Testosterone:Estradiol Ratio Changes Associated with Long-Term Tadalafil Administration: A Pilot Study

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ABSTRACT

Introduction. It has been reported that lack of sexual activity due to erectile dysfunction (ED) may be associated with testosterone (T) decline.

Aim. To investigate whether the known changes in sex hormones associated with resumption of sexual activity are sustained in the long term.

Main Outcome Measures. Primary endpoints were variations from baseline of steroid hormones: total T, free T (fT), and estradiol (E). Secondary endpoints were variations of erectile function domain scores at International Index of Erectile Function-5 (IIEF-5).

Methods. In an open-label fashion, 20 patients (mean age 54.8 ± 8.4 years) received tadalafil 10–20 mg on demand for 12 months. Exclusion criteria were those reported for phosphodiesterase inhibitors, including hypogonadism and hyperprolactinemia.

Results. Tadalafil assumption was safe and well tolerated (overall adverse effects in 15% of patients) and none discontinued medication. A significant decrease in E levels occurred at the end of the study (from 19.9 ± 9.6 to 16.6 ± 8.1 ng/dL, $P = 0.042$ vs. baseline), with parallel increase in the T:E ratio (26.3 ± 15.3 to 32.6 ± 17.7, $P = 0.05$), whereas no changes in T and fT serum levels were observed, respectively (411.4 ± 131.4 to 434.2 ± 177.1 ng/dL and 47.7 ± 15.3 to 49.9 ± 19.1 pmol/L, not significant). Interestingly, nonparametric subgroup analysis for related samples revealed that E decrease was detectable only in lean (N = 14) but not in obese (N = 6, body mass index > 27.5 kg/m^2) subjects (17.8 ± 10.1 vs. 13.5 ± 6.8, $P < 0.05$). A net increase in IIEF-5 scores was observed at the endpoint (13.7 ± 5.9 vs. 25.7 ± 2.9, $P < 0.0001$).

Conclusions. Sustained improvement in sexual function after 12 months of tadalafil administration is associated with increased T:E ratio mainly related to reduction of E levels. We hypothesize that androgen–estrogen cross-talk and possible inhibition of aromatase activity during chronic exposure to tadalafil might have a role in the regulation of erectile function. **Greco EA, Pili M, Bruzziches R, Corona G, Spera G, and Aversa A. Testosterone:estradiol ratio changes associated with long-term tadalafil administration: A pilot study. J Sex Med 2006;3:716–722.**

Key Words. Phosphodiesterase Inhibitor; Steroid Hormones; Estradiol; Testosterone; Chronic Treatment; Aromatase

Introduction

Normal erectile function requires the involvement and coordination of multiple regulatory systems and is influenced by psychological, neurological, hormonal, metabolic, vascular, and cavernosal factors, and by aging and a large variety of drugs. An alteration in any of these factors contributes to the development of erectile dysfunction (ED), although in many cases a combination of several factors is involved.

Tadalafil, sildenafil citrate, and vardenafil HCl are type 5 phosphodiesterase (PDE5) inhibitors that are effective and well tolerated for treating

ED of varied functional severity and etiology [1–3]. Tadalafil is effective up to 36 hours after dosing [4].

There are previous observations that men suffering from ED, irrespective of its etiology, have androgen levels significantly lower than normal controls, although still in the normal range [5,6], that psychological, pharmacological, and mechanical therapies are able to restore testosterone (T) levels and luteinizing hormone (LH) bioactivity [5,7], and that T supplementation improves the PDE5i therapeutical effect in patients who are not responders to sildenafil before the androgen treatment [8,9].

This study has been undertaken to determine whether variations of plasma sex steroids may occur in patients with ED after 12 months of successful exposure to tadalafil administered on demand.

Methods

Subjects and Study Design

This was a flexible, open-label, prospective study on steroid hormone fluctuations over a treatment period of 12 months with tadalafil administered on demand in patients with ED of any etiology. The 20 patients enrolled in this study met all of the following inclusion criteria: (i) age between 18 and 70 years; (ii) 3-month or longer history of ED; (iii) stable sexual relationship with a female partner for 6 months or greater; and (iv) prior response to tadalafil. Patients agreed not to use any other ED treatment and/or androgen therapy during the run-in period (before receiving the initial dose of study medication) and during the treatment phase of the study. All subjects were asked to use tadalafil on demand over the treatment period of study, had sexual activity at least four times per month, and reported it in a personalized diary (Sexual Encounter Profile, SEP), which was returned to the physician at each visit.

