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Quantitative and qualitative changes in serum luteinizing hormone after injectable testosterone undecanoate treatment in hypogonadal men

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Abstract

Aim: To clarify the immuno-active LH (I-LH) and bioactive LH (b-LH) responses and qualitative changes in the circulating LH to testosterone undecanoate (TU) injection. **Methods:** Eight men with Klinefelteri¯s syndrome were recruited for the study. They received crossover injections of TU at doses of 500 and 1000 mg. Serum I-LH and b-LH levels before and at various time intervals after TU injection were measured and the serum I-LH, b-LH, b-LH/I-LH (B/I) and testosterone/sex hormone-binding globulin (T/SHBG) ratio in LH-responders and LH non-responders were compared. **Results:** A parallel suppression of serum I-LH and b-LH was consistent with their overall high correlation between each other (r=0.84, P<0.001). Mean serum i-FSH levels were decreased by TU injection at both doses without dose-response effects. LH-responders had lower baseline serum i-LH and b-LH, and higher E_2 levels and T/SHBG ratio. There was a quantitative change in serum LH as induced by TU without qualitative change within LH-responders os LH-non-responders. **Conclusion:** A high loading dose (1000 mg) of TU is important for the initial suppression of LH. With the lower dose (500 mg), repeated injections will be required to attain such LH suppression for the purpose of fertility regulation. The lower baseline serum I-LH level may be an intrinsic characteristic of LH-responders.

1 Introduction

Although a number of testosterone (T) preparations, oral or parenteral, are available for clinical use, the rapidity of absorption, early peak release, relatively short-acting period and excessive hepatic toxicity limit their long-term application[1,2]. Recently, a new long-acting T preparation, T undecanoate (TU) has been shown to have a more favorable pharmacokinetic profile in Klinefelteri¯s syndrome patients with a significant suppression of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) after a single injection of 500 or 1000 mg, the serum LH suppression being non-uniform and varied between individuals, independent of the TU doses[3]. It is not clear whether the LH response to exogenous TU was specific to men with Klinefelteri¯s syndrome whose serum LH was relatively resistant to T suppression[4]. Recent studies showed that androgen administration is

able to modulate the amount of LH secretion as well as qualitative alteration in LH molecules[5,6]. It is unclear whether the heterogeneous response of serum LH to T suppression is associated with a qualitative change in LH molecules. Therefore, the clarification of serum LH response to TU injection would be crucial for further understanding of TU.

2 Materials and methods

2.1 Subjects

Eight Chinese men aged 16-26 years with Klinefelteri¯s syndrome, otherwise healthy, were recruited for the study. They had stopped previous hormonal therapy for their hypogonadism at least 6 weeks before recruitment. All the subjects gave written consent to participate in the study after understanding the studyi¯s purpose, benefit and possible risks.

2.2 Androgen preparation

Injectable TU (Zhejiang Xian Ju Pharmaceutical Corporation, Zhejiang, China) was available in ampules containing 250 mg of the ester in 2 mL of tea seed oil.

2.3 Study design

Eight patients were divided randomly into two groups. A prospective, crossover clinical trial with injectable TU at doses of 500 and 1000 mg (equivalent to 315 and 630 mg of T free base, respectively) was performed. The sample size was 8 for 500 mg and 7 for 1000 mg (due to personal reason, one patient only attended the 500 mg course). The first dose used for each subject was randomized. The two treatment periods were separated by an intervening washout period of 3 months. Two pre-treatment blood samples were taken for baseline estimation of serum hormones. Blood samples were allowed to clot for 24 hours before centrifugation. Serum samples were stored at -70iæ until analyzed.

