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HORMONAL EFFECTS OF GnRH AGONIST IN THE HUMAN MALE: AN APPROACH TO MALE CONTRACEPTION USING COMBINED ANDROGEN AND GnRH AGONIST TREATMENT

RONALD S. SWERDLOFF, DAVID J. HANDELSMAN and SHALENDER BHASIN
Division of Endocrinology, Department of Medicine, Harbor-UCLA Medical Center, UCLA School of Medicine, Torrance, Calif., U.S.A.

Summary—Observations that hypophysectomized men demonstrate predictable azoospermia have led to attempts to suppress gonadotropin secretion with drugs for contraceptive purposes. Testosterone enanthate, given on a weekly or bimonthly basis, failed to predictably induce azoospermia in men. Treatment with agonist analogs of GnRH significantly suppressed spermatogenesis, but led to concomitant decline in serum testosterone concentrations. To prevent GnRH agonist induced changes in libido and potency we tested regimens employing daily subcutaneous injections of 200 μg of D(Nal$_2$)^{6}GnRH in combination with 200 mg testosterone enanthate every 2 weeks. This regiment led to 86% decline in mean sperm count over the 16-week treatment period, but azoospermia was not achieved in any subject. Basal or 24 h integrated serum LH or 24 h urinary LH concentrations were not significantly suppressed by combined treatment. In order to assess whether constant infusion of GnRH agonist will lead to greater suppression of gonadal function than its intermittent administration, we administered either 20 or 200 μg of D(Nal$_2$)^{6}GnRH to 2 groups of normal male volunteers for 28 days either by single daily injection or by constant subcutaneous infusion. Serum testosterone, LH and FSH responses were not significantly different between the two modes of agonist delivery either at 20 or 200 μg dose. Marked decrease in serum testosterone and sperm counts in these studies occurred in the face of little or no change in immunoreactive LH, indicating that the antigonadal actions of GnRH agonist in the human male cannot be fully explained on the basis of downregulation of pituitary LH secretion alone. GnRH agonist treatment however, led to marked decrease in bioassayable LH concentrations suggesting secretion of a molecularly altered LH species with diminished biologic activity.

INTRODUCTION

Spermatogenesis is dependent on gonadotropic hormones [1, 2]. Observations that hypophysectomized and LH and FSH deficient men demonstrate predictable azoospermia, have led to attempts to suppress gonadotropin secretion with drugs for contraceptive purposes. To this end, several types of agents have been tested. Because of their success as female contraceptives, steroids were a logical first approach.

STUDIES WITH TESTOSTERONE ENANTHATE

In our studies with depot forms of testosterone, we utilized testosterone enanthate in sesame oil in doses as high as 200 mg each week. This proved to be relatively effective, and resulted in significant inhibition of serum LH and FSH (approx 50% of baseline) concentrations. Serum testosterone concentrations were only moderately elevated compared to baseline. The mean sperm count was suppressed by 97% [3, 4]. Almost all subjects (35 of 39) attained sperm counts of less than 5 million/cc and 60% were azoospermic. Other investigators have reported similar results with androgens alone and androgens plus progestogens [5–11]. In the studies reported in which the subjects received up to 6 months of treatment, very few adverse effects were observed. However, no regimen produced azoospermia in all the subjects tested.

STUDIES WITH GnRH AGONISTS

Rationale for combined treatment with GnRH agonist and androgen

GnRH agonistic analogs were originally developed as longer acting therapeutic agents to treat GnRH deficient patients. After early trials, it became apparent that they were poor therapeutic substitutes for authentic short acting GnRH and that, when given chronically, they had paradoxical inhibitory actions on LH and FSH secretion [12]. Subsequent studies demonstrated additional direct inhibitory effects of GnRH agonists on rat testes [13]. Reports of these antigonadal effects raised hopes of their potential application as male contraceptives and clinical trials were begun in several laboratories. Bergquist *et al.* [12] treated men for 17 weeks with 5 μg daily of Dser (TBU)^6 GnRH ethylamide with little effect on spermatogenesis, while LH, FSH and testosterone fell. Linde *et al.* [14, 15] administered 50 μg of (DTrp^6, Pro^9-*N*-ethylamide) GnRH daily and observed a fall in serum testosterone with a mean nadir of 60 ng/dl by the fourth week of treatment. Agonist treatment

