Received Date : 29-May-2016
Revised Date : 08-Jun-2016
Accepted Date : 11-Jul-2016
Article type : Original Article

Article category: Sexual Medicine

Serum levels of enclomiphene and zuclomiphene in hypogonadal men on long-term clomiphene citrate treatment

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Title

Serum levels of enclomiphene and zuclomiphene in hypogonadal men on long-term clomiphene citrate treatment

Objectives

To determine the relative concentrations of enclomiphene (ENC) and zuclomiphene (ZUC) isomers in hypogonadal men (HM) on long-term clomiphene citrate (CC) therapy. To determine whether patient age, body mass index, or duration of therapy were predictive of relative concentrations of ENC and ZUC.

Patients and Methods

Men already on CC 25 mg daily therapy for secondary hypogonadism for a minimum of six weeks were recruited to have their ENC and ZUC levels assessed. Total testosterone (T), free testosterone, estradiol, follicle stimulating hormone (FSH), and luteinizing hormone (LH) prior to initiation of and while on CC therapy were recorded for all patients. Patient demographics including age, body mass index, and medical comorbidites were recorded. Serum samples were obtained at the time of enrollment to determine ENC and ZUC concentrations.

Results

A total of 15 men were enrolled from June 2015 to August 2015. Median patient age was 36 (range 22-70) years, median body mass index 32.0 (range 21.1-40.3)kg/m^2, and median duration of treatment 25.9 (range 1.7-86.6) months. Baseline median total T, estradiol, and LH were 205.0 ng/dL, 17.0 pg/mL, and 4.0 mIU/mL, respectively. Post-treatment median total T, estradiol, and LH increased to 488.0 ng/dL 34.0 pg/mL, and 6.1 mIU/mL, respectively (all $p<0.001$). Median ENC and ZUC concentrations were 2.2 and 44.0 ng/mL, respectively. After at least six weeks of CC therapy median ZUC: ENC serum concentration ratio was 21.3. On linear regression analysis patient age, body mass index, duration of treatment and serum T levels were not predictive of ENC or ZUC concentrations.

Conclusions

Long-term CC therapy resulted in a significant alteration of ENC and ZUC concentrations, with ZUC as the predominant isomer. Given the vastly different biochemical and toxicological properties of ENC and ZUC, this study supports the need for development of a pure selective estrogen receptor antagonist for the treatment of HM.

Key Words

Serum testosterone, testosterone restoration secondary hypogonadism, clomiphene citrate, enclomiphene, zucloclomiphene

Introduction

The incidence of T deficiency increases with each subsequent decade of life; 20% of men over the age of 60 are estimated to have low T [1]. While controversy surrounding the constellation of hypogonadal symptoms, there is evidence that treatment of T deficiency can improve lean muscle mass, bone mineral density, and insulin sensitivity [2-4].Despite increased public awareness, the majority of patients with hypogonadism go untreated. This is in part due to the routes of administration, and concerns with the long term safety of exogenous T replacement therapy. Additionally, hypogonadal infertile men present a unique challenge as chronic T administration impairs endogenous T and sperm production.

An alternative to T replacement includes the off-label use of selective estrogen receptor modulators, which function by binding to the estrogen receptor to increase T production by blocking negative feedback inhibition of estradiol on the hypothalamic pituitary axis. The selective estrogen receptor modulator, CC, is a mixture of the two diasterioisomers; ENC (62%) and ZUC (38%), which act as an antagonist or agonist on the estrogen receptor and its effected tissue[5]. The *trans* isomer, ENC, has a half-life of 10.5 hours and is a potent antagonist with anti-estrogenic properties that function to stimulate T and sperm production[6]. The *cis* isomer, ZUC, has a half life of 30 days and may function as an estrogen agonist at high concentrations[6]. This becomes clinically relevant in patients who are on long-term CC therapy, as the slower metabolism leads to higher concentrations of ZUC over time.

A recent study by Fontenot et al. examined the differential effects of ENC and ZUC in male mice. The authors concluded that high dose (40 mg/kg/day) ZUC administration resulted in testicular degeneration and arrested spermatogenesis[7]. Further, while low dose (4 mg/kg/day) ZUC improved serum T, FSH, and LH; at high doses (40 mg/kg/day) ZUC negatively affected these parameters. A double-blind placebo-controlled trial by Kim et al. comparing pure ENC to T gel found that while both raised total T, T gel failed to improve mean sperm concentration in men with secondary hypogonadism[8]. This highlights the potential role of the use of a pure ENC isomer in the treatment of hypogonadal men desiring to preserve fertility.

A study evaluating the relative concentrations of ENC and ZUC in HM on long-term CC therapy has never been performed. It is our hypothesis that prolonged CC therapy will result in a shift of ENC: ZUC ratio with ZUC as the predominant isomer.

