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Treatment of Central Precocious Puberty by Subcutaneous Injections of Leuprorelin 3-Month Depot (11.25 mg)

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Depot GnRH agonists are widely used for the treatment of precocious puberty. Leuprorelin 3-month depot is currently used in adults but has not been evaluated in children. We evaluated the efficacy of this new formulation (11.25 mg every 3 months), for the suppression of gonadotropic activation and pubertal signs in children with central precocious puberty. We included 44 children (40 girls) with early-onset pubertal development in a 6-month open trial. The inclusion criteria were clinical pubertal development before the age of 8 (girls) or 10 (boys), advanced bone age, enlarged uterus (>36 mm), testosterone more than 1.7 nmol/liter (boys), and pubertal response of LH to GnRH (peak >5 IU/liter). The principal criterion for efficacy assessment, GnRH-stimulated LH peak less than 3 IU/liter, was met in 81 of 85 (95%) of the tests performed at months 3 and 6. The remaining four values were slightly above the threshold. The levels of sex steroids were also significantly reduced and clinical pubertal development was arrested. Plasma leuprorelin levels, measured every 30 d, were essentially stable after d 60. Local intolerance was noted after 10 of 86 injections (12%), and was mild in four cases, moderate in five cases, and severe in one. Among these 10 events, 4 consisted in local pain at injection's site. In conclusion, leuprorelin 3-month depot efficiently inhibits the gonadotropic axis in 95% of children with central precocious puberty studied for a 6-month period. This regimen allows the reduction of the number of yearly injections from 12 to 4. (*J Clin Endocrinol Metab* 87: 4111–4116, 2002)

GnRH AGONISTS INHIBIT the pituitary-gonadal axis and their use changed the outcome of central precocious puberty (CPP) (1). These drugs permanently stimulate the gonadotroph and densensitize the pituitary gland to hypothalamic GnRH. Therefore, constant exposure to the drug is one of the critical requirements for its efficacy. Initially, GnRH agonists were administered as daily sc injections and twice daily intranasal instillations (2, 3). These modes of administration have been replaced by monthly depot preparations, which provide a steady release of the drug between injections given intramuscularly (4, 5) or sc (5–12). Long-term results are now available for these treatments, which have been shown to restore height potential negatively affected by CPP (13–16) and to improve the quality of life of the patient by normalizing the timing of puberty (17).

A new 3-month depot formulation of leuprorelin (18–20), administered as quarterly injections, has been developed and is currently used in several countries for the treatment of prostate cancer and endometriosis (21). As the duration of treatment is often long in children with CPP (from the onset of CPP until the age at which puberty normally occurs) and young children often display strong emotional reactions to injections, the use of a slow-release formulation of this kind would increase the acceptability of the treatment and compliance with it. The aim of this study was to evaluate pituitary and gonadal inhibition in girls and boys treated for CPP by quarterly sc injections of 11.25 mg leuprorelin in the 3-month depot formulation.

Abbreviation: CPP, Central precocious puberty.

Patients and Methods

Patients

Forty-four children (40 girls, 4 boys) with CPP were included in the trial. The inclusion criteria were as follows: clinical onset of pubertal development (breasts in girls, testicular enlargement in boys) before the age of 8 in girls and 10 in boys, pubertal response of LH to GnRH (peak >5 IU/liter), plasma testosterone concentration more than 1.7 nmol/liter in boys, uterus length more than 36 mm on ultrasound examination in girls, bone age more than chronological age in both sexes, initiation of treatment before the age of 9 in girls and 11 in boys. Two patients had actual peak LH values slightly below the predefined threshold (4.8 and 4.9 IU/liter) but were maintained in this intention to treat analysis. All but one of the patients underwent a cranial computed tomography scan or magnetic resonance imaging examination at the time of diagnosis or shortly after. The study was approved by the Paris-Cochin Ethics Committee, and the patients and their parents/legal representative gave informed consent for participation.

