Serum Luteinizing Hormone Rises Within Minutes After Depot Leuprolide Injection: Implications for Monitoring Therapy

Suruchi Bhatia, MD; E. Kirk Neely, MD; and Darrell M. Wilson, MD

ABSTRACT. Objective. To find the time of the serum gonadotropin peak after depot leuprolide injection in children and to show that depot leuprolide therapy can be monitored by measuring serum luteinizing hormone (LH) immediately after injections.

Study Design. We measured concentrations of leuprolide, LH, and follicle-stimulating hormone (FSH) at multiple time points before and after the first dose of depot leuprolide in 14 pubertal children beginning therapy. Gonadotropins and sex steroids were measured again after the fourth dose.

Results. Serum leuprolide, LH, and FSH levels rose rapidly after initial injection, reaching sustained elevations at 30 to 120 minutes. The median LH level increased from 2.1 mIU/mL at baseline to a peak of 27.5 mIU/mL at 45 minutes, and FSH increased from 5.2 to 16.5 mIU/mL. After 3 months on therapy, median serum LH after depot leuprolide injection was only 0.83 mIU/mL, similar to levels observed after intravenous or subcutaneous gonadotropin-releasing hormone stimulation in comparable subjects on depot leuprolide.

Conclusion. Our pharmacokinetic data demonstrate that free leuprolide present in a depot leuprolide injection is equivalent to gonadotropin-releasing hormone in stimulating a rapid rise in serum gonadotropin concentrations. We propose that a single serum sample for LH immediately after injections.

ABBREVIATIONS. CPP, central precocious puberty; GnRH, gonadotropin-releasing hormone; LH, luteinizing hormone; FSH, follicle-stimulating hormone; IM, intramuscular; IV, intravenous; SQ, subcutaneous.

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entral precocious puberty (CPP) is the early onset of pubertal development attributable to premature gonadotropin release by the pituitary. Most commonly idiopathic, CPP can also result from central nervous system trauma or tumors. Untreated, precocious puberty can result in short stature and significant psychosocial problems for children undergoing sexual maturation years before their peers. Pulsatile secretion of endogenous gonadotropin-releasing hormone (GnRH) results in luteinizing hormone (LH) and follicle-stimulating hormone (FSH) release from the pituitary and progression of puberty, whereas the tonic stimulus of a long-acting GnRH analog causes an initial surge of gonadotropin release, followed by suppression of pituitary gonadotropin production and release. Depot leuprolide (Depot-Lupron, TAP Pharmaceuticals, Deerfield, IL), a commonly used long-acting GnRH analog, is given by monthly intramuscular (IM) injection and is slowly released into the circulation to provide such tonic suppressive levels.

Depot leuprolide consists of leuprolide enmeshed in microspheres of a glycolic and lactic acid copolymer. Free leuprolide is also present in the preparation and is absorbed into the circulation within minutes of injection, while the microspheres slowly release leuprolide, providing steady, suppressive levels. Pharmacokinetic data in adults receiving therapy for prostate and breast cancer show that free leuprolide in the depot preparation rapidly enters the serum, peaking at 45 to 60 minutes. In these studies in adult cancer patients, a rise in serum LH and FSH was documented 12 hours after injection but was not studied at earlier time points.

Pharmacokinetic data for depot leuprolide in children have not been published.

Dosage of depot leuprolide varies widely in clinical practice in children, ranging from 3.75 to 15 mg at 2- to 4-week intervals. Insufficient doses can be counterproductive, as the waxing and waning of hormone levels may actually mimic the stimulatory GnRH pulses that advance puberty. Frequent testing to ensure adequacy of therapy and to optimize the dose is important in monitoring treated patients.

Patients receiving depot leuprolide therapy are monitored clinically, through assessment of linear growth, sexual maturation, and bone age. However, these parameters may correlate poorly with the degree of suppression of gonadotropins. In addition, there is considerable lag time in bone age advancement as well as significant variability in bone age readings among examiners. Because estradiol is difficult to assay accurately, measurement of LH is the key biochemical assessment of pubertal status. Spontaneous or unstimulated LH concentrations, although potentially useful in diagnosis of CPP, have
not proven precise in monitoring pubertal suppression.\textsuperscript{11} Therefore, GnRH-stimulated LH has been the mainstay of treatment monitoring. An IV GnRH (Factrel, Wyeth-Ayerst, St Davids, PA) stimulation test, involving multiple serum samples for LH before and after an intravenous (IV) dose of GnRH, has been commonly used\textsuperscript{12} to ensure suppression of pituitary gonadotropin release during therapy, and Eckert et al\textsuperscript{13} showed the utility of a single-sample subcutaneous (SQ) GnRH stimulation test. However, persistent lack of availability of GnRH has made these tests impractical.