Table 1. Intratesticular sperm counts (million/testis) in adult male rats after chronic treatment with D-leu^{6}desGly^{10}GnRH, testosterone implants or both

Group	Day 40	Day 60
Control (10)	134 ± 7	169 ± 13
Testosterone implanted (10)	156 ± 15	122 ± 16
Analog at 200 ng	62 ± 10	44 ± 11
Analog plus testosterone	34 ± 7	9 ± 1.6

was discontinued after 6 or 7 weeks in five or eight subjects because of impotence. Libido and potency returned 2 weeks after stopping agonist therapy, but the occurrence of these symptoms represented a significant drawback to the application of GnRH agonists alone as male contraceptive agents. Of three subjects who completed 10 weeks of therapy, one was azoospermic. Recovery of spermatogenesis occurred within 10 weeks of cessation of therapy in all but the one azoospermic subject, who recovered after 14 weeks. In this regard, studies from our laboratory suggested that combined androgen and agonist treatment might lead to the development of an effective, practical male contraceptive agent. We argued that their combined use was theoretically attractive for two reasons. First, testosterone treatment would prevent the undesirable side effects of impotence and diminished libido; and second, since androgens alone are potent inhibitors of spermatogenesis in man, the addition of a second gonadotropin inhibitor might have additive or synergistic effects on the testis.

Our initial studies on combined GnRH agonist and testosterone treatment were performed in laboratory rats. Adult male Wistar rats were treated for 60 days with daily injections of GnRH analog alone, a subcutaneous testosterone implant, or analog and testosterone in combination [16, 17]. The resulting intratesticular sperm counts are shown in Table 1. These data confirmed other observations that GnRH agonist would significantly inhibit spermatogenesis and demonstrated a synergistic effect of combined androgen and GnRH agonist therapy. These data were encouraging, but emerging information indicated that the prime site of inhibitory action of GnRH agonists on the reproductive system in the rat was at testicular level while the human was affected primarily at the pituitary gland [18].

Short term studies in the human male

Clinical studies in our laboratories have utilized the GnRH agonist D(Nal$_2$)^6 GnRH (Syntex Research). Preliminary studies assessed two doses of this potent agonist on testosterone, LH and FSH secretion [19]. Daily administration resulted in an early phase of stimulation followed by a progressive decline in LH, FSH and testosterone to serum levels below baseline by day 10 of treatment. The higher dose (100 μg) was more potent in both the stimulatory and down regulatory phases. Combined treatment of a single 200 mg dose of testosterone enanthate with daily subcutaneous injections of 100 μg of GnRH agonist did not blunt the peak LH and FSH responses on day 2, but resulted in significantly lower LH responses (187 ± 30 vs 234 ± 42 mIU-day/ml) and FSH (20.6 ± 3.3 vs 32.8 ± 4.2 mIU-day/ml), as assessed by paired comparisons of the areas under the curve, from days 3–11 [20]. These studies suggested that the addition of testosterone to GnRH analog had additive inhibitory effects on LH and FSH secretion and encouraged us to test the combination in longer term studies assessing spermatogenesis.

Long term studies with the combined regimens

In the most recent studies, 7 men were given daily subcutaneous injections of 200 μg of D(Nal$_2$)^{6}GnRH in combination with 200 mg of testosterone enanthate every 2 weeks [21]. The mean sperm count declined to a nadir of 17.4 ± 6.3 million/cc (Fig. 1). However, 1 subject did not show any significant