Design of the trial and endpoints considered

The primary endpoint of this open trial was abolition of the GnRH-stimulated secretion of LH following treatment with the 3-month depot formulation of leuprorelin. Based on our normative values, and on previous experience with monthly depot leuprorelin, the criterion used for adequate gonadotropin suppression was a maximum peak LH concentration of 3 IU/liter (7, 22). Secondary endpoints included basal plasma concentrations of LH, FSH, gonadotropin α-subunit, estradiol, or

testosterone, and leuprorelin, determined monthly. The clinical progression of pubertal signs was evaluated every 3 months. Plasma testosterone levels less than 1 nmol/liter and estradiol levels less than 73 pmol/liter, the upper limits of normal values in prepubertal children (22) were used as thresholds for adequate suppression. The trial lasted 6 months (two injections).

Methods

Treatment. Leuprolide acetate (Des-Gly^{10}-(D-Leu^{6})-LH-RH ethylamide acetate), incorporated into polylactic acid microcapsules was used in the 3-month long-acting formulation (18–20) (Takeda Chemical Industries Ltd., Osaka, Japan). It was suspended in 2 ml of vehicle just before use and injected sc every 90 d, at a dose of 11.25 mg, into children weighing more than 20 kg. A half-dose had been planned for children weighing less than 20 kg but was not required for the patients included, all of whom weighed more than 20 kg.

Hormone determinations. Plasma leuprorelin concentration was determined by RIA, using a polyclonal antiserum developed by Takeda Chemical Industries Ltd.. Leuprorelin, labeled with ^{125}iodine by the lactoperoxidase method, was used as a tracer. The detection limit was 35 pg/ml. The intraassay coefficients of variation were 7.7 and 4.1% for concentrations of 87 and 254 pg/ml, respectively. Serum immunoreactive LH and FSH concentrations were measured by immunoradiometric assays using two monoclonal antibodies (Coatria, Biomérieux, Marcy-l'Etoile, France). The detection limits were 0.12 and 0.1 IU/liter for FSH and LH, respectively. The intraassay coefficients of variation were: 4.3 and 2.9% for concentrations of 1.5 and 15.3 IU FSH/liter, respectively, 4.1 and 2% for concentrations of 1.5 and 14.9 IU LH/liter, respectively. Plasma immunoreactive free gonadotropin α-subunit concentration was determined by RIA, as previously described (23), using the first immunoreactive preparation-human CG α-subunit 75/569 as a standard. The detection limit was 0.04 ng/ml. The intraassay and interassay coefficients of variation were 6.2 and 11.5%, respectively, for a concentration of 0.3 ng/ml, and 4.0 and 7.2%, respectively, for a concentration of 3.5 ng/ml. Estradiol and testosterone concentrations were determined by RIA, with detection limits of 7 pmol/liter and 0.1 nmol/liter, respectively. During GnRH tests (100 μg/m^2), plasma LH and FSH concentrations were determined at baseline and 20, 40, 60, and 90 min after iv injection. We used the Sempé normative data for height and weight (24). Bone age was determined by the method of Greulich and Pyle (25).

Statistics. Results are presented as means ± 1 SD. We used the Wilcoxon signed rank test to compare values at individual time points with baseline values and the Friedman test applied to repeated values for a global analysis of progression during the trial. Statistical tests were performed with the SAS software package (SAS Institute Inc., Cary, NC) (26).

Results

Initial characteristics of the patients (Table 1)

All patients had clinical signs of puberty before the age of 8 yr in girls and 10 yr in boys. Mean age at treatment initiation was 8.2 ± 0.7 yr in girls and 10.8 ± 0.3 yr in boys. There was a 1.1 ± 0.5 (0.3 to 2.3) yr time lag between the onset of clinical puberty and the initiation of treatment. Growth rate was accelerated in all children in whom it could be accurately recorded (8.3 ± 1.7 cm/yr, n = 30). In all patients, bone age was greater than chronological age (mean bone age advance: 2.3 ± 0.9 yr). Weight adjusted for height was more than 120% in 2 boys and 6 girls (18% of the patients). In two patients, the peak LH response to GnRH was less than 5 IU/liter, but this value was within the confidence interval of the result (4.8 and 4.9 IU/liter, respectively) given the coefficient of variation of the assay. They were maintained in the analysis because other criteria for precocious puberty were present. Plasma testosterone concentration was high (>5.2 nmol/liter) in all the boys, whereas estradiol concentration in the girls was more variable, ranging from 20.9 to 179.8 pmol/liter (Tables 2 and 3). CPP was secondary to hypothalamic hamartoma in one girl and considered idiopathic in all other children. Sixty-eight percent of the patients were of Caucasian origin, and 20% had been adopted.

