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Endocrinological and clinical evaluation of two depot formulations of leuprolide acetate in pre- and perimenopausal breast cancer patients

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Abstract *Purpose*: To evaluate the endocrinological and clinical activity of a new slow-release formulation of leuprolide acetate in breast cancer patients. *Methods*: A total of 50 pre- or perimenopausal patients with early- or late-stage breast cancer who were candidates for endocrine treatment were included in the study and randomly allocated to receive either 3.75 mg of leuprolide acetate every month or 11.25 mg of leuprolide acetate every 3 months. Patients were treated until disease recurrence or progression or for a maximum of 24 months. Treatment outcome, side effects, and serum levels of gonadotrophins, estradiol, progesterone, and Δ4-androstenedione were analyzed at different time points. *Results*: In all, 23 patients were allocated to the monthly formulation and 27, to the 3-monthly formulation. The median time on treatment was comparable. There was no evidence of any difference in clinical outcome or drug-induced side effects, hot flushes being recorded in about 50% of patients in both groups. Altogether, 35 patients were actively menstruating at the beginning of treatment; all of them became amenorrhoic after 3 months and remained so until treatment with leuprolide was continued, irrespective of the allocated treatment. All endocrine parameters, particularly estradiol levels, were suppressed to a similar extent. *Conclusions*: The present results indicate that the two formulations exert a comparable estrogen-suppressive effect and warrant further study of the 3-monthly formulation of leuprolide acetate in breast cancer patients.

Key words Breast cancer · LH-RH analogues · Leuprolide acetate

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Introduction

Oophorectomy represents an established treatment for premenopausal patients with advanced breast cancer. Median response rates of 10–14 months following surgical castration have been reported in about one-third of patients [16], and similar clinical response rates have been achieved following radiation ablation of ovarian function [17]. Because neither surgery nor pelvic irradiation is devoid of significant morbidity, increasing in-

terest is being paid by clinicians to pharmacological maneuvers. Luteinizing hormone-releasing hormone (LH-RH) analogues probably represent the most appropriate pharmacological approach to advanced breast cancer in premenopausal women. Indeed, the chronic administration of these drugs produces a persistent reduction in estradiol to levels in the range of postmenopausal values [10]. The safety and clinical efficacy of treatment with LH-RH analogues has been demonstrated by a number of individual studies [4, 9, 11, 12, 14, 20]. Moreover, the efficacy of these drugs has been shown to be comparable with that of oophorectomy or ovarian irradiation, with side effects being similar and without any major morbidity [3, 19]. More recently, the clinical efficacy of gonadal ablation in the adjuvant setting was demonstrated by a large meta-analysis [6]. Although the most appropriate duration of adjuvant treatment with LH-RH analogues is not yet known, it is clear that prolonged administration of these compounds, i.e. for 2 years or longer, is probably required. Therefore, the development of depot formulations of these compounds represents an attractive area of research. Indeed, it is expected that the new formulations might allow better compliance with pharmacological treatment in patients who might be candidates for long-term treatment with these drugs, independently of their disease stage. Leuprolide (D-leu^6-des Gly^{10}-Pro^9-NH Et-LHRH) is a potent analogue of human gonadotrophin-releasing hormone that has been proved to be highly effective in patients with advanced breast cancer [7]. A study comparing the endocrine activity of 3.75 or 7.5 mg of a slow-release formulation of leuprolide given once every 4 weeks has shown the capability of both formulations to suppress estrogens to levels within the postmenopausal range. This study demonstrated that the slow-release formulation of leuprolide was effective, with no major disadvantage being found for the use of the 3.75-mg rather than the 7.5-mg dose [5]. More recently, a depot formulation of leuprolide acetate containing 11.25 mg of the active drug has been developed. This formulation releases the drug for at least 3 months at the same daily dose that is released by the monthly formulation. The pharmacodynamic and pharmacokinetic properties of the 3-monthly formulation have been studied in dogs and rats, and no major difference has been observed with respect to the monthly formulation [18].

The main purpose of the present study was to investigate the pharmacodynamic activity of the monthly versus the 3-monthly formulation of leuprolide acetate in comparable groups of breast cancer patients. To limit selection biases, treatment was allocated on a random basis.