Exclusion criteria for this study were: (i) history of clinical hypogonadism or other endocrine disorders; (ii) history of angina under treatment with nitrates or instable angina, history of myocardial infarction within 6 months prior to the screening visit, any supraventricular arrhythmia, any evidence of congestive hearth failure, noncontrolled hypertension (blood pressure >170 mm Hg systolic and >100 mm Hg diastolic); (iii) cancer chemotherapy; (iv) antiandrogen therapy or any current treatment with drugs that may confound the interpretations of study results; (v) clinically significant or uncontrolled medical or psychiatric conditions; (vi) radical prostatectomy; and (vii) past or current history of drug or alcohol abuse. The protocol and the informed consent were approved by the local ethical committee.

On-demand dosing refers to intermittent tadalafil administration prior to expected sexual activity with a maximum frequency of one dose per day. Initial dose was 10 mg with the possibility of titrating the drug up to 20 mg or decrease to 5 mg. Thirty doses were dispensed at each scheduled visit to patients during the treatment period of the study; further distributions of study drug were performed in extra visits in case of patients' request after visit 3, visit 4, and visit 5.

Overall, six visits were performed for each patient: a screening visit (visit 1) to evaluate patients for inclusion criteria; a baseline visit after 1 month of run-in period (visit 2); a control visit every 3 months of treatment (visit 3, visit 4, visit 5); and a last visit at the end of the study (visit 6).

At visit 1, informed consent was obtained; patients were evaluated for inclusion in the study and finally entered a 28-day run-in period in which no ED and/or androgen therapy was allowed. After this period, patients entered the study (visit 2) and received treatment with 10 mg of tadalafil (30 tablets) with the possibility of titrating up to 20 mg or down to 5 mg at the following visit depending on efficacy and adverse events, respectively. At visit 3, visit 4, visit 5, and visit 6 patients were evaluated for safety parameters and for titration of the study medication and were dispensed new doses of the drug. During each visit International Index of Erectile Function-5 (IIEF-5) questionnaire was administered and SEP diary cards were distributed to each subject, clinical evaluation and posttreatment investigations were taken, returned tablets were collected, and correspondence of the diaries with the attempts at sexual intercourse was assessed.

Clinical laboratory measurements including serum chemistry, hematology, urinalysis, and hormones levels (total T, free T [fT], estradiol [E], sex hormone-binding globulin [SHBG], and LH) were performed at visit 2 (baseline), visit 3 (3 months), and visit 6 (12 months), along with an electrocardiogram evaluation just at baseline visit, and were compared with 10 age-matched healthy controls. Safety evaluation was performed by recording clinical adverse events at each visit.

Although in our study each patient served as his own control, to evaluate the hormone profile at baseline, hormonal values were compared with

Table 1 Characteristics of ED patients enrolled in the study

Patients	N = 20	Comorbidities	
Mean age (years)	54.8 ± 8.4	Hypertension	6
BMI (kg/m^2)		Diabetes mellitus	6
<27.5	14	Depression	3
>27.5	6	Dyslipidemia	6
ED etiology		No. of tablets/month after study entry	10.3 ± 1.7
Organic	10	Preferred dose	
Psychogenic	4	20 mg	11
Mixed	6	10 mg	9
Duration of ED history (years)	2.6	Sex intercourse/month after study entry	8.1 ± 1.1

those of an age-matched control group of healthy, sexually active volunteers, with their informed consent.

Diagnostic Evaluation

In addition to sex hormones, serum basal levels of prolactin and thyroid-stimulating hormone were measured to exclude hyperprolactinemia and thyroid disorders.

