avait comme but d'examiner cette possibilité.
Chez 18 volontaires normozoospermiques, du TU a été administré (40 mg 3x/j) pendant 6 jours avec des dosages sériques de TU, testostérone totale (T), DHT, E_2, SHBG, FSH, LH et PRL avant et à la fin du traitement. Les hormones stéroïdes (T, E_2, TU) ont également été mesurées dans le plasma séminal. Dans un groupe de 7 hommes ayant une azoospermie non obstructive préalablement diagnostiquée, les concentrations de TU, T et E2 ont été mesurées dans des fragments de tissu de la biopsie testiculaire effectuée avant ICSI et à la fin du même traitement.
Une augmentation marquée des taux sériques de DHT (moyenne de 148 %, $P < 0{,}001$) a été retrouvée après le traitement alors que T, E_2, FSH, LH, PRL SHBG n'ont pas sensiblement changé. Des quantités mesurables de TU ont été retrouvées dans le sérum de tous les hommes, mais pour

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six cas seulement dans le plasma séminal (11.1 ± 8.0 ng/mL) et pour l'ensemble d'entre eux, dans le sperme recueilli 7-8 heures après la prise de la dernière gélule de TU. Dans le liquide de dilution des fragments de tissu testiculaire, ont été retrouvées des quantités non détectables de TU, tandis que les valeurs moyennes pour les concentrations de E_2 et T ont été évaluées à 92.5 ± 54.3 pg/mL et 43.8 ± 16.3 ng/mL. Des corrélations positives entre les concentrations de TU et E_2, T ou DHT ont été retrouvées dans les échantillons de sérum ($P < 0.01$, 0.02 et 0.002), ainsi que entre E_2 et T ($P < 0.01$) ou E_2 et DHT ($P < 0.001$) ou T et DHT ($P < 0.001$).
Il est conclu que du TU a été identifié et mesuré pour la première fois dans le plasma séminal d'un pourcentage considérable (33 %) des hommes prenant ce traitement et sa présence a été associée pour tous les hommes à une hausse marquée de la concentration de DHT, un promoteur de la fonction épididymaire, en l'absence d'un effet sur l'activité de l'hypophyse et des gonades. Sur cette base, apparaît légitime de retenir un possible effet bénéfique du TU sur la fonction épididymaire.

Mots clés Undécanoate de testostérone · Dihydrotestostérone · Plasma séminal · Sang périphérique

Introduction

The effects of testosterone undecanoate (TU) on pituitary and testicular basal or stimulated hormone secretion have been studied in detail and no compromising actions of its administration have been demonstrated at a dose of 120 mg/day [1-3]. Moreover, TU administration as an auxiliary of tamoxifen citrate therapy in patients with idiopathic oligozoospermia (I.O.) has resulted in a significant improvement of sperm parameters and a marked increase of the functional sperm fraction. Additionally, this combination significantly increased the number of pregnancies in subfertile couples with male factor problem related to I.O. [4].

The beneficial effect of TU on sperm parameters has been attributed to the action of this agent mainly on accessory glands since direct effects of TU either on Leydig cells or on seminiferous tubules have not been convincingly demonstrated. In this regard, it would be of interest to seek evidence for TU's presence in the different compartments and biological fluids of the male reproductive tract.

This piece of work was designed to investigate the possibility of TU's presence in the various compartments of the male genital apparatus and particularly those responsible for spermatogenesis and sperm maturation these including seminal fluid and testicular tissue. Moreover, the effects of TU on the main biological promoter of accessory glands, dihydrotestosterone (DHT), were also evaluated.

Clinical material and methods

Patients

A total of 18 healthy men, aged 22-58 years, volunteered for this study. The protocol included the following steps (Table 1): (a) initial evaluation with sperm analysis and hormone assessment, (b) administration of TU (40 mg in castor oil capsules taken immediately before meals, t.i.d.) for 6 days, and (c) final evaluation on day 7 of treatment in a manner similar to that of the initial assessment. Time of sample collection was 08.00-10.00 with sperm delivery following blood sampling by an average of less than 1 h and 7-8 h after the last TU capsule was taken. In a small number of cases (4) ejaculation on final evaluation occurred 3-4 h after the last TU capsule, whereas in the remaining volunteers the seminal samples were collected some 7-8 h after the last dose of medication was taken.