Patients and Methods

Patient selection

We enrolled male patients who were already on CC 25 mg daily therapy for secondary hypogonadism to have their ENC and ZUC levels drawn. All patients were on generic CC therapy at the time of enrollment. Inclusion criteria included a pre-treatment baseline morning T level less than 300 ng/dL prior to initiation of treatment for secondary hypogonadism, body mass index less than 42, and duration of treatment with CC for a minimum of six weeks. Duration of treatment was defined as time from initiation of CC therapy to date that ENC and ZUC levels were drawn. Exclusion criteria included history of androgen deprivation therapy, history of venous thromboembolism, chronic opioid use, use of steroids within previous three months, polycythemia, elevated prostate specific antigen, or treatment with exogenous T or an aromatase inhibitor within the previous three months of having ENC and ZUC levels drawn.

The study was performed with approval of the Albany Medical Center Institutional Review Board, and written patient consent. Patient characteristics including age, body mass index, and medical comorbidites were recorded. Pre- and post-treatment labs including total T, free T, estradiol, FSH, and LH were obtained by retrospective chart review. Pre-treatment labs were defined as most recent labs obtained prior to initiation of treatment for hypogonadism. Post-treatment labs were defined as hormone levels drawn while on CC therapy and on or before the date that ENC and ZUC levels were drawn. ENC and ZUC concentrations were assessed at a single time point after patients had been on CC therapy for a minimum of six weeks using liquid chromatography-mass spectrometry with a Kinetex XB-C18 reverse phase column.

Statistical analysis

Minitab 17 software (Minitab Inc., State College, PA, USA) was used to perform all statistical calculations, with $p < 0.05$ considered statistically significant. A two-tailed t-test (paired) was used to compare pre-treatment and post-treatment values of total T, free T, estradiol, FSH, and LH in patients on CC therapy. Linear regression analysis was performed to assess for predictors of ZUC:ENC ratio. The regression model included patient age, body mass index, duration of therapy, and percent change in median pre- and post-treatment total T, free T, estradiol, FSH, and LH levels.

Results

A total of 15 men were enrolled at the Urological Institute of Northeastern New York between June 2015 and August 2015. All patients were on CC 25 mg daily. Median patient age was 36 years (range 22-70), median body mass index 32.0 kg/m^2(range 21.1-40.3), and median duration of treatment 25.9 months (range 1.7-86.6). Of the 15 patients enrolled, three patients had previously been on anastrozole, two of which were preceded by exogenous T replacement therapy. The most frequently reported concomitant medical conditions are summarized in Table 1; erectile dysfunction (60.0%), and hypertension (40.0%) were the most commonly reported conditions. One patient had a history of prostate cancer treated successfully with radical prostatectomy in 2004; he had an undetectable prostate specific antigen and no evidence of disease on follow-up at time of enrollment into the study.

Patients on CC experienced an increase in median total T from 205.0 ng/dL to 488.0 ng/dL pre- and post-treatment, respectively (p=0.0001). One patient demonstrated a paradoxical decrease in total T pre- and post-treatment. Patients saw an increase in median free T pre- and post-treatment, 6.3 ng/dL and 16.3 ng/dL, respectively (p=0.0180). Median estradiol increased from 17.0 pg/mL pre-treatment to 34.0 pg/mL post-treatment (p=0.0001). Similarly, both FSH and LH also increased in patients on CC therapy, median values are summarized in Table2.

Median ENC and ZUC concentrations were2.2 ng/mL and 44.0 ng/mL, respectively. Median ZUC:ENC ratio was 21.3; results are summarized in Table 3. On linear regression analysis neither patient age, body mass index, duration of CC therapy, or total T levels were predictive of either ENC or ZUC concentrations. Linear regression analysis results are summarized in Table 4.

Discussion

In the present study we quantified ENC and ZUC concentrations in patients on long-term CC therapy. After treatment with CC for greater than six weeks, the two isomers were present in vastly different concentrations that did not correlate to patient hormone levels, age, body mass index, or duration of CC therapy.

In this study we are the first to report ENC and ZUC concentration in men on long-term CC therapy. Much of the existing literature for relative isomer concentration is in women on CC for anovulation. Ghobadi et al. previously reported on ENC and ZUC concentration after a single oral dose of 50 mg CC in anovulatory women with polycystic ovarian disease[9]. Levels were drawn at CC at 1, 2, 4, and 6 hours after the dose, and found that mean maximum ENC and ZUC concentrations were 5.0 and 15.0 ng/mL, respectively. Another study by Ghobadi et al. of 39 anovulatory women treated for 4 days in a stepwise fashion with 50, 100, and 150 mg of CC if the patient did not respond to the previous dose. Blood samples were taken on days 2, 8, and 21 in each cycle of treatment between 1 and 24 hours after the dose of CC. At the end of three cycles mean ZUC:ENC ratio was approximately 125 and 75 for responders versus non-responders, respectively, although these values were not statistically different[10]. It is difficult to draw a comparison to our findings of median ENC concentration, median ZUC concentration, and ZUC:ENC ratio of 2.2 ng/mL, 44.0 ng/mL, and 21.3, respectively, given the different duration of therapy and doses administered. Both studies, however, support our finding that CC therapy alters ENC:ZUC ratio making ZUC the predominant isomer present. This finding is concerning giving the potential deleterious effects of ZUC on the male reproductive system through inhibition of the hypogonadal-pituitary-axis.