Suppression of gonadotropins and gonadal steroids during leuprorelin treatment (Tables 2 and 3, Figs. 1 and 2)

Leuprorelin was administered sc, at a dose of 11.25 mg, at baseline and at month 3. The mean initial dose was therefore 368 ± 69 (186 to 511) μg/kg. One patient was lost to follow-up after the first injection and another left the study after completing the evaluation at M3 because the family considered the protocol too demanding. Therefore, 42 of the 44 (95%) patients completed the trial. Peak GnRH-stimulated LH concentration decreased in all patients at M3 and remained low at M6 (Fig. 1, Table 2, $P < 0.0001$ for the comparison of M3 and M6 with baseline by the Wilcoxon test; $P < 0.0001$ by the Friedman test for repeated values). In 81 of the 85 (95%) GnRH tests, peak LH values were below the thresh-

TABLE 1. Initial characteristics of the patients

	Girls	Boys
No.	40	4
Age at clinical onset of puberty (yr)	7.1 ± 0.7 (5; 8.1)	9.6 ± 0.4 (9.2; 10)
Cause of puberty (n, %):		
Idiopathic	39 (97%)	4 (100%)
Associated with a CNS lesion	1 (3%)	
At initiation of therapy:		
Age (yr)	8.2 ± 0.7 (5.4; 9)	10.8 ± 0.3 (10.3; 11)
Pubertal stage (n, %)	B2: 9 (23%)	G3: 3 (75%)
	B3: 29 (74%)	G4: 1 (25%)
	B4: 1 (3%)	
Height (SD score)	1.9 ± 1 (−0.7; 3.8)	1.4 ± 0.9 (0.2; 2.5)
Growth rate (cm/yr)	8.2 ± 1.5 (5.6; 11.1)	9.2 ± 3.3 (6; 12.6)
Weight (SD score)	2.2 ± 1.6 (−0.4; 6.6)	2.6 ± 2.3 (0.7; 5.9)
Weight for height (%)	106 ± 14 (79; 146)	119 ± 18 (103; 143)
Bone age (yr)	10.3 ± 1 (8.3; 12)	12.6 ± 0.8 (12; 13.5)
Bone age — chronological age (yr)	2.3 ± 0.9 (0.3; 4.3)	2 ± 0.7 (1.2; 2.8)
Uterus length (mm)	43 ± 8 (25; 63)	

Results are given as means ± SD (range). CNS, Central nervous system.

TABLE 2. Basal and GnRH-stimulated gonadotropin concentrations

	LH (IU/liter)			FSH (IU/liter)		Gonadotropin α-subunit
	Basal	Peak	Peak values ≤3 IU/liter, n (%)	Basal	Peak	
Before treatment (n = 44)	1.1 ± 0.9	15.6 ± 10.9 (4.8; 46.6)	0	3.8 ± 2	10.6 ± 4.5	0.4 ± 0.2
Month 1 (n = 43)	0.6 ± 0.3			1.2 ± 0.7		1.7 ± 0.9
Month 2 (n = 43)	0.5 ± 0.3			1.4 ± 0.8		1.4 ± 0.9
Month 3 (n = 43)	0.4 ± 0.2	1.3 ± 0.8 (0.2; 3.9)	40 (93)	1.1 ± 0.6	1.9 ± 1.5	1.2 ± 0.5
Month 4 (n = 42)	0.4 ± 0.2			0.9 ± 0.5		1.2 ± 0.5
Month 5 (n = 42)	0.4 ± 0.2			1.2 ± 0.6		1.0 ± 0.4
Month 6 (n = 42)	0.3 ± 0.2	1.3 ± 0.9 (0.3; 4.9)	41 (98)	1.3 ± 0.8	2.2 ± 1.6	1.1 ± 0.4

Results are given as means ± SD (range).