We hypothesized that depot leuprolide contains enough free leuprolide to cause a rapid rise in serum gonadotropin concentrations in unsuppressed patients. If true, measurement of gonadotropins after therapeutic injection could prove to be useful in the monitoring of treatment efficacy.

**STUDY DESIGN**

**Participants**

All children starting depot leuprolide therapy to halt gonadotropin-mediated pubertal development were invited to participate in this study. Enrollment criteria included clinical and laboratory evidence of central puberty in both girls and boys. Fifteen patients of 16 starting depot leuprolide therapy between February 1999 and August 2000 agreed to be part of the study. No samples were obtained from 1 participant because of venous access difficulties. Written informed consent was obtained from the parents of each participant before enrollment, as well as written assent from all children over 7 years of age. The study was approved by the Stanford University Administrative Panel on Human Subjects in Medical Research.

**Procedures**

Participants received their first dose of 7.5 mg of depot leuprolide IM in the General Clinical Research Center. An IV catheter was placed in an antecubital vein after EMLA cream (lidocaine and prilocaine 2.5%) had been applied to minimize discomfort. Blood was withdrawn through the IV catheter at times and prilocaine 2.5%) had been applied to minimize discomfort.

**RESULTS**

**Historical Data**

Because GnRH was unavailable, IV or SQ GnRH stimulation could not be performed in study participants. For comparative purposes historical data showing LH concentrations in response to IV and SQ GnRH stimulation tests at diagnosis and during depot leuprolide therapy have been included. Some of these data, comparing IV and SQ GnRH testing in the diagnosis of CPP, were previously published.\textsuperscript{13} Previously unpublished data for patients on therapy include LH concentrations after standard IV GnRH stimulation performed at the 3-month follow-up visit and LH after SQ GnRH stimulation between 3 to 6 months.

**Statistics**

All data are graphed as a concentration of each analyte, LH or FSH, versus time relative to the injection. The median value is shown on each graph. Correlations between body weight and peak leuprolide concentration and between peak leuprolide concentration and peak LH and FSH were calculated according to the Spearman method (SAS, Cary, NC). Median peak LH after depot leuprolide and median peak LH and IV and SQ GnRH stimulation tests were compared by Wilcoxon rank-sum test. \( P \) values of less than 0.05 were considered significant.

**TABLE 1.** Subject Characteristics

<table>
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<tr>
<th>Study ID</th>
<th>Age (Year)</th>
<th>Gender</th>
<th>Diagnosis</th>
<th>Weight (kg)</th>
<th>Tanner (BG/P/A)</th>
<th>LH at Diagnosis* (mIU/mL)</th>
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<td>2.8</td>
</tr>
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</table>

BG/P/A indicates breasts or genitalia/pubic hair/axillary hair.

* Spontaneous LH by immunochemiluminometric assay.

† LH after SQ GnRH stimulation.
and had onset of pubertal changes before 8 years of age (Table 1). The 3 oldest participants (05, 09, and 10) were pubertal and began therapy with depot leuprolide in an effort to slow bone maturation and allow for additional linear growth. Participant 09 had an elevated baseline concentration of FSH.

**Serum Leuprolide**

Serum leuprolide concentration showed an immediate rise after IM injection of depot leuprolide, with levels detectable at the first time point of 7.5 minutes (Fig 1). The median leuprolide level peaked at 60 minutes (44.8 ng/mL) and then declined. There was an inverse correlation between participant body weight and maximal serum leuprolide after IM injection ($r = -0.53$, $P = .0492$, Spearman).

**Serum Gonadotropins**

Both LH (Fig 2) and FSH (Fig 3) showed a rapid rise after IM depot leuprolide injection. The median LH level at baseline was 2.1 mIU/mL and promptly rose to 5.9 mIU/mL at 7.5 minutes. Median peak LH increased to 12 and 23 mIU/mL at 15 and 30 minutes, respectively, and reached a plateau of approximately 26 mIU/mL at all time points from 45 to 120 minutes. FSH rose less rapidly than LH, from a median value of 5.2 mIU/mL at baseline to 5.8 mIU/mL at the first time point, and to 8.9 and 12.5 mIU/mL at 15 and 30 minutes, respectively. In contrast with the plateau of LH levels, peak FSH continued to rise gradually throughout the time course, from 13.5 mIU/mL at 45 minutes to 16.5 mIU/mL at 120 minutes.