The ratio of ZUC:ENC ranged from 1.43 to 88.3 in our patient population. Review of ENC and ZUC values individually reveals that, with the exception of a few patients, there was little variation in ENC concentration with 93% of patients demonstrating an ENC concentration less than 15 ng/mL and 80% with an ENC concentration less than 4 ng/mL. In contrast, ZUC concentration demonstrated a high degree of interpatient variability. ZUC concentration ranged from 27.6 to 109.0, with 73% of patients having a ZUC concentration between 20 and 60 ng/mL. This was surprising given the fact that ZUC has a half life of nearly 30 days compared to ENC, which is 10.5 hours. While variation in duration of CC therapy is a potential explanation for this disparity, even in the subset of patients on CC for more than 2.5 years ZUC concentration ranged from 35.9 to 91.

Only one patient in our study experienced a paradoxical decrease in serum T, from 254 to 222 ng/dL while on CC therapy. He had no prior history of treatment for hypogonadism and was on CC therapy for 50 days – representing the shortest duration of any patient in the study. The patient's ENC and ZUC concentration levels were comparable to those whose serum T responded to CC therapy at 2.2 and 28.4 ng/mL, respectively. After CC therapy was discontinued, he was subsequently treated with testosterone cypionate, to which he did respond. Previous studies have also demonstrated that a small percentage of men may develop a paradoxical decrease in T while on CC, and in some may be associated with an adverse effect on sperm morphology[11-14]. Mazzola et al. reported their data in HM on CC which showed that factors predictive of CC response (defined as increase in serum T ≥ 200 ng/dL at ≥ 6 months) included mean testicular volume, and LH level[15].

We were not able to show any predictors of ENC or ZUC concentrations or ZUC:ENC ratio including duration or therapy, patient age body mass index, or percent change in serum T, free T, estradiol, FSH, and LH. Clinical studies have aimed at classifying the metabolism of CC, which may account for the variable concentrations of ENC and ZUC in our study. Murdter et al. demonstrated that CC is primarily metabolized by the cytochrome p450 enzyme 2D6 which catalyzes hydroxylation of ENC and ZUC. They found that healthy female volunteers who showed extensive metabolism of ENC compared to ZUC had a 10 times higher area under the curve of ZUC compared to ENC in contrast to women who exhibited poor metabolism of the drug secondary to a nonfunctional cytochrome p450 2D6 enzyme as evidenced by an equal area under the curve for both isomers[16].

In our study we noted a statistically significant increase in total T, estradiol, FSH, and LH in patients on CC therapy, which is consistent with previous studies[14, 15, 17, 18]. Moskovic et al. previously demonstrated long-term efficacy of CC therapy after 3 years mean serum T levels 582 ± 227 ng/dL; LH and estradiol also saw a sustained increase even at 3 years [19]. More than half of the patients in our study were on CC for at least two years. Clinical studies using pure *trans* isomer, ENC, in men with secondary hypogonadism have also demonstrated improvement of serum T, FSH, and LH[8, 20-22]. A randomized, double-blind, placebo-controlled trial by Kim et al. demonstrated that one of the advantages of treating men with secondary hypogonadism with ENC versus T gel was that mean sperm concentration failed to improve in patients on T gel at 16 weeks[8]. Additionally, while both groups saw an increase in mean serum T levels at 16 weeks, patients in the ENC group experienced greater improvement in T levels, 445.8 ng/dL versus 350.6 ng/dL ($p<0.001$), for the ENC and T gel groups respectively.

While we assessed changes in hormonal parameters while on CC, we did not address the effect of ZUC:ENC ratio on symptomatic relief, although previous studies have documented the ability of CC to provide symptomatic relief with short- and long-term use[14, 19, 23]. Some authors have postulated that symptomatic relief of hypogonadal symptoms may in fact be related to estrogen levels[23, 24], which may give selective estrogen receptor modulators an advantage when compared to aromatase inhibitors in the treatment of symptomatic HM.