2.4 Hormone assays

2.4.1 RIA

Reagents for RIA of T, estradiol (E_2) and prolactin (PRL) were supplied by the Diagnostic Products Corporation, USA. All samples from one subject were analyzed in the same assay. The lower detection limits of the assays were 0.14 nmol/L, 3.7 pmol/L and 20 mIU/L for T, E_2 and PRL, respectively. The intra-assay coefficient of variation for serum T, E_2 and PRL was 7.6%, 5.8% and 6.2%, respectively. The mean inter-assay coefficient of variation was less than 10% for all the three hormones. The serum sex hormone-binding globulin (SHBG) was measured by the Nichols Institute (San Juan Capistrano, CA) with a double antibody RIA kit supplied by the Diagnostic System Laboratories (Webster, TX). The lower detection limit was 5.0 nmol/L. The mean intra-assay and inter-assay coefficient of variation for serum SHBG was 4.0% and 8.8%, respectively.

2.4.2 Fluoroimmunoassay

The I-LH and I-FSH were measured by time-resolved fluoroimmunoassay (Delfia, Wallac Oy, Finland). The lower detection limit was 0.018 IU/L and 0.016 IU/L for LH and FSH, respectively. All samples from one individual were analyzed in the same assay. The mean intra- and inter-assay coefficients of variation were 4.4% and 9.3% for LH and 5.9% and 8.7% for FSH, respectively.

2.4.3 LH Bioassay

The in vitro bioassay of LH was a modification[7-9] of the procedure described by van Damme *et al*[10] and Dufau *et al*[11]. This assay is based on the measurement of T production by the dispersed Leydig cells isolated from immature male Swiss Webster mice (34-45 day-old). T was measured by RIA using reagents supplied by the

Diagnostic System Laboratories (Webster, TX). LER 907 was used as the reference standard. Serial dilutions of serum samples containing high LH levels were shown to be parallel to the standard curve. All the serum samples were run in duplicate. The minimal detection limit of b-LH was 0.04 mIU/tube and the intra-assay and inter-assay coefficients of variation were 8.4% and 24%, respectively.

2.5 Data analysis and statistics

Based on the suppression/normalization of their i-LH levels, patients were grouped as LH responders and LH-non-responders. This classification was assigned irrespective of the doses administered. Four patients fell in each of the two categories. The B/I ratio was calculated and compared. Results were expressed as the meaniÀSEM, and were analyzed by correlation, linear regression and paired or unpaired *t*-test, as required. $P<0.05$ was considered as significant.

3 Results

3.1 Serum I-LH, I-FSH, T, E_2 and PRL levels

In the 4 LH responders, TU suppressed the nadir of serum I-LH levels to the upper normal threshold of eugonadal men (Figure 1). Serum I-LH levels at the baseline were significantly lower in LH responders than in non-responders. There were marked differences between those who response with a decline of I-LH following TU administration (Figure 2). Mean serum I-FSH levels were decreased by TU injection at both doses without dose response effects. There was no apparent difference in serum T (Figure 3) and FSH levels between the two groups. However, serum E_2 levels were slightly higher ($P>0.05$) in LH responders (Figure 4). Serum PRL levels remained unchanged in both groups throughout the study period.

Figure 1. Serum I-LH levels before and after crossover injection of TU at doses of 500 mg and 1000 mg to 8 men with Klinefelteri¯s syndrome. Solid symbols (LH responders), open symbols (LH non-responders). .....Upper normal limit.

Figure 2. Comparison of serum I-LH levels (meaniÀSEM) between LH-responders and LH-nonresponders. $^bP<0.05$ vs LH-responders.

Figure 3. Comparison of serum T levels (meaniÀSEM) between LH-responders and LH-nonresponders.

Figure 4. Comparison of serum E_2 levels (meaniÀSEM) between LH-responders and LH-nonresponders.

3.2 SHBG and T/SHBG ratio

A comparison of serum SHBG levels and T/SHBG ratio between the two groups is shown in Table 1. Serum SHBG level was relatively higher at baseline in both groups than that after TU administration. It decreased after TU injection, especially in the LH responders and remained within the normal range throughout the study period. The T/SHBG ratio was significantly higher in LH responders, although serum T levels were almost the same between the two groups. No significant differences in serum SHBG levels were found at different time points between the two groups as well as between the baseline and various time points in either groups. A weak negative correlation ($r=-0.36$) between T/SHBG and LH levels was observed.