Peak leuprolide concentration did not correlate with peak LH ($r = -0.015$, $P = .96$, Spearman) or peak FSH ($r = 0.15$, $P = .61$), suggesting that the amount of leuprolide in serum is far in excess of the amount needed to fully stimulate gonadotropin release.

Comparison of peak LH at onset of therapy with historical controls: Maximal LH concentrations after the first dose of depot leuprolide were compared with those seen after either IV or SQ GnRH stimulation in historical controls at the time of diagnosis (Fig 4). The median peak LH level after depot leuprolide (27.5 mIU/mL) was similar to that after IV GnRH (27.2 mIU/mL, $P = .98$, Wilcoxon) or SQ GnRH (22 mIU/mL, $P = .54$).

**Serum Gonadotropins During Therapy**

At the 3-month follow-up visit 13 of 14 participants had a history and clinical examination consistent with adequate suppression of puberty. Participant 07 presented to the follow-up visit with complaints of cyclical abdominal pain and breast tenderness and showed clinical evidence of pubertal progression. Laboratory data at that time included an FSH of 7 mIU/mL and estradiol of 1.1 ng/dL. Her LH of 5.1 mIU/mL after the fourth dose of depot leuprolide was substantially higher than the LH con-
centrations for all the other, clinically well-suppressed, participants. Her dose of depot leuprolide was increased to 11.25 mg IM every 4 weeks, and repeat testing after 3 months on the higher depot leuprolide dose showed a postinjection LH of 0.94 mIU/mL. Although highlighted in Fig 5, her values were excluded for the calculation of median values for treated participants. For all other participants, median serum LH after the 7.5 mg depot leuprolide injection was 0.83 mIU/mL. Median FSH was 2.2 mIU/mL.

Comparison of LH During Therapy With Historical Controls

Median LH after dose 4 for the study group was compared with a group of patients on therapy for CPP previously assessed with IV or SQ GnRH stimulation tests after similar durations of therapy (Fig 5). The median LH values after depot leuprolide, IV GnRH ($P = .17$), and SQ GnRH ($P = .22$) were similar (0.83, 0.6, and 0.54 mIU/mL, respectively).

DISCUSSION

A reliable laboratory method of monitoring therapeutic efficacy is needed for children receiving GnRH agonists. The dose needed to adequately suppress puberty varies considerably. Inadequate therapy can lead to clinical progression and continued advancement of bone maturation. On the other hand, excessive dosing is costly: recent pharmacy retail pricing at our hospital shows that a 3.75-mg dose of depot leuprolide costs a patient $643, 7.5 mg costs $855, and 11.25 mg costs $1598. More importantly, overtreatment may suppress endogenous growth hormone secretion and decrease growth velocity and bone density accrual below that expected in the prepubertal period. A convenient and reliable method of monitoring could help the practitioner to avoid undertreatment of patients with CPP and also might permit judicious lowering of depot leuprolide dosage in well-controlled patients, with subsequent testing performed to confirm pubertal suppression at the lower dose.

Clinical scoring systems have been devised in an attempt to standardize assessment of CPP patients on therapy, but most continue to rely on subjective measures such as change in bone age. Although careful clinical assessment is crucial in follow-up of these patients, an objective and prompt laboratory measure of treatment success or failure is needed. The GnRH stimulation test provides just such a measure. IV administration remains the standard, but SQ GnRH testing is reliable and more convenient. However, availability of GnRH has been severely restricted in recent years, and GnRH testing requires an additional injection for the child.

Other laboratory measures for assessing therapy have been proposed. Cook et al reported that overnight LH sampling detects treatment failure. This method is expensive and time-consuming, and therefore is not likely to be performed as frequently as might be desirable in treatment monitoring. Twenty-four-hour urine collection for gonadotropins has not proven reliable in discerning treatment failure. An ultrasensitive estradiol assay has been very promising but has proven to be technically difficult. Salerno et al have reported serum sampling for gonadotropins and estradiol 12 hours after dosing with depot-triptorelin, another GnRH analog. Although this method also relies on the stimulatory effect of free GnRH agonist particles in the depot preparation, sampling 12 hours after the treatment dose is inconvenient as well as unnecessary. Finally, short-acting GnRH analogs, such as nafarelin (nasal delivery) and SQ leuprolide, can be used as substitutes for GnRH in testing, but neither therapeutic standards nor time of peak response have been established, and an additional medication is still required.