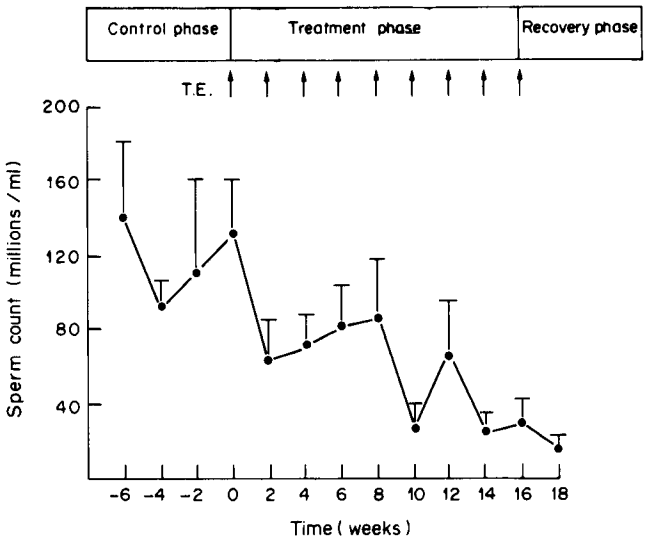

Fig. 1. Effect of combined treatment with testosterone and GnRH on sperm count. The data are mean ± SEM $n = 7$. One subject did not show significant suppression as assessed by regression analysis. No subjects became azoospermic. Reproduced with permission from [20].

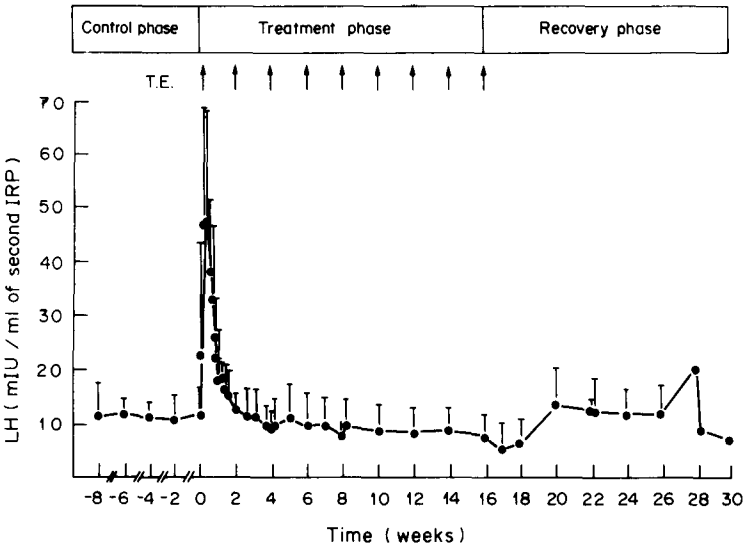

Fig. 2. Effect of combined treatment with testosterone and GnRH on basal LH concentrations. Data are mean ± SEM $n = 7$. Reproduced with permission from [20].

suppression of sperm count as assessed by regression analysis. If this subject were excluded, the mean sperm count in the remaining 6 subjects declined by more than 90%. While no patients became azoospermic, 1 patient had sperm counts below 50,000/ml of semen. Thus, this combined regimen of intermittent agonist injection with bimonthly injections of testosterone did not induce azoospermia. These results are similar to those of Evans *et al.* [22] who also noted only partial suppression of spermatogenesis with a combined regimen that employed subsuppressive dose of testosterone [24].

Basal serum LH concentrations after an initial phase of stimulation, declined progressively to baseline by day 28 but were not significantly below baseline by the end of the treatment period (Fig. 2). Basal FSH concentrations declined more rapidly than LH to 40–50% of baseline by the end of second week and stayed decreased thereafter. Following discontinuation of treatment, both LH and FSH returned to baseline within 4–6 weeks without a significant rebound.

Detailed LH responses to GnRH agonists on day 0, 1, 28 and 112 were assessed by multiple blood sampling over 24 h period. On day 1, LH concentrations rise promptly and stay elevated for the entire 24 h period. In contrast, the LH responses on days 28 and 112 are markedly blunted with no significant rise in LH after agonist injection, indicating significant down regulation of pituitary LH secretion.