TABLE 3. Estradiol (girls) and testosterone (boys) levels

	Estradiol (pmol/liter)	% values <73 pmol/liter	Testosterone (nmol/liter)	% values <1 nmol/liter
Before treatment	n = 40, 77 ± 48 (21; 180)	55%	n = 4, 8 ± 2 (5.5; 10.8)	0%
Month 1	n = 39, 26 ± 7 (10; 51)	100%	n = 4, 1.7 ± 0.7 (0.7; 2.2)	25%
Month 2	n = 39, 22 ± 7 (9; 48)	100%	n = 4, 0.3 ± 0.3 (0.1; 0.7)	100%
Month 3	n = 39, 26 ± 11 (7; 66)	100%	n = 4, 0.7 ± 0.3 (0.2; 1.1)	75%
Month 4	n = 38, 22 ± 7 (8; 32)	100%	n = 4, 0.7 ± 0.3 (0.1; 1.1)	75%
Month 5	n = 38, 22 ± 7 (8; 59)	100%	n = 4, 0.3 ± 0.1 (0.2; 0.6)	100%
Month 6	n = 38, 26 ± 7 (12; 48)	100%	n = 4, 0.7 ± 0.7 (0.2; 2)	75%

Results are given as means ± SD (range).

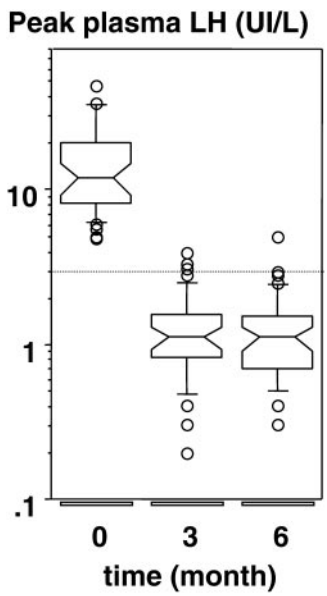

FIG. 1. Peak GnRH stimulated plasma LH. *Horizontal lines* indicate the 10th, 25th, 50th, 75th, and 90th percentiles. Individual values lying outside this interval are also shown; the *horizontal dotted line* represents the 3 IU/liter threshold.

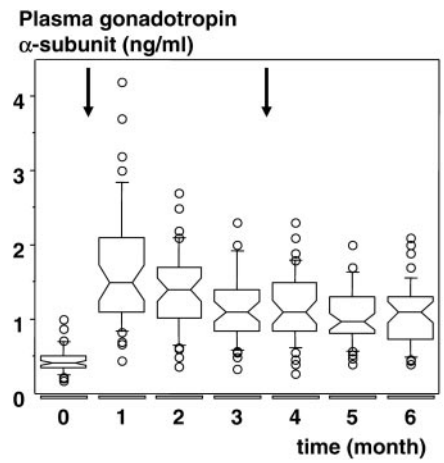

FIG. 2. Plasma gonadotropin α-subunit levels. *Horizontal lines* indicate the 10th, 25th, 50th, 75th, and 90th percentiles. Individual values lying outside this interval are also shown; the *arrows* represent leuprorelin injections.

old of 3 IU/liter (one patient: 3.9 and 4.9 IU/liter at M3 and M6, 2 patients: 3.3 and 3.1 IU/liter at M3 and <3 IU/liter at M6). Peak GnRH-stimulated FSH concentration also decreased significantly ($P < 0.0001$ in the Friedman test for repeated values). Basal plasma LH and FSH concentrations were also significantly lower than baseline levels at M1 and remained stable thereafter.

Concentrations of gonadal steroids significantly decreased during treatment (Table 3, $P < 0.0001$ for changes in plasma estradiol concentration in the Friedman test). All 231 estradiol determinations in patients on treatment were less than 73 pmol/liter, *vs.* only 55% of those performed before treatment. Testosterone was still detectable in the plasma of boys at month 1 (median: 1.7 nmol/liter). From months 2–6, in 17 of the 20 determinations, testosterone concentration was less than 1 nmol/liter (one patient: 1.11 nmol/liter at M3, one patient: 1.11 nmol/liter and 2.0 nmol/liter at M4 and M6).

The concentration of gonadotropin α-subunit in plasma increased, as expected (23), due to the differential regulation of subunits mRNAs, in response to the action of the GnRH agonist (27, 28). A maximum mean value was observed at M1 and a plateau after M2 or M3 (Fig. 2), with a large overlap between treated and untreated values.