Table 1. Serum SHBG level and T/SHBG ratio between LH responders and non-responders. meaniÀSEM. $^bP<0.05$, between the two groups.

	LH responders		LH non-responders	
	SHBG (nmol/L)	T/SHBG	SHBG (nmol/L)	T/SHBG
Pretreatment				
(Week 0)	30.67iÀ7.26	0.21iÀ0.16	33.86iÀ6.79	0.25iÀ0.08
Treatment				
Week 1	18.87iÀ0.13	3.36iÀ0.18^b	25.29iÀ5.17	2.07iÀ0.34^b
Week 8	18.00iÀ4.04	0.92iÀ0.52	27.00iÀ4.28	0.53iÀ0.12

Normal range of serum SHBG in adult men is 6-44 nmol/L.

3.3 LH bioactivity

A parallel suppression of serum b-LH and I-LH was consistent with an overall high correlation (r=0.84, P<0.001) between them (Figure 5). b-LH levels at the baseline of the two groups were almost at the same level, whereas i-LH levels at baseline were different. After TU injection, b-LH significantly decreased in the LH responders and gradually returned to baseline levels 8 weeks after treatment (Figure 6). The B/I ratio, an index of relative LH biopotency, fluctuated slightly within each group. In LH responders, the B/I ratio maintained a higher level throughout the observation period except at week 7 (Figure 7). There was no correlation (r=-0.14) between serum T levels and B/I ratio, and between T and serum i-LH or b-LH levels. A weak negative correlation (r=-0.43) between T/SHBG and b-LH levels existed.

Figure 5. Correlation analysis between serum I-LH and b-LH. y=4.54+0.18x.

Figure 6. Comparison of serum LH bio-activity (meaniÀSEM) between LH-responders and LH-nonresponders. ^{b}P<0.05 vs LH-responders.

Figure 7. Comparison of serum LH B/I ratios (meaniÀSEM) between LH-responders and LH-nonresponders.

4 Conclusions

Administration of supraphysiological dose of T causes profound suppression of spermatogenesis by the depletion of gonadotropins and endogenous T[12,13]. A decrease in the level of gonadotropins, especially LH, would be a criterion for evaluating the potency of male hormonal contraceptives. The results derived from pooled LH responders and non-responders showed obvious heterogeneity in the two groups. Of considerable interest is the significant difference in serum i-LH baseline levels between the two groups. LH responders who had lower i-LH levels before the first injection also had lower levels before the second injection, irrespective of the dose used. These findings indicate that a lower baseline i-LH might be an intrinsic characteristic of LH responders. Since these patients already had lower baseline i-LH levels, serum LH could be easily suppressed by TU. As LH plays an important role in male reproduction[8,9,14,15], we propose that the present study, focusing on the changes in LH after TU injection, will provide a baseline data for the future development of male hormonal contraceptives. In this study, the lower baseline and nadir levels of serum I-LH before the second injection suggests that in order to produce profound suppression of the serum i-LH, TU injection should be given every 4-6 weeks before the serum LH returns to the baseline.

Many studies have shown that administration of exogenous T to men can result in a dose-dependent decrease in LH[16-19]. Unexpectedly, higher serum T levels were not seen in LH responders in our study. This finding seemed not to be a reasonable interpretation for the results when taking into consideration serum SHBG and T/SHBG ratio. An increase in T/SHBG ratio seems justifiable for LH-responders who do not have higher serum T levels, as the increase in T/SHBG may be the result of a decreased serum SHBG that induces a rise in the MCR of T. An inverse relationship between SHBG and MCR of T has been proved by earlier studies[20]. Thus, it is not difficult to explain why higher serum T levels were not seen in LH responders. It has been reported that a fall in SHBG leads to an increase in free T[21]; whether other endocrine parameters have a strong negative feedback regulation on LH, independent of the total T concentration, is still unknown.