Effects of combined treatment on 24 h integrated LH and FSH responses on days 1, 10, 28, 56 and 112 were assessed by calculating the areas under the curve on these days above day 0 baseline. The stimulatory effects of the agonist abate by day 28. Despite this loss of stimulatory response, only a very modest inhibition of integrated LH and FSH responses below baseline can be seen even as late as day 112. Another method of assessing integrated LH secretion is to measure urinary LH, which has been shown to accurately reflect 24 h LH secretion. 24 h Urinary LH in subjects treated with the combined regimen, after an initial phase of stimulation return to baseline by day 10–28 but do not show significant inhibition below day 0 baseline at any time (Fig. 3). Thus, integrated immunoreactive LH secretion, assessed by measuring the areas under the curve or by 24 h urinary LH, shows no significant inhibition during the 16 week treatment period. In light of these observations, it is intriguing that the sperm counts fell by approx 90% in 6 out of 7 subjects. In separate studies in subjects treated with similar dosages of GnRH agonist alone for 28 days, serum testosterone concentrations were noted to decrease far more than could be explained by the modest decrease in serum gonadotropins, measured by standard radioimmunoassays. These data indicate that the antigonadal effects of the GnRH agonist in the human male cannot be fully explained on basis of down regulation of pituitary LH secretion alone, additional mechanisms need to be invoked. Preliminary data

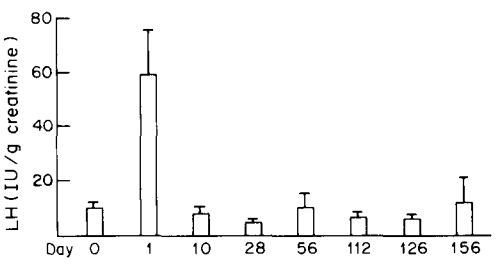

Fig. 3. Effect of combined treatment with testosterone and GnRH on urinary LH concentrations. Data are mean ± SEM $n = 7$. Reproduced with permission from [20].

from our and other laboratories suggest that GnRH agonist treatment may result in secretion of qualitatively different LH species with diminished biologic activity [24]. Other possibilities include loss of pulsatile pattern of gonadotropin secretion, direct inhibition at the gonadal level and secretion of a circulating inhibitor of LH action.

Reasons for incomplete suppression of spermatogenesis

The failure of combined regimens employing intermittent injections of GnRH agonist appears related at least in part to incomplete suppression of gonadotropins because complete hypophysectomy predictably leads to azoospermia. This may be due to several factors including the possibilities that: (i) the human male may be biologically resistant to the inhibitory effects of the GnRH agonist; (ii) the dose of the agonist could have been inadequate and (iii) the regimens of intermittent daily injection may fail to provide constant and optimum blood levels.

Of these, the latter appeared likely since the serum concentrations of the GnRH agonist, measured by radioimmunoassay decline to undetectable levels by 12 h after the agonist injection. Furthermore, studies in rhesus monkey have shown that constant infusion of GnRH agonist results in far greater suppression of gonadotropins and spermatogenesis than regimens of intermittent injection [25].

Studies with constant infusion of GnRH agonist in the human male

Short Term Studies

In order to assess if constant infusion of GnRH agonist will also lead to greater suppression of gonadal function than its intermittent administration, we administered either 20 or 200 μg of nafarelin to two groups of normal volunteers for 28 days either by a single daily injection or by constant infusion by means of a portable infusion device (Autosyringe).

Two hundred microgram group. Serum LH response to 200 μg of nafarelin administered as a single daily injection or as a constant infusion is shown in Fig. 4. Basal or 24 h integrated LH responses were not significantly different in the two groups. Multiple sampling on day 1 (the first treatment day) revealed that the temporal pattern of LH response on day 1 was strikingly different, in the 2 groups. Serum LH concentrations reached a peak at 1 h in the single daily injection group, while in the constant infusion group, serum LH concentrations rose slowly to a peak between 12–16 h.

Basal and integrated serum FSH responses to 200 μg of nafarelin were not significantly different in the two groups.

Serum testosterone concentrations showed consistent suppression in all the 5 subjects in the constant infusion group, while 2 out of the 5 subjects receiving single daily injections did not have significant suppression. Serum testosterone concentrations fell to castrate range (less than 50 ng/dl) in 2 out of 5 subjects in the constant infusion group in contrast to only 1 subject receiving daily injections. Although both the mean basal (213 ± 116 vs 510 ± 190 ng/dl) and 24 h integrated ($-15{,}032 \pm 1447$ vs -3932 ± 6188 ng-h/dl) testosterone concentrations were lower in the constant infusion group, these differences did not approach statistical significance (Fig. 5).