In an unrelated group of 7 cases with previously established non-obstructive azoospermia who were similarly treated with TU, testicular sperm extraction procedures took place in the context of an ICSI procedure 7-8 h after the test TU capsule (08.00-10.00) and tissue samples were kept frozen until evaluation.

Endocrine evaluation included measurements of FSH, LH, PRL, total testosterone (T), estradiol (E_2), DHT, SHBG and TU in blood samples, T, E_2 and TU in seminal plasma specimens and the same steroids in post-TU testicular tissue (all morning samples). In the latter cases, tissue samples after homogenization and extraction were kept frozen at −20°C

Table 1 Protocol of the study

I. Initial evaluation
  hormone assays
    peripheral blood: FSH, LH, PRL, T, DHT, E_2, SHBG
    seminal fluid: T, TU, E_2
  sperm collection
↓
II. TU 40 mg t.i.d. x 6 days
↓
III. Final evaluation (7^{th} day)
  hormone assays
    peripheral blood: FSH, LH, PRL, T, DHT, TU, E_2, SHBG
    seminal fluid: T, TU, E_2
  sperm collection*

* sperm collection after the last dose: 3-4 hours (n:4) or 7-8 hours (n:14)

and after defrosting they were weighted and freezed in N_2. Extraction with 5 mL of diethylether for 2 min and centrifugation for 10 min at 1200 × *g* were the next steps and the ether extracts were dried under air. Lastly, 0.5 mL of phosphate buffer was added to the dried extract of each sample before the RIA assays. Due to a laboratory mishappening, seminal plasma specimens for DHT assessment were destroyed.

The protocol of the study has been formulated in accordance to Helsinki's declaration of 1995 as modified in 2000 and was pre-registered according to the appropriate guidelines [5]. Moreover, the protocol proposed has been approved by both Hospitals Ethics and Research Committee.

All volunteers have been properly informed for the purpose of the study and the procedure involved and gave their written and informed consent.

Methods

Sperm analysis was carried out before and at the end of TU treatment following the WHO criteria [6].

Assays of FSH, LH, PRL, E_2, and SHBG were made using AutoDelphia kits B017-201, B031-101, B018-301, B056-101, and B070-101, respectively (PerkinElmer, Inc., Wallac Dy, Turku-Finland). The standards for FSH–LH have been calibrated against the 2nd IRP of pituitary FSH/LH for human bioassay (78/549) and for PRL against WHO 3rd International Standard for Prolactin (coded 84/500). Assays for T and DHT were made by resident assays employing capillary gas chromatography with mass spectrometry using positive ion chemical ionization and applying ammonia as reaction gas in multiple ion detection (MID) mode. In this MID mode, the trimethylsilane (TMS) derivative of T was measured at *m/z* 361.2, the TMS derivative of DHT was measured at *m/z* 363.2, the TMS derivative of deuterated T was measured at *m/z* 364.2, and the TMS derivative of DHT was measured at *m/z* 366.2.

GC–MS analysis was done on a HP 5890 series II Gas Chromatograph (Agilent Technologies, Netherlands B.V.) equipped with a CTC Autosampler (CTC Analytics), a fused-silica capillary column [(DB-17, length = 20 m, ID = 0.18 mm, film-thickness = 0.30 μm (J&W Scientific)], and connected to a Finnigan MAT TSQ 7000 mass spectrometer. Inter-assay and intra-assay variation coefficients for T were 6.4% and 7.2%, respectively, the corresponding figures for DHT being 7.2% and 3.7%.

TU and its internal standard ($[^2H_3]$-TU 538) were isolated from human serum and seminal plasma by solid phase extraction. The extracts were quantified by liquid chromatography coupled to mass spectrometry (LC–MS) using electrospray ionization in multi-reaction monitoring (MRM) mode. In the MS/MS mode the protonated $[M+H]^+$ molecules of TU and $[^2H_3]$-TU are used as precursor ion and measured as product ion at *m/m* 270.0 and *m/z* 273 [small deviations in the masses (*m/z* ± 0.2) may occur due to tuning differences].

LC–MS analysis was done on a HP 1100 (Applied Biosystems) equipped with a CTC Autosampler (CTC Analytics), an analytical column (Supelcosil LC-8-DB, length = 50 mm, ID = 4.6 mm, particle size = 5 μm), and connected to an Applied Biosystems API 3000 mass spectrometer. TU in serum: Inter-assay precision was <10.0% (taken from assay validation) and intra-assay precision was <11.9% (taken from ba study).

