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Comparison of the Suppressive Capacity of Different Depot Gonadotropin-Releasing Hormone Analogs in Women*

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ABSTRACT

Different depot GnRH analogs (GnRH-A) are currently used for the reversible suppression of the pituitary-ovarian axis in several reproductive and neoplastic disorders in women. In spite of anecdotal reports of incomplete suppression by some depot GnRH-A, this issue has never been systematically investigated in adult women. Thus, we elected to study 40 normally cycling women with male-related infertility or benign reproductive disorders; each group of 10 subjects received a different GnRH-A for 3 months: buserelin (group B; 300 μg, sc, every 12 h, as a control), goserelin (group G; 3.6 mg, sc, every 28 days), leuprorelin (group L; 3.75 mg, im, every 28 days), and triptorelin (group T; 3.75 mg, im, every 28 days). Depot GnRH-A was administered by one of the investigators. GnRH tests (100 μg, iv) were performed before treatment (cycle day 7; test A) and on treatment days 57 (*i.e.* 1 day after the third depot GnRH-A; test B) and 84 (*i.e.* 28 days after the third depot GnRH-A; test C). Immunoreactive (i) LH levels were measured with an ultrasensitive immunochemiluminometric assay. Profound suppression of the iLH response to the GnRH test occurred in all subjects during treatment. Conversely, FSH levels in the third month of treatment tended to be higher in the depot GnRH-A groups than in group B, and this difference achieved statistical significance ($P < 0.05$) in groups G and L during test C. In GnRH test B, while the mean estradiol (E_2) level was less than 75 pmol/L (<20 pg/mL) in all group B subjects, individual E_2 levels were greater than 75 pmol/L in five patients receiving depot GnRH-A (two in group G, one in L, and two in T). Finally, individual E_2 levels during test C were greater than 75 pmol/L in only two patients of group G, who also reported vaginal spotting. Thus, we conclude that in adult women, 1) iLH was profoundly suppressed in the third month of administration of all GnRH-A tested; 2) FSH suppression with depot GnRH-A was less marked than that with high-dose short-acting sc buserelin; and 3) signs of an incomplete block of ovarian function can be present in the third month of depot GnRH-A administration, particularly when goserelin is employed. (*J Clin Endocrinol Metab* **77:** 130–133, 1993)

THE USE of GnRH analogs (GnRH-A) for the treatment of benign and neoplastic disorders of the human reproductive system has gained general acceptance worldwide (1). The more recent introduction of depot GnRH-A formulations that remain active over at least a 4-week period has further increased acceptability and improved compliance with this form of therapy. However, because of the relatively low daily GnRH dosages released by depot formulations, it is possible that the existing standard regimens employed may be inadequate to ensure complete pituitary suppression and gonadal steroid reduction into the castrate range. This issue is of particular importance when hormone-dependent neoplastic disorders are treated. Incomplete pituitary-ovarian suppression during GnRH-A treatment has been anecdotally reported in adults and suggested in children treated with depots (2) and intranasal short-acting preparations (3); however, this issue has never been systematically investigated in women. Furthermore, because of the availability of different depot GnRH-A, results inferred from studies performed with a single GnRH-A may not be generally applicable. Thus, we elected to study pituitary-ovarian suppression in adult women who received three different depot GnRH-A formulations and to compare it to treatment with a short-acting GnRH-A given sc at a high dose.

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Subjects and Methods

Patient population

A total of 40 women participated in the study. All subjects were less than 36 yr old and had regular menstrual cycles of 26- to 32-day duration. The majority of patients (n = 27) presented male-related infertility, 11 were treated for endometriosis, and 2 had a small uterine leiomyoma. Informed consent was obtained from all patients. All subjects underwent a basal clinical and endocrine evaluation (Table 1); no between-group differences were found in body mass index (BMI) or serum levels of LH, FSH, PRL, estradiol, testosterone, GH, TSH, free T_4, or glucose. Fasting serum insulin levels were significantly higher ($P < 0.05$) in group G than in group B; however, none of the individual insulin values were above normal.