Patients and methods

A total of 50 pre- or perimenopausal patients with hystologically or cytologically proven breast cancer were recruited into the study. Perimenopause was defined by the absence of regular menses for less than 12 months at the time of trial entry. Previously hysterectomized patients were eligible, provided that they were less than 55 years old or had basal follicle-stimulating hormone (FSH) levels of less than 50 mIU/ml. Primary tumors were to be either estrogen receptor (ER)-positive or of unknown ER status. Positivity of ER status was defined by levels of at least 10 fmol/mg of protein when the charcoal-dextran method was employed or by a positive immunostaining of 10% or more of cell nuclei when the immunohistochemical method was employed. For the purpose of the study, patients surgically treated for breast cancer or with locally advanced and/or metastatic disease who were considered to be candidates for hormone therapy by the local investigators were recruited. Patients previously treated with adjuvant chemotherapy were eligible, provided that they were regularly menstruating at the time of trial entry. All patients gave their written informed consent before randomization. This study was approved by the ethics committees of the Istituto Nazionale per la Ricerca sul Cancro in Genoa and of the other participating centers.

Allocated treatments

Patients were randomly allocated to receive either an i.m. injection of 3.75 mg of leuprolide acetate every month or an i.m. injection of 11.25 mg of leuprolide acetate every 3 months. In actively menstruating patients, treatment had to be started in the follicular phase of the cycle (possibly at the end of menstrual bleeding). In all patients it had to be continued until disease progression or for a maximum of 24 months. Both drug formulations were provided by Takeda Italia Farmaceutici S.p.A. (Rome, Italy).

Clinical evaluations

At the first visit the medical and breast cancer history were recorded and a complete physical examination was done, including staging of carcinoma. For metastatic patients, measurable or evaluable lesions were documented and taken as a point of reference for assessment of the objective response according to UICC criteria [8]. All patients were clinically evaluated every 3 months to rule out disease recurrence (in patients with early breast cancer) or progression (in patients with advanced disease). Toxicity was evaluated at each visit on the basis of WHO directives [13]. If an adverse event not included in the WHO list was observed, its severity was arbitrarily assigned a score ranging from 1 to 4.

Hormone evaluations

Hormone assessments were done at entry (baseline value), after 1 week, after 3 weeks, after 1 month, after 3 months, at 1 week following the administration at the 3rd month, and at 6 months after the beginning of treatment. Blood samples were taken in the morning before 10 a.m. and were centrifuged for 15 min at 3500 rpm within 1 h of collection. The serum obtained was fractionated into individual aliquots of 1.5 ml, which were kept at least at −20 °C until they were transferred to the clinical pathology laboratory of the coordinating center where all hormone evaluations were carried out. The serum concentrations of LH, FSH, estradiol (E_2), and progesterone (Pg) were measured using immunodiagnostics kits (Boehringer, Mannheim, Germany). Tests were performed by an ES300 multibatch analyzer that uses solid-phase assays with biotinylated antibodies in streptavidin-coated polystyrene tubes as a catcher and peroxidase-conjugated antibodies as a tracer. Diammonium 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonate) (ABTS) was the chromogen. The assay's precision and accuracy were assessed by determination of the intra- and interassay coefficients of variation (CVs) using two controls at different concentrations of analyte (low and high) included in the kits. Serum Δ4-androstenedione (Δ4-a) was determined with a radioimmunoassay kit (Ares Serono Diagnostics, Friburg, Germany). In brief, serum was added to ^{125}I-labeled androstenedione in coated tubes with rabbit anti-androstenedione antibodies. The intra- and interassay CVs were determined using controls of two levels of

Table 1 Patients' demography

	Monthly leuprolide (n = 23)	3-Monthly leuprolide (n = 27)
Median age in years (range)	41 (35–52)	42 (35–54)
Median weight in kg (range)	63.5 (50–88)	65.5 (41–95)
Premenopausal	16 (69.6%)	19 (70.4%)
Perimenopausal	7 (30.4%)	8 (29.6%)
Stage I–III	22 (95.6%)	24 (88.8%)
Stage IV	1 (4.4%)	3 (11.2%)
ER-positive	15 (65.2%)	20 (74.1%)
ER-negative/PgR-positive	3 (13.1%)	1 (3.7%)
ER status unknown	5 (21.7%)	6 (22.2%)
Previous chemotherapy		
Yes	18 (78.3%)	19 (70.4%)
No	5 (21.7%)	8 (29.6%)

analyte (low and high) included in the kit. The intra- and interassay CVs were below 10% for all hormone determinations. Hormone concentrations were given in picograms per milliliter for E_2, in nanograms per milliliter for Pg and Δ4-a, and in milliunits per milliliter for LH and FSH.