Inter- and intra-assay coefficients of variation for FSH were 3.3% and 1.7%, for LH 5.7% and 2.6%, for PRL 6.3% and 4.2%, for E_2 8.1% and 5.4%, and for SHBG 8.8% and 2.8%, respectively.

Statistics

Analysis of the data was carried out using Mediscale and Excel statistical programs. Sperm data were expressed as median (25th, 75th percentiles), whereas hormone values as mean ± SD.

Results

Sperm analysis results were all within the normal range for the group of normozoospermic volunteers (median – 25th, 75th percentile: 116.1 total cells × 10^6/mL – 56.2, 213.4) and showed no marked variation as a result of TU treatment (140.2 total cells × 10^6 mL – 39.1, 221.7).

Regarding the hormonal measurements the results were as follows.

Peripheral blood

TU was found in measurable amounts in peripheral blood while on medication in concentrations relatively higher than those of T (Table 2). On the other hand, DHT showed a remarkable rise following TU treatment (average 148%, $P < 0.001$), whereas T and E_2 showed non-significant changes as a result of this medication. With regard to the pituitary hormones, FSH, LH, and PRL showed no significant variations as a result of this treatment (Table 2). Moreover, SHBG remained also unchanged, signifying the lack of a marked effect of TU on its regulation as expected since E_2 levels did not change either.

Evaluation of the relationships among the hormonal parameters studied showed positive correlations between TU and E_2, T or DHT ($P < 0.01$, 0.02, and 0.002) in peripheral blood while on treatment. Moreover, significant

Table 2 Serum concentrations before and while on TU

I. Steroid hormones				
	T (ng/mL)	DHT (ng/mL)	E_2 (pg/mL)	TU (ng/mL)
pre-TU n:18	4.9 ± 1.6[+]	0.46 ± 0.20	27.6 ± 8.0	not assayed
on-TU n:18	4.2 ± 1.7	1.14 ± 0.70*	26.9 ± 5.4	12.7 ± 1.1
II. Peptide hormones-SHBG				
	FSH (IU/L)	LH (IU/L)	PRL (ng/mL)	SHBG (nmol/L)
pre-TU n:18	6.0 ± 0.7	5.0 ± 2.0	5.1 ± 3.7	30.0 ± 13.8
on-TU n:18	5.1 ± 2.5	4.0 ± 2.0	4.1 ± 2.1	26.0 ± 1.7

[+] mean ± SD, * difference from pre-TU: P < 0.001

inter-relationships between serum E_2 and T ($P < 0.01$) or DHT ($P < 0.001$) and T and DHT ($P < 0.001$) were also noted.

Seminal plasma

The concentration of T and E_2 in seminal plasma showed little variation in the subgroups studied (Table 3). On the other hand, TU was variable being below the sensitivity of the method in most samples but measurable in 6 cases, coming from the subgroup of sperm delivery 7-8 h after taking the last TU capsule. Of interest was the finding that higher serum TU values were noted in men with measurable amounts of this agent in seminal plasma than in those with undetectable levels (13.8 ± 2.1 ng/mL n = 6 vs 11.6 ± 1.4 n = 12, $P < 0.05$). On the other hand, serum TU values in men with short or long time of ejaculation from the last TU capsule (3-4 h vs 7-8 h) were not different (13.2 ± 1.7 vs 12.1 ± 1.3 ng/mL).

Table 3 Seminal plasma T, TU and E_2, in the subgroups studied

	T (ng/mL)	TU (ng/mL)	E_2 (pg/mL)
pre-TU			
a.	0.54 ± 0.26[×]	not assayed	68.2 ± 23.2
b.	0.62 ± 0.29	not assayed	75.1 ± 28.3
c.	0.63 ± 0.34	not assayed	72.2 ± 30.4
on-TU			
a.	0.64 ± 0.25	<0.1*	67.0 ± 33.3
b.	0.68 ± 0.33	<0.1**	72.1 ± 19.0
c.	0.73 ± 0.32	11.1 ± 8.0[+]	70.7 ± 21.0

[×] mean ± SD.

* undetectable, 3-4 hours after last TU capsule (n:4).

** undetectable, 7-8 hours after last TU capsule (n:8).

[+] detectable, 7-8 hours after last TU capsule (n:6).