Protocol

Subjects were admitted to the out-patient section of the Reproductive Medicine Unit of Bologna University on the seventh day of a spontaneous menstrual cycle. On that day, a standard GnRH challenge test was performed (test A); 100 μg GnRH were administered iv, and blood samples for the determination of LH, FSH, and estradiol (E_2) were drawn 15 min before and 0, 15, 30, 60, and 120 min after the GnRH bolus. Immediately after this initial test, GnRH-A administration was begun. Buserelin (Suprefact, Hoechst Italia, Milan, Italy) was self-administered by the patients sc at a dose of 300 μg twice a day for 84 days (group B). Conversely, all depot GnRH-As were injected by one of the investigators under controlled conditions at 28-day intervals, three times, according to standard suggested regimens: goserelin (Zoladex, ICI Italia, Milan

TABLE 1. Baseline clinical and endocrine evaluation of subjects

GnRH analog	Age (yr)	Wt (kg)	Ht (cm)	MC (days)	BMI (kg/m^2)	LH (IU/L)	FSH (IU/L)	PRL ($\mu g/L$)	E_2 (pmol/L)	P (nmol/L)	T (nmol/L)	GH ($\mu g/L$)	TSH (mU/L)	Free T_4 (pmol/L)	Glucose (mmol/L)	Insulin (pmol/L)
Buserelin	33 ± 1	58 ± 1	162 ± 1	28.5 ± 0.3	22 ± 1	7.9 ± 3.6	7.3 ± 1.0	12 ± 1	341 ± 110	2.5 ± 0.5	2.4 ± 0.4	2.2 ± 0.7	0.8 ± 0.1	17 ± 2	4.9 ± 0.2	36 ± 6
Triptorelin	30 ± 1	59 ± 3	162 ± 2	29.0 ± 0.4	22 ± 1	9.8 ± 1.8	7.1 ± 1.0	14 ± 2	240 ± 55	2.2 ± 0.4	2.5 ± 0.2	2.3 ± 0.5	1.2 ± 0.1	18 ± 1	5.1 ± 0.2	51 ± 4
Leuprorelin	30 ± 1	62 ± 4	165 ± 1	29.0 ± 0.4	23 ± 1	6.8 ± 0.9	7.1 ± 1.0	10 ± 1	299 ± 64	1.9 ± 0.1	3.1 ± 0.2	3.0 ± 1.3	1.1 ± 0.1	17 ± 1	4.7 ± 0.2	52 ± 6
Goserelin	32 ± 1	65 ± 3	168 ± 1	29.4 ± 0.5	23 ± 1	6.3 ± 1.6	7.0 ± 0.8	9 ± 1	237 ± 24	2.9 ± 0.8	2.8 ± 0.1	1.5 ± 0.4	1.2 ± 0.2	19 ± 1	4.7 ± 0.3	78 ± 7[a]

MC, Menstrual cycle length; P, progesterone; T, testosterone.
[a] $P < 0.05$ vs. the Buserelin group.

Italy), 3.6 mg, sc (group G); leuprorelin (Enantone, Takeda Italia Farmaceutici, Rome, Italy), 3.75 mg, im (group L); and triptorelin (Decapeptyl, Ipsen, Milan, Italy), 3.75 mg, im (group T). Two additional standard GnRH tests were performed on days 57 (test B) and 84 (test C) from the beginning of GnRH-A administration in all subjects (including group B), *i.e.* 1 and 28 days after the third depot GnRH-A dose in groups G, L, and T. Clinical parameters (*e.g.* hot flashes, menstrual bleeding, and other side-effects) were recorded by the patient and reported monthly.