In this study we did not control for the source of CC; all patients were prescribed generic CC, which they obtained from their own pharmacy. There may have been some variation in CC composition, which according to the United States Pharmacopoeia contains between 30% and 50% of the ZUC isomer[25]. Future studies should include a single source of CC and measurement of both ENC and ZUC isomers. Additional limitations of the study include ENC and ZUC concentrations being drawn at

a single time point, as it may be valuable to trend change in concentrations over time, although half-life information has previously been established in women[26]. Serum ENC and ZUC concentrations were drawn between the hours of 0900 and 1500. Patients were asked when they took their last dose of CC; 11 of the 15 patients reported taking their CC in the morning, defined as between 0600 and 0900. Within our small patient cohort, analysis of ENC and ZUC concentration did not correlate with time from CC administration to lab draw.

To our knowledge this is the only study evaluating ENC and ZUC levels in hypogonadal men on long-term CC therapy. While we acknowledge that it represents a small cohort of patients with a single measurement of relative isomer concentrations, we believe that it warrants further investigation given the potential deleterious effects of high doses of ZUC on testicular function and spermatogenesis.

Conclusion

Although CC was administered in a 3:2 ENC:ZUC racemic mixture, prolonged treatment resulted in a 1:21 ENC:ZUC ratio of serum concentration. Patient age, body mass index, duration of treatment and hormonal levels were not predictive of ENC or ZUC concentrations. Our study provides strong evidence for further studies investigating the affects of pure ENC isomer versus CC in the treatment of HM.

Acknowledgements

Analysis of ENC and ZUC concentrations were performed by Repros Therapeutics.

Conflicts of Interest

Dr. McCullough reports non-financial support and other from Repros, during the conduct of the study; other from Pfizer, other from Endo, non-financial support from Lilly, non-financial support from Antares, other from Mero Biopharma, other from International Medical Devices, other from Lipocine, outside the submitted work; .

Dr. Fontenot is an employee of Repros Therapeutics receiving a salary and stock.

Dr. Helo reports non-financial support from Repros Therapeutics, during the conduct of the study; .

Dr. Wiehle reports In addition, Dr. Wiehle has a patent 7,737,185 issued, a patent 7,368,480 issued, a patent 7,173,064 issued, a patent EPO 1487612.8 - 1451 pending, a patent EPO 14807612.8 - 1453 pending, a patent USP2011261722013 pending, and a patent USP2011361831542 pending.

All other authors have nothing to disclose.

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Tables

Table 1. Most frequently reported medical conditions

Medical condition	n (%)
Erectile dysfunction	9 (60.0)
Hypertension	6 (40.0)
Hyperlipidemia	4 (26.7)
Infertility	4 (26.7)
Diabetes mellitus	2 (13.0)
Prostate cancer	1 (6.7)

Table 2. Hormone levels pre- and post-treatment on clomiphene citrate

	Median pre-treatment (IQR)	Median post-treatment (IQR)	% Median change pre-and post-treatment	p value
Total testosterone (ng/dL)	205 (188-253)	488.0 (401-617)	138.0	0.0001
Free testosterone (ng/dL)	6.3 (5.1-9.1)	16.3 (11.5-35.3)	143.3	0.0180
Estradiol (pg/dL)	17.0 (11.0-24.9)	34 (25-48)	92.3	0.0001
FSH (mIU/mL)	3.9 (2.8-5.1)	9.8 (4.5-13.0)	85.7	0.0030
LH (mIU/mL)	4.0 (2.2-5.7)	6.1 (5.0-12.8)	156.0	0.0028

Abbreviations: FSH, follicle stimulating hormone; IQR, interquartile range; LH, luteinizing hormone.

Table 3. Enclomiphene and zuclomiphene concentrations in patients on clomiphene citrate

	Median concentration (IQR)	Range
Enclomiphene (ng/mL)	2.2 (1.5-3.9)	0.6-37.3
Zuclomiphene (ng/mL)	44.0 (35.9-75.1)	27.6-109.0
Zuclomiphene:Enclomiphene ratio	21.3 (11.3-29.8)	1.4-88.3

Abbreviations: IQR, interquartile range.

Table 4. Correlation of baseline characteristics, treatment duration, and median post-treatment hormone levels with zuclomiphene:enclomiphene ratio

	Regression coefficient (95% CI)	p value
Age (years)	0.2 (-1.2, 1.6)	0.77
Body mass index (kg/m^2)	1.43 (-1.8, 4.6)	0.35
Duration of clomiphene citrate therapy (days)	0.01 (-0.02, 0.03)	0.60
Post-treatment testosterone (ng/dL)	-0.06 (-0.17, 0.04)	0.20
Post-treatment free testosterone (ng/dL)	0.12 (-0.21, 0.45)	0.44
Post-treatment estradiol (pg/dL)	1.12 (-0.36, 2.75)	0.11
Post-treatment FSH (mIU/mL)	0.97 (-1.47, 3.42)	0.39
Post-treatment LH (mIU/mL)	-2.40 (-6.82, 2.02)	0.25

Abbreviations: FSH, follicle stimulating hormone; LH, luteinizing hormone.