Circulating levels of leuprorelin (Fig. 3)

Leuprorelin was detectable in 96% of plasma samples at month 1, 98% of plasma samples at month 6 and all plasma samples at the other time-points tested. Mean plasma leu-

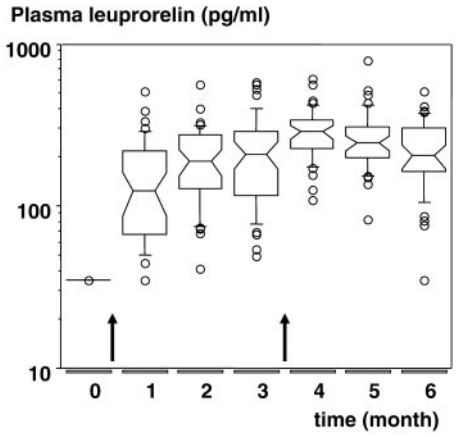

FIG. 3. Plasma leuprorelin levels. *Horizontal lines* indicate the 10th, 25th, 50th, 75th, and 90th percentiles. Individual values lying outside this interval are also shown; the *arrows* represent leuprorelin injections.

prorelin concentration increased during the first 60 d after the injection and then reached a plateau, although a slight rise in plasma leuprorelin levels was observed 1 month after the second injection ($P < 0.005$). No relationship was found between leuprorelin concentration and dose, expressed in micrograms per kilogram or with gonadotropin α-subunit.

Clinical efficacy, effects on growth and skeletal maturation

The clinical signs of pubertal development regressed or stabilized in all patients. Breast development, as reflected by Tanner staging, regressed in 39 and 41% of girls at M3 and M6, respectively, and was stable in the remainder. Two girls had minimal withdrawal bleeding episodes: after the first injection of leuprorelin for both and also after the second injection for one. Uterus length decreased from 43 ± 8 mm at baseline to 39 ± 8 mm at M3 and 38 ± 6 mm at M6 ($P = 0.0004$ by the Friedman test for repeated measurements). Growth rate decreased from 8.3 ± 1.7 cm/yr before treatment to 6.6 ± 2.0 cm/yr during the 6 months of treatment ($P < 0.001$ by the Wilcoxon test). Bone age progressed by 5 ± 7 months during the 6-month trial.

Tolerance

The general tolerance was excellent. Two side effects were reported with some consistency: transient headaches in 16 patients (36%) and hot flushes in 3 (7%). Several gastrointestinal symptoms were reported during the trial (abdominal pain in 10 patients, nausea in 2, vomiting in 4, diarrhea in 2). In all cases, these symptoms were transient and resolved spontaneously or with symptomatic medication.

Local tolerance was carefully noted by the patients and families and recorded at each visit. After the first injection, 6 patients (14%) had a local reaction reported to be mild (n = 4) or moderate (n = 2). These reactions consisted of pain (n = 3), swelling (n = 2) or erythema (n = 1). After the second injection, 4 patients (9%) had a local reaction reported to be severe (n = 1) or moderate (n = 3) consisting of pain (n = 2), swelling (n = 2) or erythema (n = 2). One of the patients had local side effects after both injections (reported to be moderate and severe, respectively); it was therefore decided to stop treating her with leuprorelin 3-month depot after month 6.

Discussion

In this study, we showed that leuprorelin 3-month depot, at a dose of 11.25 mg per 3 months, in children weighing more than 20 kg, was effective at suppressing gonadotropin secretion in most children with CPP, during a 6-month trial. The pharmacodynamic profile of this formulation was similar to that previously observed with the monthly depot formulation of the same drug. Several aspects of the study require further discussion: the dose used, and whether the monthly and 3-month formulations are equivalent in children with CPP, in terms of both tolerance and efficacy.