Assays

All samples of each test were run in the same assay. LH and FSH were measured with an enzyme immunofluorometric assay (IFMA; Tosoh Corp., Hayakawa, Japan). The sensitivity of both the LH and FSH IFMAs was 0.5 IU/L. The intra- and interassay coefficients of variation (CVs) of the FSH IFMA at low (~~3.5 IU/L), medium (~~9.3 IU/L), and high levels (~~56.0 IU/L) of the standard curve were, respectively, 2.1% and 3.4%, 5.3% and 6.4%, and 6.0% and 7.1%. The intra- and interassay CVs of the LH IFMA at low (~~1.8 IU/L), medium (~~8.5 IU/L), and high levels (~~66.1 IU/L) of the standard curve were, respectively, 10.2% and 24.4%, 6.8% and 4.9%, and 7.1% and 8.0%. As the majority of LH values for GnRH tests B and C were near or below the detection limit of IFMA, LH measurements in all of these samples were repeated with an immunochemiluminometric assay (ICMA; Ciba Corning Diagnostics Corp., Medfield, MA). The sensitivity of the LH ICMA was 0.08 IU/L. The intra- and interassay CVs of the LH ICMA at low (~~0.9 IU/L), medium (~~4.5 IU/L), and high levels (~~55.0 IU/L) of the standard curve were, respectively, 4.1% and 7.7%, 2.4% and 4.9%, and 1.9% and 4.5%. E_2 was measured in unextracted serum by RIA (Diagnostic Products Corp., Los Angeles, CA); the sensitivity of this RIA was 18 pmol/L. The intra- and interassay CVs of the E_2 RIA at low (~~154 pmol/L), medium (~~513 pmol/L), and high levels (~~3534 pmol/L) of the standard curve were, respectively, 11.0% and 14.7%, 7.0% and 9.6%, and 6.0% and 7.4%.

The gonadotropin and E_2 response to the GnRH challenge tests were assessed as the mean ± SE, and statistical differences between groups were assessed with an unpaired Student's *t* test (two-tailed).

Results

Clinical

All subjects admitted to the study completed the 3-month treatment period. The standard side-effects of GnRH-A therapy (hot flashes, vaginal dryness, *etc.*) were reported by all patients in the second and third months of drug administration. Menstruation or light vaginal bleeding was reported by most patients in the first month of GnRH-A, but amenorrhea was present in the second and third months of treatment in all but two subjects, who reported vaginal spotting; both of these patients belonged to group G.

Endocrine

The mean gonadotropin and E_2 levels during GnRH tests A, B, and C are shown in Table 2. All subjects had a response to the basal GnRH test (test A) appropriate for the mid- to late follicular phase of the normal menstrual cycle. Mean FSH levels during test A were higher ($P < 0.05$) in groups G and L than in group B; however, none of these values was outside the normal range. The immunoreactive (i) LH response to GnRH was profoundly reduced in all subjects during test B (57 days into GnRH-A treatment) and remained suppressed during test C. The FSH response to the GnRH test was also diminished during GnRH-A treatment, although to a lesser extent than LH. Between-group differences in FSH levels during test B were not significant. Conversely,

TABLE 2. Gonadotropin and E_2 levels during GnRH tests

	LH (IU/L)		FSH (IU/L)	E_2 (pmol/L)
	IFMA	ICMA		
GnRH test A				
Buserelin	13.6 ± 3.0	NM	8.4 ± 0.8	330 ± 114
Triptorelin	23.8 ± 5.3	NM	13.1 ± 2.5	147 ± 22
Leuprorelin	27.0 ± 8.3	NM	13.4 ± 1.8[a]	345 ± 73
Goserelin	25.4 ± 8.4	NM	12.0 ± 1.3[a]	297 ± 84
GnRH test B				
Buserelin	0.7 ± 0.2	0.83 ± 0.35	5.4 ± 0.9	29 ± 4
Triptorelin	0.6 ± 0.1	0.51 ± 0.10	4.3 ± 0.6	51 ± 15
Leuprorelin	0.8 ± 0.1	0.63 ± 0.20	6.7 ± 0.8	26 ± 7
Goserelin	0.5 ± 0.1	0.31 ± 0.08	5.4 ± 1.2	40 ± 11
GnRH test C				
Buserelin	0.7 ± 0.1	0.37 ± 0.09	4.1 ± 0.5	26 ± 4
Triptorelin	0.8 ± 0.1	0.28 ± 0.05	5.5 ± 0.7	33 ± 7
Leuprorelin	0.7 ± 0.1	0.64 ± 0.15	7.0 ± 0.6[a]	22 ± 4
Goserelin	0.6 ± 0.1	0.27 ± 0.07	7.5 ± 1.4[a]	44 ± 15

NM, Not measured.
[a] $P < 0.05$ vs. the Buserelin group in the same GnRH test.