T is converted to estradiol (E_2) by aromatase. Previous studies have demonstrated that E_2 has an important role on the negative feedback regulation of serum LH[22-24]. In the present study, the E_2 levels were relatively higher in LH responders, which may indicate a higher conversion rate of T to E_2. This finding also suggests that 1) the suppressive effect of T on serum LH might be partially mediated by E_2, and 2) a combination of supraphysiological dose of T with higher physiological levels of E_2 might have a synergistic effect on the negative feedback regulation. This finding raises further concern in the application of T/estrogen combination for the enhancement of LH/FSH suppression in male contraceptive research.

Our results demonstrated that both I-LH and b-LH were similarly suppressed. However, some inconsistent changes between I-LH and b-LH were also seen (Figure 5). When I-LH value was over 30 IU/L, relative higher b-LH activity was not seen, indicating that these LH molecules are more immuno-active but less biologically active. In contrast, at points where b-LH value .

In contrast to the significant difference in baseline I-LH levels between the two groups, their baseline b-LH levels were similar. Thus, B/I ratio at the baseline was lower in LH non-responders. LH non-responders may was over

200 IU/L, these LH molecules were found to be more bio-active but less immuno-active. These findings suggest that there are differences in modification of LH molecules.

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have a relatively higher ratio of biologically inactive LH molecules (deglycosylated form) or LH subunits before TU treatment. In both groups the pattern of b-LH suppression curve was similar to that of I-LH. The B/I ratio was slightly fluctuating within each group, which indicated that TU might be inducing a quantitative change in serum LH without any qualitative change. This finding is not in consistence with previous reports that T enhanced LH biopotency and increased B/I ratio[6]. A possible explanation for the enhancement of b-LH activity after T therapy was attributed to the fact that the synthesis of the abnormal LH molecules could have been altered by T or its metabolites within the pituitary since gonadal steroids were known to modify the process of transcription[25]. However, Bagatell reported that administration of T or E_2 to patients with idiopathic hypothalamic hypogonadism, who were treated with GnRH infusion until their gonadotropins reached the normal range, resulted in decreased levels of b-LH and i-LH in the same ratio[22]. In interpreting whether T treatment will induce an increase in B/I ratio or not, the hormonal status of patients, such as hypo- or hypergonadotropic hypogonadism, and the type and dose of androgen administered should be considered carefully.

The present study confirmed that men with Klinefelteri¯s syndrome, who had a higher baseline level of I-LH, had a higher resistance of I-LH to T treatment. In KlInefelteri¯s syndrome, androgen replacement therapy was still efficacious even without normalization of serum I-LH and FSH[4]. Therefore, during androgen replacement therapy in Klinefelteri¯s syndrome, the usefulness of serum i-LH assessment remains questionable. In this situation, the efficacy of androgen replacement therapy may be estimated mainly through improvement of symptoms and signs, clinical chemistry and metabolic parameters. Klinefelteri¯s syndrome had a lower resistance of I-LH to exogenous T, thus serum I-LH, T (T/SHBG) and E_2 would be appropriate parameters for assessing the adequacy of androgen replacement therapy.

In summary, serum I-LH suppressive response to exogenous TU in men with Klinefelteri¯s syndrome was heterogenous. LH responders had a lower baseline serum I-LH, and higher T/SHBG and E_2 levels during TU treatment. These endocrine parameters may be responsible for a decrease in serum I-LH. In both LH responders and non-responders, TU induced a quantitative change in serum LH without any qualitative change. The authors suggest that a higher initial dose of TU injection (1000 mg) is important for adequate suppression of LH. Repeated injections with a lower dose (500 mg) should be given (every 4-6 weeks) before LH returns to the baseline in order to produce appropriate LH suppression for the purpose of male fertility regulation.

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