Statistical analysis

The primary aim of this trial was to investigate the estrogen-suppressive effects of two different depot formulations of leuprolide acetate. Although this was an open trial and randomization was used mainly to minimize selection biases, a comparison between the endocrine profiles of the two formulations was performed to determine whether there was any contraindication to further study of the 3-monthly formulation in a larger group of patients. Student's t-tests for two independent samples were performed between formulations for each hormonal parameter on study at the different time points. The Friendman two-way analysis of variance by ranks was used to test for changes between pretreatment levels of any hormone on study and levels determined at 1, 4, 12, and 24 weeks, respectively. Descriptive statistics were calculated for clinical efficacy parameters and adverse effects [1, 15]. On the basis of previous experience [5], the 3.75-mg monthly formulation was expected to yield a 3-month mean E_2 level of about 20 pg/ml. This level is mainly intermediate between the ranges of E_2 levels that can be commonly measured in postmenopausal women with the assay employed in our study (95% percentile values between 11 and 46 pg/ml). Therefore, it was estimated that 20 evaluable patients per arm at 3 months could be sufficient for the detection of an absolute difference of about 7.5 pg/ml between the two formulations at a P value of 0.05 or less and a power equal to 80%. Even in this case the mean E_2 level achieved by treatment with the 3-monthly formulation at 3 months would be in the range of postmenopausal values. Because a certain percentage of dropout was expected at 3 months, we arbitrarily decided to close study entry after the inclusion of 50 patients.

Results

Clinical efficacy and safety results

In all, 23 patients were allocated to the monthly formulation and 27, to the 3-monthly formulation, of whom 13 and 16 remained on treatment at the time of the present analysis. The median time on treatment was comparable, independently of the allocated formulation. The main characteristics of the study patients are summarized in Table 1. Four patients were enrolled despite their ER-negativity because they had a Pg receptor (PgR)-positive assay. All patients were evaluable for toxicity and for clinical efficacy on an "intention to treat" basis. There was no evidence of any difference in clinical outcome, irrespective of the leuprolide formulation. Common adverse events are shown in Table 2. There was no significant difference in the incidence of adverse events between groups, hot flushes being the most common adverse event reported in both groups. Three patients in the 3-monthly group and two in the monthly group experienced grade 1–2 leukopenia that was not considered to be drug-related because all of them had received prior chemotherapy. A slight increase in body weight during treatment was observed in both groups, the median weight increase being 1.4 (range 0–12) kg in the monthly formulation group and 1.6 (range 0–10) kg in the 3-monthly group. This difference was not statistically significant. No major side effect was noted at the injection site, whatever formulation was used.

Effects on menstrual activity

Altogether, 35 patients were actively menstruating when they started leuprolide treatment, 16 in the monthly formulation group and 19 in the 3-monthly formulation group. Seven of these patients were menstruating after the 1st month of treatment (four in the monthly formulation group, i.e., 25%, and three in the 3-monthly formulation group, i.e., 16%; nonsignificant difference). However, all patients became amenorrhoic after 3

Table 2 Patients with adverse events of any grade on the WHO scale

	Monthly leuprolide (n = 22)[a]	3-Monthly leuprolide (n = 26)[a]
Hot flushes	12 (54.5%)	14 (53.8%)
Breast tenderness	–	1 (3.9%)
Headache	8 (36.4%)	4 (15.4%)
Irritability	2 (9.0%)	1 (3.9%)
Dysuria	1 (4.5%)	2 (7.7%)
Leukopenia	2 (9.0%)	3 (11.5%)

[a] 1 patient in each group refused treatment soon after randomization

Table 3 Hormonal changes observed during treatment with either monthly or 3-monthly leuprolide[a, b]