The ratio of blood to seminal plasma concentrations was not different in the 3 subgroups after TU treatment (for T, a: 6.2 ± 1.3, b: 6.7 ± 2.3, and c: 5.8 ± 2.7; for E_2, a: 0.39 ± 13, b: 0.36 ± 21, and c: 0.37 ± 19). In the case of TU, the concentration ratio between the two biological fluids was 1.14 ± 0.36 in those men with detectable levels in seminal plasma (subgroup c), this being indicative of the relatively higher presence of TU in the latter fluid. No marked changes as a result of TU administration were noted for T and E_2 levels.

Testicular tissue

Determination of E_2, T, and TU in dilution fluid from testicular tissue extracts showed mean values of 92.5 ± 54.3 pg/mL for E_2, 43.8 ± 16.3 ng/mL for T but no detectable TU levels (<0.01 ng/mL).

Discussion

The present study has elucidated to a certain degree the ways by which TU exerts its effect on the functional ability of some important compartments of the male reproductive tract and, indirectly, on sperm quality in man. To start with, TU was detected in a number of seminal plasma samples in concentrations comparable to those of peripheral blood. This finding was indicative of the ability of this lipophilic androgen to find its way through circulation into the biological fluids contained in the ejaculation. Obviously, a uniform pattern of TU's presence in the seminal plasma could not be demonstrated but only in part of the cases and this, theoretically, might have been related to factors like poor absorption of the compound, unknown clearance conditions in the reproductive tract, local tissue pathology or faulty compliance. Certainly, the higher serum TU values seen in men with measurable than those with undetectable in seminal plasma TU levels might have been a contributing, although not decisive, factor. On the other hand, the fact that TU was not detected in seminal samples ejaculated shortly after taking the medication (3-4 h) may be indicative of the time needed for this agent to find its way into the relevant biological fluid. Other parameters determining the specific pharmacokinetics of TU may have been also involved in bringing about the presence or absence of this agent from seminal plasma. In the same context, it was of interest that TU was not detected in testicular tissue extracts indicating that no appreciable part of this androgen was present, mostly, in seminiferous tubules. The precise reason for this failure cannot be postulated and factors like methodology might have been involved. However, as an implication of the negative finding, it follows that the seminal plasma TU

measured in the relevant subgroup was probably not of testicular origin.

Of importance was also the observation that the peripheral blood to seminal plasma concentration ratio in those cases with simultaneous presence of TU in both fluids was close to one (1.14 ± 0.36). Indeed, it appears that the average TU concentration in the positive seminal fluid samples was approximately 87.4% of that of peripheral blood, this being indicative of a very active transfer mechanism of this agent from circulation to this fluid product of the genital system. In such conditions one may speculate for TU's entrance also in other compartments of the tract including epidydimis. This part of the tract is particularly active in promoting sperm maturation [7] and its functional activity is mostly DHT-dependent [8,9]. Unfortunately, the failure to demonstrate the presence of TU in all seminal samples obscures the picture.

An important finding of this study was the rise of blood DHT following TU treatment. This was a very marked change (147.8% rise) and, probably, biologically significant in affecting accessory gland function and thus improving sperm quality. Indeed, although DHT's effects on human spermatogenesis are not clearly established [10-13], on the other hand it is well known that these androgen exerts important biological actions on accessory gland function and particularly epididymis [14] and, therefore, a beneficial effect on sperm parameters can be anticipated in oligozoospermic men on treatment with the combination of TU and tamoxifen citrate (TMX). Indeed, marked improvements of acrosine, aniline, and free l-carnitine levels were noted in a properly designed trial [3]. In this context, it was unfortunate that DHT measurements in seminal plasma were not available. However, evidence from other studies indicates that this steroid is present in measurable amounts in this fluid [15-18]. Moreover, earlier studies had demonstrated an increase of peripheral plasma DHT following TU administration in dyspermic men [19]. In this context, our data lend support to those findings and substantiate the hypothesis for a biological effect of this hormone at epididymal level.

Finally, regarding the lack of an effect on pituitary activity and particularly on LH, this was to be expected since earlier studies have shown that short-term TU administration at the dosage employed did not compromise basal gonadotropin secretion [2], whereas the absence of an effect on SHBG was probably related to the short duration of treatment as well as the lack of any T and E_2 changes.