We used a fixed dose of 11.25 mg in children weighing more than 20 kg. With the 1-month depot, we have previously shown that 3.75 mg injections (roughly 120 μg/kg) are efficient in almost all children with CPP (7). The commonly recommended dose for the 1-month depot contrasts between the European Union (3.75 mg or 120 μg/kg) and the United States (300 μg/kg) (1, 9, 29, 30). Tanaka *et al.* (6) have shown that the minimum effective dose in children is 30 μg/kg. Similarly, the recommended dose of the 1-month depot for patients with advanced prostate cancer is 7.5 mg in the United States and 3.75 mg in the European Union and Japan (31, 32). The 3-month release formulation was then evaluated for use in patients with prostate cancer (33–36), endometriosis (37), and breast cancer (38). In particular, several randomized studies have shown an equivalence between the 1-month and 3-month preparations at the respective doses of 3.75 and 11.25 mg (33, 37, 38). Based on these data, we used a single dose of 11.25 mg in the children in this study, whose mean weight was 30.6 kg; this dose therefore corresponds to approximately twice the dose per kilogram used in adults.

Although we did not perform a randomized trial, the use of similar inclusion criteria, hormonal assays and endpoints made it possible to compare the results for the 3-month depot with those previously obtained for the 1-month depot (7). We observed the expected stabilization of clinical signs of puberty and a decrease in growth rate. Using a cutoff corresponding to the 50th percentile of normal prepubertal children, we observed 95% inhibition of LH responses with the 3-month depot, *vs.* 85 and 98% at 3 and 12 months with the 1-month depot form. The only patient showing incomplete inhibition, with an LH peak of 4.9 IU/liter at 6 months, continued to be treated with leuprorelin 3-month depot after the study and showed a reduction in LH peak at 12 months (1.8 IU/liter). The lack of correlation between dose, expressed in micrograms per kilogram, and LH peak or gonadotropin α-subunit concentration indicates that in most patients, this fixed dose was largely sufficient to inhibit the pituitary gonadal axis. In girls, estradiol levels were more rapidly and uniformly suppressed with the 3-month depot than with the 1-month depot (100% *vs.* 94–97% of values <73 pmol/liter). In boys, although too few patients were studied for definitive conclusions to be drawn, testosterone levels were not adequately decreased after 1 month in 3 of 4 patients, whereas this was the case for only 2 of the 9 patients treated with the 1-month form. However, subsequent mea-

surements were satisfactory (85% of values for testosterone concentration less than 1 nmol/liter, the other 15% being only marginally above this threshold). In adult patients with prostate cancer treated similarly with 3-month depot leuprorelin, all plasma testosterone concentrations at 4 wk were below a threshold of 1.7 nmol/liter (34). It is unclear whether this difference reflects the use of different thresholds, different assays, or differences between adults and children (adrenal androgens). Leuprorelin levels were sustained throughout the study, and trough levels were similar to those observed previously with the 1-month form (3.75 mg SR): 217 ± 51 pg/ml at 3 months and 163 ± 37 pg/ml at 12 months (7) *vs.* 229 ± 137 pg/ml and 229 ± 103 pg/ml at 3 and 6 months with the 3-month form.

Cutaneous intolerance to depot leuprorelin or other GnRH agonists has been reported before (7, 10, 39, 40), affecting 3–13% of patients, with 4% of patients affected in our study with the 1-month depot formulation (7). In the clinical trial presented here, 9 of the 44 patients (20%) reported local intolerance consisting of local pain only in 4 cases. Five patients (11%) had moderate (n = 4) or severe (n = 1) local intolerance, leading to treatment withdrawal in one case. In adults, randomized trials have concluded that the 3-month form results in a higher (41) or equal (37, 38) frequency of local adverse events than the 1-month form. Whatever the frequency, clinicians and families using these treatments should be aware of the possibility of local reactions, in some cases severe, which should lead to treatment with the 3-month formulation being stopped.

In conclusion, leuprorelin 3-month depot efficiently inhibits the pituitary gonadal axis in 95% of children studied with CPP during a 6-month trial. Further follow-up should evaluate the long term efficacy of this formulation in CPP. As with the 1-month depot, careful follow-up of cutaneous tolerance is required and the treatment should be stopped if a severe cutaneous reaction occurs. Similarly, the efficacy of treatment should be assessed regularly by GnRH testing with precise criteria for adequate inhibition. Reduction of the number of yearly injections from 12 to 4 should improve the acceptability of and tolerance to treatment in children with central precocious puberty.

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