In our study, we have shown to our knowledge the first pharmacokinetic data of serum leuprolide and

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**Fig 4.** Peak stimulated LH concentrations (mIU/mL) in untreated participants: data for study participants receiving their first dose of depot leuprolide (column 1; $N = 14$) compared with historical data for girls with precocious puberty tested with IV GnRH (column 2; $N = 16$) and/or SQ GnRH (column 3; $N = 14$) stimulation tests. Bar marks median values.

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**Fig 5.** Stimulated LH concentrations (mIU/mL) in treated participants at dose 4 (3 months): data for participants receiving their fourth dose of depot leuprolide (column 1; $N = 13$), compared with historical data for girls with precocious puberty tested with IV GnRH (column 2; $N = 10$) and SQ GnRH (column 3; $N = 12$) stimulation tests after 3 to 6 months on depot leuprolide therapy. Bar marks median values. Participant 07 highlighted because of treatment failure and not included in calculation of median for suppressed patients.
gonadotropin concentrations after IM depot leuprolide in children. Serum leuprolide, LH, and FSH concentrations all rise very rapidly after a 7.5-mg dose of depot leuprolide in pubertal children. The free leuprolide available in IM depot leuprolide functions interchangeably with GnRH in stimulation of pituitary LH release, and in a rapid time course comparable with that induced by IV GnRH. Peak LH levels 10-fold higher than basal levels are sustained from 30 to 120 minutes after depot leuprolide injection, allowing a single sample obtained at any time point in this range to accurately represent the maximal postdepot concentration.

Excluding the 1 transient treatment failure, the median peak LH after the fourth injection of depot leuprolide was 0.83 mIU/mL, indicating nearly complete suppression of pituitary LH release. This value is similar to those observed after IV and SQ GnRH stimulation testing in patients during depot leuprolide therapy (Fig 5). We cannot exclude the possibility that LH concentrations stimulated by depot leuprolide during therapy are slightly higher than those stimulated by GnRH, as has been observed in comparison of 500 µg SQ leuprolide and 100 µg IV GnRH at the time of diagnosis of CPP. However, this argument is undermined by the indistinguishable LH levels stimulated by 7.5-mg IM depot leuprolide and 100-µg IV GnRH in our studies at diagnosis (Fig 4), implying that both medications at those doses maximally stimulate LH release.

As with the IV and SQ GnRH stimulation tests, in most participants depot-stimulated LH levels at the 3-month therapeutic follow-up were suppressed to the 0.5- to 1.0-mIU/mL range, a figure that is clearly dependent on the assay used, which in this case is an immunochemiluminometric assay. Based on our data, we tentatively use an LH level of <3 mIU/mL as the cutoff for treatment adequacy in our routine clinical practice. The proposed upper limit is consistent with prepubertal norms after GnRH stimulation using this LH assay. It remains to be seen whether follow-up LH levels of 1.5 to 3.0 mIU/mL represent a lesser degree of therapeutic suppression, possibly a transient elevation during the early months of therapy. The proposed threshold needs to be evaluated in more participants for a longer duration of therapy before being put into widespread use. We are also at this time unable to discern an appropriate lower limit of LH indicating excessive dosing.

CONCLUSION

We have demonstrated from pharmacokinetic data that depot leuprolide functions like GnRH itself in promptly stimulating an LH surge in children. We believe that a single serum sample for LH drawn 30 or more minutes after a treatment dose of depot leuprolide is an accurate and reliable method to assess treatment efficacy, in a manner directly comparable with GnRH testing. Measurement of GnRH-stimulated peak LH or LH/FSH ratio, or possibly of spontaneous LH alone by an ultrasensitive assay, will still be needed for the diagnosis of CPP. In contrast, the simple method of obtaining serum LH after depot leuprolide appears to suffice for treatment monitoring. Some clinicians may wish to continue using GnRH-stimulation tests for initial monitoring to establish satisfactory suppression. However, given our intermittent difficulties in obtaining GnRH and the relative laboriousness of other tests, the depot leuprolide stimulation test is a reasonable solution for long-term monitoring of depot therapy. Its convenience allows the test to be used spontaneously and frequently, facilitating more careful management of children with CPP.

ACKNOWLEDGMENTS

The investigation was supported, in part, by Human Health Service grant M01-RR00070, General Clinical Research Centers, National Center for Research Resources, National Institutes of Health, Child Health Research Fund, and TAP Pharmaceutical Products Inc.

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DOI: 10.1542/peds.109.2.e30

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