By and large, it appears that TU administration in addition to its presence in seminal fluid amplifies the bioavailability of DHT in peripheral blood and hence in accessory glands. These observations are of great relevance for men treated with TU and TMX for I.O., a sperm abnormality very often associated with a compromised androgen bioactivity [15, 20]. Moreover, these findings advance the understanding of complimentary actions of TMX and TU in the empiric management of I.O. with this combination. It appears that TU's contribution is probably exerted through its augmenting effect on androgen bioactivity at epidydimal and accessory glands level thus improving the local environment and sperm maturation.

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References

1. Gooren LJ (1987) Androgen levels and sex functions in testosterone-treated hypogonadal men. Arch Sex Behav 16:463–73
2. Adamopoulos DA, Nicopoulou S, Kapolla N, et al (1995) Endocrine effects of testosterone undecanoate as a supplementary treatment to menopausal gonadotropins or tamoxifen citrate in idiopathic oligozoospermia. Fertil Steril 64:818–24
3. Adamopoulos DA, Nicopoulou S, Kapolla N, et al (1997) The combination of testosterone undecanoate with tamoxifen citrate enhances the effects of each agent given independently on seminal parameters in men with idiopathic oligozoospermia. Fertil Steril 67:756–62
4. Adamopoulos DA, Pappa A, Billa E, et al (2003) Effectiveness of combined tamoxifen citrate and testosterone undecanoate treatment in men with idiopathic oligozoospermia. Fertil Steril 80:914-20
5. A fifth amendment of the Declaration of Helsinki (2000) Lancet 356(9236):1123–7
6. World Health Organization WHO laboratory manual for the examination of human semen and semen-cervical mucus interactions. 4th ed. 1999.
7. Cooper TG (2007) Sperm maturation in the epididymis: a new look at an old problem. Asian J Androl 9:533–9
8. Bilinska B, Wiszniewska B, Kosiniak-Kamysz K, et al (2006) Hormonal status of male reproductive system: androgens and estrogens in the testis and epididymis. In vivo and in vitro approaches. Reprod Biol 1:43–58
9. Robaire B, Seenundun S, Hamzeh M, Lamour SA (2007) Androgenic regulation of novel genes in the epididymis. Asian J Androl 9:545–53
10. Verhoeven G (1999) Spermatogenesis and spermatogenic control: a state of the art. Verh K Acad Geneeskd Belg 61:417–32
11. Jarow JP, Zirkin BR (2005) The androgen microenvironment of the human testis and hormonal control of spermatogenesis. Ann NY Acad Sci 1061:208–20
12. Robaire B, Henderson NA (2006) Actions of 5 alpha-reductase inhibitors on the epididymis. Mol Cell Endocrinol 250:190–5
13. Komori S, Kasumi H, Sakata K, Koyama K (2007) The role of androgens in spermatogenesis. Soc Reprod Fertil 63:25–30
14. Elzanaty S, Giwercman YL, Giwercman A (2006) Significant impact of 5alpha-reductase type 2 polymorphisms on sperm concentration and motility. Int J Androl 29:414–20
15. Zalata A, Hafez T, Verdonck L, et al (1995) Androgens in seminal plasma: markers of the surface epithelium of the male reproductive tract. Int J Androl 18:271–7
16. Anderson RA, Kelly RW, Wu FC (1997) Comparison between testosterone enanthate-induced azoospermia and oligozoospermia in

a male contraceptive study. V. Localization of higher 5 alpha-reductase activity to the reproductive tract in oligozoospermic men administered supraphysiological doses of testosterone. J Androl 18:366–71
17. Amory JK, Wang C, Swerdloff RS, et al (2007) The effect of 5alpha-reductase inhibition with dutasteride and finasteride on semen parameters and serum hormones in healthy men. J Clin Endocrinol Metab 92:1659–65
18. Horst HJ, Erdmann T (1980) Recovery of free androgens in the rat prostate in vivo and in vitro after treatment with orally active testosterone undecanoate (TU). Horm Metab Res 12:541–5
19. Comhaire F (1990) Treatment of idiopathic testicular failure with high-dose testosterone undecanoate: a double-blind pilot study. Fertil Steril 54:689–93
20. Facchinetti F, Comitini G, Genazzani A, et al (1987) Seminal fluid androgen levels in infertile patients. Int J Fertil 32:157–61