	LH (mIU/ml) Mean (±S.E.)		FSH (mIU/ml) Mean (±S.E.)		E_2 (pg/ml) Mean (±S.E.)		Pg (ng/ml) Mean (±S.E.)		Δ4-a (ng/ml) Mean (±S.E.)	
	Monthly	3-Monthly	Monthly	3-Monthly	Monthly	3-Monthly	Monthly	3-Monthly	Monthly	3-Monthly
Baseline	14.8 (±3.3)	15.4 (±2.7)	26.0 (±5.8)	33.2 (±7.1)	110.1 (±32.8)	61.2 (±17.2)	2.3 (±1.14)	0.3 (±0.04)	2.3 (±0.3)	2.2 (±0.3)
Week 4	0.9 (±0.18)	1.4 (±0.33)	5.2 (±0.8)	7.0 (±0.6)*	16.7 (±2.2)	25.7 (±6.3)	0.15 (±0.05)	0.11 (±0.04)	1.8 (±0.26)	1.9 (±0.26)
Week 12	0.4 (±0.07)	0.7 (±0.15)	7.9 (±0.9)	8.8 (±0.9)	18.3 (±2.7)	23.3 (±4.4)	0.08 (±0.03)	0.22 (±0.1)	2.0 (±0.29)	1.9 (±0.26)
Week 24	0.4 (±0.08)	0.4 (±0.09)	7.1 (±0.7)	7.7 (±0.6)	16.7 (±3.6)	17.8 (±5.2)	0.06 (±0.03)	0.12 (±0.03)	2.1 (±0.35)	2.2 (±0.64)

* $P < 0.0001$
[a] No significant difference ($P > 0.05$) between values recorded for the two study groups at each point
[b] See text for P values between baseline and on-treatment hormonal levels within each study group

Fig. 1 Mean hormone levels of E_2 as determined before and during treatment with monthly and 3-monthly injections of leuprolide acetate

Fig. 2 Mean hormone levels of Pg as determined before and during treatment with monthly and 3-monthly injections of leuprolide acetate

Fig. 3 Mean hormone levels of FSH as determined before and during treatment with monthly and 3-monthly injections of leuprolide acetate

months and remained so until treatment with leuprolide was discontinued, irrespective of the allocated formulation.

Hormone results

In all, 44 patients (20 in the monthly formulation group and 24 in the 3-monthly formulation group) were

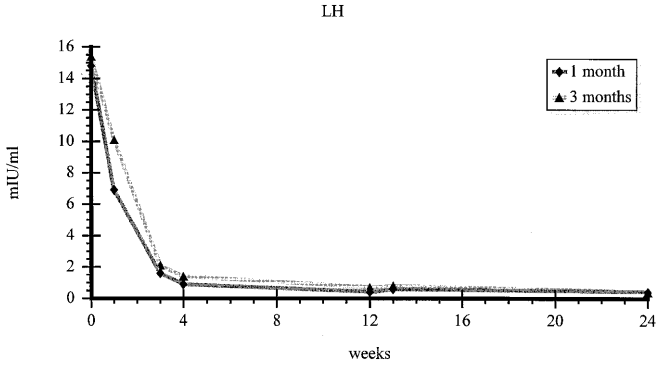

Fig. 4 Mean hormone levels of LH as determined before and during treatment with monthly and 3-monthly injections of leuprolide acetate

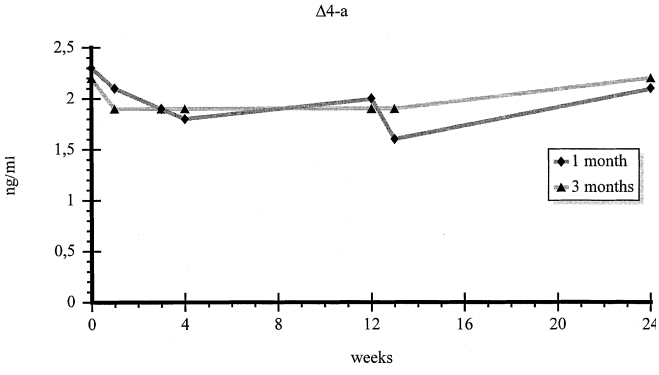

Fig. 5 Mean hormone levels of Δ4-a as determined before and during treatment with monthly and 3-monthly injections of leuprolide acetate

available for hormone evaluations. The mean levels of E_2, Pg, FSH, LH, and Δ4-a determined before and during treatment are shown in Table 3 and in Figs. 1–5.