Twenty microgram group. The results of administering 20 μg of nafarelin either by single daily injection or constant infusion were similar to those obtained with 200 μg dose. Serum LH and FSH responses in the constant infusion group were not different from those receiving single daily injections.

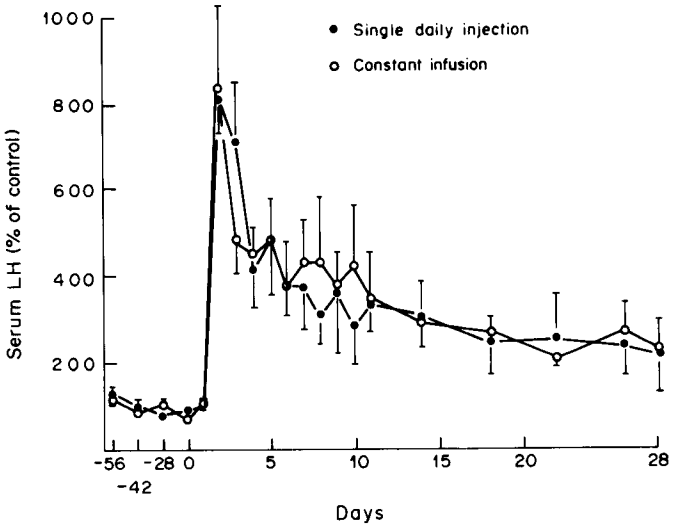

Fig. 4. Serum LH response to 200 μg of nafarelin acetate administered by single daily injection or constant subcutaneous infusion. Basal serum LH concentrations are expressed as percent of control. Data are mean $\pm$ SEM $n = 5$.

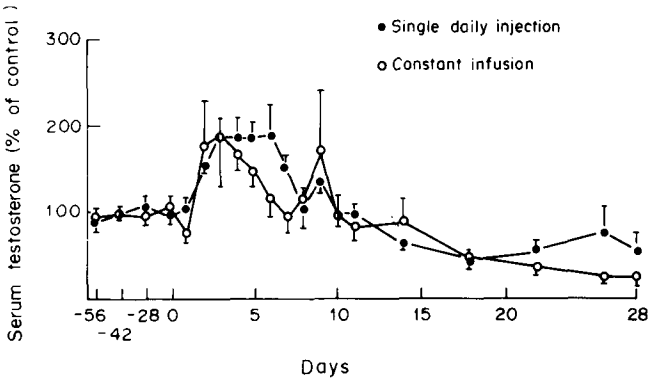

Fig. 5. Serum testosterone response to 200 μg of nafarelin acetate administered by a single daily injection or constant infusion. Serum testosterone concentrations are expressed as percent of control. Data are mean $\pm$ SEM $n = 5$.

Mean serum testosterone concentrations were not statistically different in the two groups.

Thus, constant infusion of GnRH agonist in the human did lead to more consistent suppression of serum testosterone but its effects were not as striking as reported in the rhesus monkey. Decline in serum testosterone in both the groups occurred in face of little or no change in serum LH suggesting that the antigonadal effects of intermediate term GnRH-A treatment in man cannot be fully explained on the basis of downregulation of gonadotropin secretion alone.

Long term studies with constant infusion

In order to determine if constant infusion of a higher dose of GnRH-A would result in greater suppression of spermatogenesis in man, we administered 400 mcg of D(Nal$_2$)^6 GnRH daily by constant subcutaneous infusion by an insulin pump with bimonthly injections of 200 mg T.E. to 7 normal men for 16 weeks. These studies are still in progress but 5 subjects have completed the treatment phase. Sperm counts fell in all 5 subjects from control of 54.2 ± 8.0 to a nadir of 6.4 ± 3.2 million/ml (mean $\pm$ SEM) during week 14–16. Four subjects had sperm counts of less than 10 million/ml and 1 subject became azoospermic. Serum LH and FSH, after an initial period of stimulation, declined but did not fall below baseline by d112. These data are similar to those of Schurmeyer *et al.* [26] who were also unable to induce azoospermia in all the subjects with a comparatively lower dose of buserelin.