All hormone assays were performed at the Research Laboratories of the Department of Medical Pathophysiology of the University "La Sapienza" of Rome. Blood samples were collected in the morning after an overnight fast; the period of storage at –80°C varied from 0 to 12 months. All samples obtained from the same subject were measured in the same assay. All determinations were performed in duplicate. Serum concentrations of total T were measured by radioimmunoassay (RIA) using a commercial kit (Diagnostic System Laboratories, Webster, TX, USA). The intra- and interassay coefficients of the total T assay were 7.5% and 11% at the normal adult male range, which in our laboratory was 300–1000 ng/dL. Serum fT was measured by RIA of the dialysate after an overnight equilibrium dialysis using the same RIA reagents as in the total T assay. The coefficient of variation for fT recovery for increasing doses of total T in the adult male ranged from 10% to 18%. The intra- and interassay precisions of fT were 14% and 17%, respectively, at the normal adult male range, which in our laboratory was 200–700 pmol/L. Estradiol was measured with chemiluminescence (provided by Architect Systems, Abbotts Diagnostics, Germany), with a normal adult male range of 0–30 ng/dL; SHBG levels were measured by immunoradiometric assay (Radim SpA, Pomezia, Italy) with an intra- and interassay coefficient of variation below 6% at the normal adult range, which in our laboratory was 9–55 nmol/L; LH, prolactin, and thyroid-stimulating hormone were measured by direct chemiluminescence (ADVIA Centaur, Bayer Co, Germany) with an intra- and interassay coefficient of variation between 6% and 3% at the normal adult range, which in our laboratory is 1.5–9 IU/L, 2–17 ng/mL, and 0.35–5.5 mIU/L, respectively.

Statistical Analysis

Results have been expressed as means ± standard deviation. Results were considered statistically significant if the two-tailed P value was <0.05. A subgroup analysis was performed in the treatment group according to different body mass indexes (BMI) by using Wilcoxon signed ranks test.

Results

The demographic characteristics of the study groups at baseline (N = 20) are shown in Table 1. The mean age of patients was 54.8 years (range: 35–65), while the mean duration of ED history was 2.6 years. The ED etiology was clinically determined by investigators and reported as organic in 50%, mixed in 30%, and psychogenic in 20% of patients. The comorbidities reported by patients reflect those commonly seen in ED patients in clinical practice; in this study the most common comorbidities were hypertension (N = 6), diabetes mellitus (N = 6), depression (N = 3), and dyslipidemia (N = 6).

Tadalafil assumption was safe and well tolerated and none discontinued medication due to adverse events (Table 2). However, side-effects were

Table 2 Adverse events reported by patients with ED (N = 20) during 1-year tadalafil assumption

	1 Month		12 Months	
	N	%	N	%
Dyspepsia	3	15	1	5
Headache	2	10	0	—
Back pain	1	5	0	—
Flushing	1	5	0	—
Total	7	35	1	5

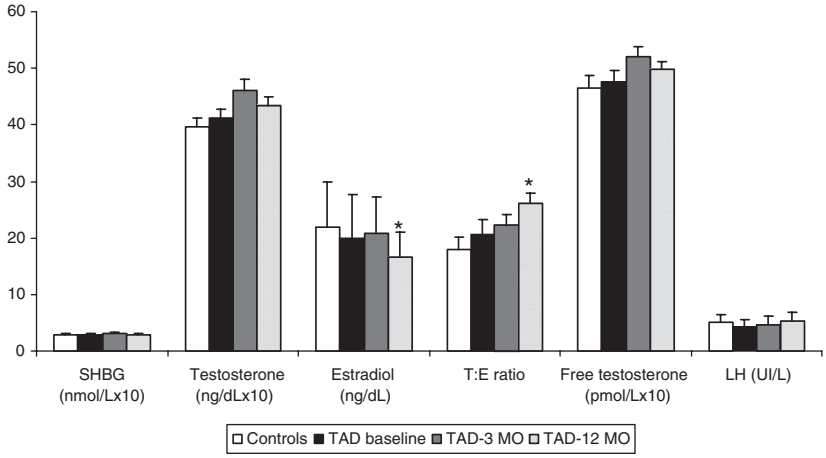

Figure 1 Hormonal variations after long-term tadalafil (TAD) treatment in men (N = 20) with ED. Values reported have been converted by using standard International Conversion Factors. $P = 0.05$ vs. baseline. MO = months.

greater after the first month of tadalafil assumption (overall adverse events in 30% of patients, mainly dyspepsia, back pain, and headache), were mild in intensity, and tended to resolve over time (10% after 12 months). The preferred dose was 20 mg (N = 11), while the remaining nine patients stayed with the 10 mg dose. Each patient assumed a mean of 10.3 ± 1.7 tablets per month throughout the entire period of study, which resulted in 8.1 ± 1.1 vs. 1.2 ± 0.7 sexual satisfactory attempts for intercourse when compared with baseline ($P < 0.0001$, data not shown).