The mean LH levels recorded at baseline did not differ between groups and were in the range found in the late follicular phase of the cycle, just before or during the ovulatory peak. This indicated that the majority of the women included in this trial had retained a normal ovulatory function, although most of them had previously been treated with chemotherapy. In both groups, LH levels fell to mean levels of about 2 mIU/ml by week 3 and were further suppressed by week 4. These values are consistent with an effective suppression of ovarian steroidogenesis. There was little variability in the LH levels after week 4 and no difference between formulations at any time point considered for this analysis up to week 24. Values recorded at weeks 4, 12, and 24 were significantly lower than pretreatment levels in both the monthly leuprolide group ($P = 0.00001$, $P = 0.00002$, and $P = 0.0002$, respectively) and the 3-monthly leuprolide group ($P = 0.000007$, $P = 0.0001$, and $P = 0.0001$, respectively).

FSH was also strikingly suppressed by week 4 with respect to baseline values ($P = 0.003$ in the monthly group and $P = 0.0001$ in the 3-monthly group) and remained so thereafter up to week 24, although a non-significant trend toward an increase from week 4 to week 24 was evident in both groups. Again, there was no significant difference between the two formulations at any time point considered.

The mean baseline E_2 levels differed in the two groups, although the difference was not statistically significant. This probably reflects the variability in the day of the phase of the cycle on which treatment was started. This is confirmed by the yet higher mean baseline E_2 levels found in patients allocated to the monthly formulation, also after the exclusion of perimenopausal women (data not shown), and suggests that by chance, more women in the monthly leuprolide group probably started their treatment in the late follicular phase or even in the early luteinic phase. Of the 20 evaluable women in the monthly formulation group, 11 had E_2 basal levels higher than 46 pg/ml, i.e., the 95% percentile value of our laboratory range for postmenopausal women, as compared with 10 of 24 evaluable women in the 3-monthly formulation group (difference not significant). E_2 levels were significantly suppressed by as early as week 3 in both groups at levels within the range of our laboratory postmenopausal levels and were further suppressed, showing little variability, up to week 24. The differences observed with respect to pretreatment values at weeks 4, 12, and 24 were highly significant in women allocated to the monthly formulation ($P = 0.003$, $P = 0.0002$, and $P = 0.001$, respectively) as well as women allocated to the 3-monthly formulation ($P = 0.003$, $P = 0.005$, and $P = 0.012$, respectively). Again, there was no difference between formulations at any time point considered for the present analysis.

Pg baseline levels also differed in the two groups, the difference being close to significance ($P = 0.08$). Again, this suggests that more women allocated to monthly leuprolide started their treatment in a later phase of the menstrual cycle. However, both formulations appeared strongly to suppress Pg levels by as early as week 3, the values showing a low degree of variability thereafter up to week 24; again, no difference was evident between formulations at any time point considered.

There were few consistent changes in Δ4-a levels during treatment and there was no marked difference between formulations. Indeed, the mean on-treatment values remained within normal ranges at all times.

Discussion

This study was designed with the main objective of determining whether there were differences in the E_2-suppressive effects of two depot formulations of leuprolide acetate, which would indicate that the 3-monthly formulation was ineffective. The data suggest that the two formulations do not markedly differ. All the important parameters, i.e., LH, FSH, E_2, and Pg, were suppressed to a comparable extent, the pattern of suppression being

very similar to that previously found with the 3.75-mg monthly formulation of leuprolide [5]. Moreover, all the patients who were actively menstruating at the time of trial entry became amenorrhoic by week 12 and remained so thereafter, irrespective of the allocated treatment. In the present study there was no indication of an early increase in LH, FSH, or E_2 levels as previously seen in some studies with other LH-RH agonists [2, 20]. However, this was probably due to the first of the blood samples' being drawn after only 7 days of treatment. By contrast, an early surge in Pg levels was recorded by us after 1 week, indicating that both formulations induced some patients to ovulate. However, the lack of this effect at week 13 and the strong suppression of Pg levels thereafter indicate that complete inhibition of ovulation was achieved and maintained in both groups. The observed disparity in the effects on gonadotrophins is similar to that previously reported following long-term monitoring of LH-RH agonists [5, 20] and probably reflects the release of inhibin by the ovaries as a response to reduced FSH secretion. There was a small and statistically nonsignificant fall in mean Δ4-a values obtained with both formulations between week 1 and week 4, followed by a trend toward a slight increase thereafter. These data confirm that the adrenal glands are the major source of circulating androgens, even in premenopausal patients, and that adrenal function is not influenced by LH-RH agonists. In conclusion, both of the formulations employed in this study appear to be safe and to exert a comparable estrogen-suppressive effect. The present findings warrant further clinical testing of the 3-monthly formulation in premenopausal breast cancer patients.

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