Mechanisms of action of GnRH agonist in the human male

Molecular Heterogeneity and Biologic Activity of LH after GnRH Agonist Treatment

While it is clear that chronic treatment with GnRH agonist leads to down-regulation of pituitary gonadotropin secretion, the decrease in immunoreactive LH following intermediate term (4–12 weeks) GnRH agonist treatment cannot fully explain the disproportionately greater decline in serum testosterone and sperm counts; suggesting that additional mechanisms must be operative. In separate studies, patients with prostate cancer were treated with 1 mg of D(leu)^{6}desGly^{10} GnRH EA (leuprolide) for 8 weeks. While serum LH as measured by immunoassay declined from 11.8 ± 2.7 (mean $\pm$ SEM) to 9.8 ± 1.9 mIU/ml of second IRP after 8 weeks of agonist treatment, bioassayable LH decreased from 38.3 ± 11 to 5.8 ± 0.7 mIU/ml of a second IRP. The bioassayable to immunoassayable (B/I) LH ratio decreased from 3.20 ± 0.59 to 0.69 ± 0.11 (Fig. 6). These data suggest that GnRH agonist treatment

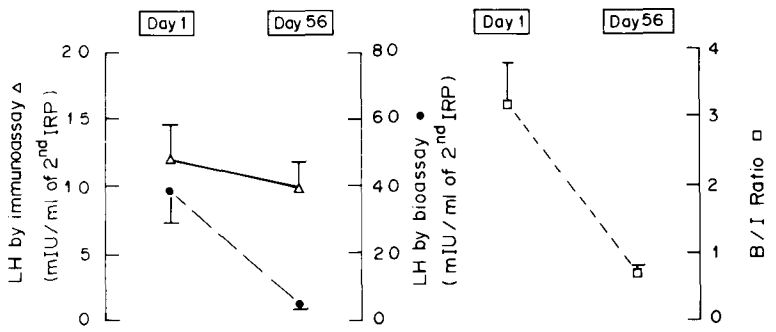

Fig. 6. Serum LH concentrations measured by radioimmunoassay and bioassay and the bioassayable to immunoassayable (B/I) LH ratios after 8 weeks of GnRH agonist treatment. Data are mean $\pm$ SEM $n = 9$.

leads to secretion of a qualitatively different LH species with diminished biologic activity. Sera from 4 of these patients before and after 8 weeks of agonist treatment were subjected to gel filtration on polyacrylmide agarose column (Ultrogel AcA 54) with fractionation range of 5,000–80,000. No significant shift in LH peak was noted after treatment. Chromatograms of the sera on HPLC Bio Sil TSK-125 column also did not show significant shift in LH peak. Chromatofocusing of urinary LH, however revealed significant shift in the isoelectric point of the predominant LH species to more acid range. These data indicate that GnRH agonist treatment results in secretion of LH species that differ significantly in their charge but not in their molecular weights suggesting a change in carbohydrate side chain. These data are consistent with our hypothesis that GnRH agonist alter post-translational glycosylation of LH.

Do GnRH Agonists Exert Direct Gonadal Effects in the Human Male?

The question whether GnRH agonists exert a direct gonadal effect in the human male remains unresolved. "GnRH receptors" have never been unequivocally demonstrated in the human or primate testis although some investigators have reported the presence of low affinity binding sites.

We measured serum concentrations of progesterone, 17-hydroxyprogesterone, androstenedione, pregnenolone, 17-hydroxypregnenolone, androstanediol and testosterone in normal men treated with 200 μg of GnRH agonist for 4 weeks. Only the concentrations of 17-hydroxyprogesterone showed consistent and marked decline after GnRH agonist treatment with significant increase in the ratio of progesterone to 17-hydroxyprogesterone. Since, testis is the source of over 95% of circulating 17-hydroxyprogesterone, these data suggest inhibition of testicular 17α-hydroxylase.

On the other hand, serum testosterone response to exogenous hCG or LH after GnRH agonist treatment has not been found to be significantly different from pretreatment response in a number of published studies [22, 27]. However, large pharmacologic doses of hCG and LH were used in all these studies and therefore, a masked gonadal effect can not be excluded. Studies are currently underway to critically evaluate this question.

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