The mean hormonal levels as well as T:E ratio of the study group were comparable to controls at baseline.

Figure 1 shows pre- and posttreatment hormonal variations and comparison with controls. A significant decrease in E levels in the treated patients at the end of the study (from 19.9 ± 9.6 to 16.6 ± 8.1 ng/dL, $P = 0.042$ vs. baseline), with parallel increase in the T:E ratio, was found (26.3 ± 15.3 to 32.6 ± 17.7, $P = 0.05$; Figure 2), whereas no changes in T and fT serum levels, LH, SHBG, and prolactin (data not shown) levels were observed, respectively.

Interestingly, nonparametric subgroup analysis for related samples revealed that E decrease and T:E ratio increase were detectable only in lean (18.8 ± 9.8 vs. 13.7 ± 6.7, $P = 0.019$; and 38.5 ± 17.8 vs. 29.2 ± 15.9, $P = 0.05$, respectively, N = 14) but not in obese (N = 6, BMI > 27.5 kg/m^2) treated subjects, without significant changes from baseline in the BMI. Furthermore, E decrease was maintained even after exclusion of patients with pathological E levels at baseline (15.8 ± 6.4 vs. 13.0 ± 6.4, $P = 0.039$; N = 12). After the overall treatment period of 12 months, a net increase in the IIEF-5 scores was observed (13.7 ± 5.9 vs. 25.7 ± 2.9, $P < 0.0001$).

Discussion

In this study we show for the first time a reduction in E levels in patients with ED after chronic expo-

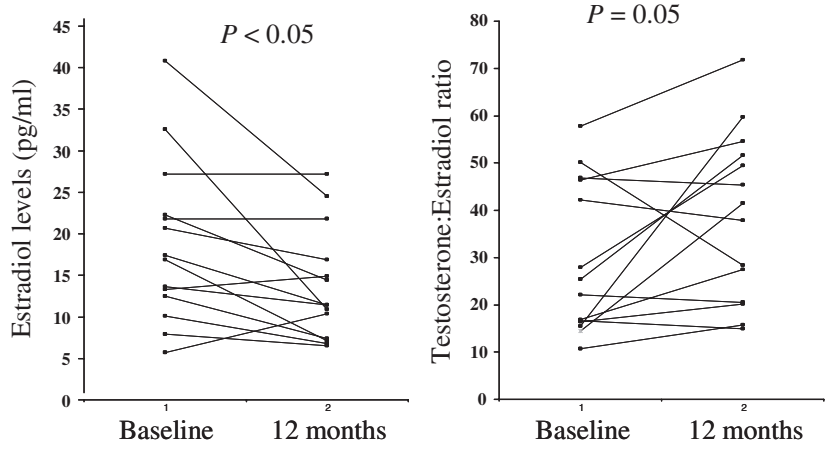

Figure 2 Estradiol and testosterone:estradiol variations after long-term tadalafil treatment in lean men (N = 14) with ED.

sure to tadalafil and a concomitant increase in the T:E ratio. This hormonal pattern was found mostly in lean subjects, independent of the etiology of ED, comorbodities, and concomitant drug treatments. We also show that increased T:E ratio was not due to increase in T serum levels, as previously reported in hypogonadal men after resumption of satisfactory sexual activity [6], but rather to possible effects of tadalafil on aromatase activity.