FSH levels during test C were significantly greater ($P < 0.05$) in groups G and L than in group B. Mean E_2 levels were markedly suppressed in all groups during both tests B and C; no between-group difference in mean E_2 levels was found in either test. However, inspection of individual mean E_2 concentrations (Fig. 1) showed that five patients had E_2 levels greater than 75 pmol/L (>20 pg/mL) during GnRH test B; none of these patients belonged to group B, two were in group G, one was in group L, and two were in group T. BMI was not increased in these patients. During test C, all subjects had mean E_2 levels below 75 pmol/L, except two patients in group G who presented mean E_2 levels of 150 ± 10 and 120 ± 20 pmol/L, respectively. In GnRH test C, FSH levels were also greater (10.4 ± 0.2 and 10.6 ± 0.4 IU/L; $P < 0.001$) in these patients than in group B. These two patients were the same subjects who reported vaginal spotting in the second and third months of GnRH-A administration; one of them had been treated for a small intramural uterine leiomyoma (2.5 cm in diameter) that further diminished to about 1.5 cm by the end of the third month of GnRH-A administration.

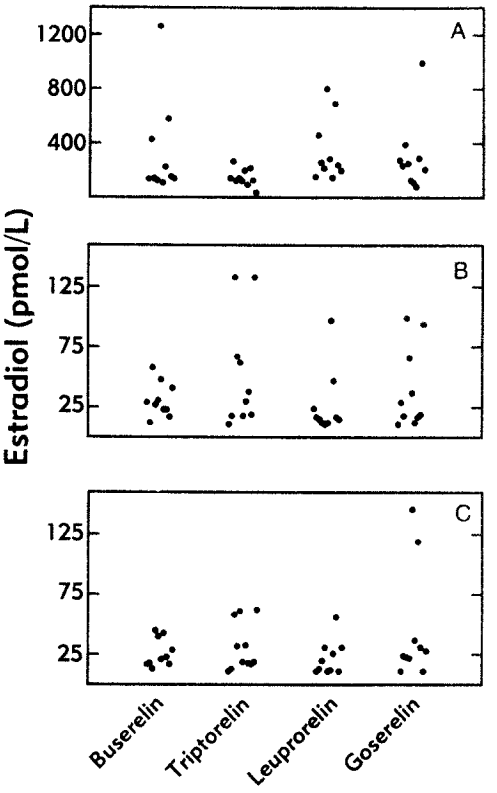

FIG. 1. Serum E_2 levels during GnRH test A (*upper panel*), test B (*middle panel*), and test C (*lower panel*). Each *point* represents mean E_2 levels (six values) during the test in each of the patients treated with different GnRH analog formulations. Notice that five and two subjects had mean E_2 values above 75 pmol/L during tests B and C, respectively.

Discussion

The introduction of GnRH-A bound to lactic-glycolic acid copolymers that slowly release these drugs over a period of 4 weeks or more has simplified this form of therapy and improved patient compliance. Although these formulations have the advantage of constant drug release, administering depot GnRH-A is less flexible than the administration of short-acting GnRH-As. In spite of anecdotal reports of different clinical efficacies of depots, no systematic studies that address this issue have been published. Thus, we elected to investigate the suppressive capacity of three depot GnRH-A (goserelin, leuprorelin, and triptorelin) given in the standard regimen suggested by the manufacturers and to compare it to that of short-acting buserelin, administered sc at the dose we previously showed to be adequate for profound pituitary and ovarian suppression in women (4). We chose to more closely investigate the third month of GnRH-A treatment, *i.e.* a time when complete pituitary-ovarian suppression should be achieved by any analog. We performed a standard GnRH test (100 μg, iv) 1 and 28 days after the third depot GnRH-A injection, *i.e.* at the moment of peak GnRH-A release from microcapsule depots (5, 6) and at the limit of theoretical efficacy of depot GnRH-A, respectively. Depot GnRH-A was personally injected by one of the investigators to insure compliance and a correct mode of administration. Physician's administration was, of course, impossible for the patients treated twice a day with short-acting buserelin; however, we found that the most profound pituitary-ovarian suppression was achieved with this regimen, thus indirectly confirming patient compliance and the adequacy of this regimen for the control group.

Profound LH suppression was achieved in all patients by the beginning of the third month of treatment. Several LH determinations during tests B and C were so low that all serum samples of these two tests were run again in a more sensitive ICMA, which confirmed that serum LH was unresponsive to exogenous GnRH and that no significant difference existed between buserelin and the depot groups. FSH decreased, as expected, from pretreatment levels to test B and C; however, the mean FSH value in the depot groups tended to be higher than that in group B during test C, and this difference achieved statistical significance in groups G and L. Finally, mean serum E_2 levels appeared to be profoundly suppressed during both tests B and C to below the "castration threshold" of 75 pmol/L (20 pg/mL) in all of the analog groups. However, inspection of individual data

showed that during GnRH test B, five patients (one in group L and two each in groups G and T) had mean E_2 levels greater than 75 pmol/L, *i.e.* a level indicative of residual ovarian steroidogenic activity; none of these patients was overweight. Increased E_2 levels on the day after the third depot GnRH-A administration may be related to a residual agonistic activity of the peak GnRH-A levels encountered immediately after microencapsulated depot GnRH-A administration (5, 6). By test C, only two patients (both in group G) had mean E_2 levels above 75 pmol/L and reported persistence of vaginal spotting in the second and third months of treatment, thus indirectly confirming the secretion of biologically relevant amounts of estrogens. One of these patients had received GnRH-A therapy for a uterine leiomyoma. Although, uterine leiomyomas are known to affect endometrial proliferation by the diffusion of locally produced estrogens (7, 8), it is unlikely that this disorder caused this late bleeding, as the patient had no irregular bleeding before treatment, and the tumor was small (2.5 cm), located well within the uterine wall, and shrank significantly during therapy to a diameter of about 1.5 cm.

Scant information exists on the different efficacies of various depot and nondepot GnRH-A preparations. Rossmanith *et al.* (9) reported that over a 6-month period, triptorelin is less effective than goserelin in reducing testosterone levels in normally cycling women. Parinaud *et al.* (10) compared short-acting buserelin, leuprorelin, and triptorelin in an *in vitro* fertilization program and found comparable pregnancy rates, while buserelin was more effective in suppressing iFSH levels. In a preliminary report, Lindner *et al.* (11) found no difference in the suppressive capacity of the same four GnRH-A employed in our study. It is unclear why depot goserelin was less effective in the suppression of ovarian steroidogenetic function in our study. Goserelin appears to be potent (12) and highly effective in suppressing gonadotropin and gonadal steroid secretion (13). However, a recent study (14) reported that at least 9% of 119 patients treated for endometriosis with goserelin for a 6-month period showed persistence of uterine bleeding episodes. Pharmacodynamic studies of goserelin release from its rod-shaped lactic-glycolic acid copolymer carrier indicate that serum goserelin levels reach peak levels about 2 weeks after implantation and are lowest in the early and late periods of administration (15–17); this pattern contrasts with the analog release dynamics of microencapsulated GnRH-A formulations, characterized by peak analog levels hours within the depot injection, followed by stable serum analog levels for a period of at least 28 days (5, 6). Although the lack of specific measurements of serum GnRH-A levels prevented us from documenting it, this release pattern may have affected the clinical efficacy of depot goserelin. Mean FSH levels during test C were significantly higher in group G and markedly elevated in the two patients who reported spotting in the third month of analog treatment. Greater FSH-induced aromatase activity (18) may have contributed to increased E_2 in some GnRH-A-treated patients.

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