A key role of androgens in sexual function has been assumed. Testosterone is the main androgen involved in the regulation of the male sexual function. The deficiency of androgens may represent one of the major causes of ED. On the other hand, the increase of E plasma levels might be important because it also plays a role in determining sexual dysfunction and symptoms of aging [10]. Testicular E synthesis is very low in men. It is predominantly the product of peripheral aromatization of testicular and adrenal androgens. Aromatase activity is found in gonadic tissue and brain, but in humans this enzyme activity can also be detected in placental, adipose tissue, and fetal liver [11–13]. Testicular and adrenal steroidogenesis declines with aging; the total plasma levels of E do not decrease owing to increased SHBG and aromatization in fat tissue [14,15]. It is traditionally called a female hormone; E has significant effects in males. Favorable effects on bone and brain have been identified [16], whereas an interference with sexual function and behavior has been described [17]. Recent studies show that increased E levels are associated with venous occlusive disorders in patients with organic ED [18], and that the aromatase inhibition in elderly men with mild hypogonadism normalizes serum T and fT levels and improves sexual function [19]. Furthermore, in men with hypogonadism, a low dose of clomiphene citrate is effective in improving T:E ratio, thus giving more long-term benefit for the management of ED in hypogonadal patients [20].

In our study we observed a significant decrease of plasma E levels after a treatment period of 12 months with tadalafil on demand at a flexible dosage (5, 10, and 20 mg), with a significant increase in T:E ratio, although no changes in T and fT serum levels were observed. In agreement with previous studies [6,7], the improvement of sexual function (significant increase in the IIEF-5 scores) in our small series of patients was associated with a tendency to increase of T and fT serum levels after 3 months of treatment with PDE5i whereas after 12 months of treatment this trend was not maintained and we observed no changes in T and fT levels compared with baseline, although IIEF-5 scores were maintained with excellent overall efficacy scores. Finally we observed no changes in SHBG and LH levels. It should be recognized that our results obtained on a small cohort of patients (20 patients with normal baseline T levels compared with other published cohorts of patients with baseline T levels in the lower range) cannot be compared at all with hundreds of published cases; but, it is reasonable that the reduction in E levels should be confirmed also in the long term and from larger studies.

Interestingly, nonparametric subgroup analysis for related samples revealed that E decrease and T:E ratio modification were detectable only in lean but not in obese (BMI > 27.5 kg/m^2) treated subjects (see Figure 2), with no differences in T:E ratios among the different subgroups at baseline, due to the small number of subjects examined, and without any variation in the BMI at endpoint. Several studies showed that the expression of aromatase is tissue-specific and may be different between visceral and subcutaneous fat deposits, being highly expressed in the former. The observation that after 12 months of treatment with tadalafil on demand at flexible dosage determines a significant decrease of E levels and a significant increase of T:E ratio might be more important than total serum T increase and may be due to a possible interference of tadalafil action with phosphodiesterase isozymes that may interfere with aromatase pathway. The evidence that E decrease was detectable only in lean but not in obese subjects may be due to the larger amount of aromatase in visceral and subcutaneous adipose tissue in the latter, with consequently increased aromatase activity that characterizes obese subjects, and may be accounted for by the number of overall pills consumed (1 every 3 days). Also, the hypothesis that 1 year of tadalafil treatment depresses aromatase activity in lean but not obese subjects leading to the altered T:E ratio and that the suppression of E may be responsible for the improvement in IIEF scores may be accounted for by the fact that in our series there were six obese diabetic subjects who reported partial responses to tadalafil treatment in terms of sexual satisfaction. Further studies are ongoing to confirm the possible relationship between chronic type 5 phosphodiesterase inhibition and aromatase activity in human adypocites in vitro because as it stands there has been no clear-cut demonstration on such interplay.

In conclusion, in this study we demonstrated that long-term administration of tadalafil is associated with increase in T:E ratio mainly due to significant reduction of E levels. Also, lack of tachyphylaxys in efficacy due to prolonged tadalafil use suggests that this is an effective and well-tolerated therapy in men of different age and broad ED etiologies and has a favorable cardiovascular safety profile [21]. Whether higher E levels are involved in the pathogenesis of ED in our series of patients has not been investigated. Any presumable effect of PDE5i on aromatase activity should be further confirmed with both in vitro and in vivo studies because it may open a new research avenue, for example, atherosclerosis, prostate disease, breast cancer, and osteoporosis. This is specially true in view of chronic use of these medications for treating organic ED refractory to on-demand therapy [22].

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Conflict of Interest: None.

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