A Single Luteinizing Hormone Determination 2 Hours after Depot Leuprolide Is Useful for Therapy Monitoring of Gonadotropin-Dependent Precocious Puberty in Girls

VINICIUS N. BRITO, ANA C. LATRONICO, IVO J. P. ARNOLD, AND BERENICE B. MENDONÇA

Unidade de Endocrinologia do Desenvolvimento, Laboratório de Hormônios e Genética Molecular LIM/42, Disciplina de Endocrinologia, Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, 05403-900 São Paulo, Brazil

Long-acting GnRH analogs represent the standard treatment for gonadotropin-dependent precocious puberty. The aim of this study was to determine the hormonal parameters for monitoring the adequacy of depot leuprolide acetate treatment in girls with clinical and hormonal diagnosis of gonadotropin-dependent precocious puberty. Eighteen girls were treated monthly with 3.75 mg depot leuprolide acetate. Adequate hypothalamic-pituitary-gonadal axis suppression during treatment was achieved in 16 of the 18 girls according to the clinical parameters and prepubertal LH levels. In these 16 well-controlled girls, the LH peak after a classical GnRH test was compared with a single LH measurement obtained 2 h after depot leuprolide acetate administration before and during GnRH analog treatment. Before therapy, the mean ± SD LH peak after a classical GnRH test was 18.4 ± 11.2 IU/liter (ranging from 7–41.5 IU/liter), and it was 22.6 ± 8.3 IU/liter 2 h after the first depot leuprolide dose (ranging from 10–35.3 IU/liter). During therapy, the mean ± SD of LH peak after classical GnRH test was 1.4 ± 0.6 IU/liter (ranging from <0.6 to 2.3 IU/liter), and it was 2.7 ± 1.9 IU/liter (ranging from 0.7–6.6 IU/liter) 2 h after depot leuprolide. The LH peak after a classical GnRH test and that 2 h after depot leuprolide administration correlate significantly before and during treatment. In conclusion, we established the LH cut-off values for an adequate depot leuprolide therapy as an LH peak below 2.3 IU/liter after a classical GnRH test or below 6.6 IU/liter 2 h after depot leuprolide. The latter measurement may replace the classical GnRH test as a reliable and convenient tool for monitoring therapy in female gonadotropin-dependent precocious puberty. (J Clin Endocrinol Metab 89: 4338–4342, 2004)

THE CLASSICAL GnRH stimulation test has been often used for the differential diagnosis between gonadotropin-dependent and -independent precocious puberty in children of both sexes as well as in monitoring the efficacy of depot GnRH analog treatment in patients with gonadotropin-dependent precocious puberty (GDPP). However, this test requires a clinical investigation unit or out-patient testing area and multiple blood sampling, which may be cumbersome, especially in young children, besides being painful and expensive (1–5). In addition, the lack of commercial availability of synthetic GnRH has made this test infeasible (6). Therefore, other reliable tools for diagnosing and evaluating therapy efficacy that could be easily performed on an out-patient clinic basis are desirable in clinical practice.

Long-acting GnRH analogs represent the standard treatment for GDPP. The depot leuprolide acetate, a GnRH analog frequently used in the treatment of precocious puberty, consists of leuprolide encased in microspheres of a glycolic and lactic acid copolymer, which are responsible for its slow release. Free leuprolide is also present in the preparation and is absorbed into the circulation within minutes after the injection (1, 7). Based on this last feature, Bhatia et al. (6) recently demonstrated that LH peak levels were sustained from 30 min to 2 h after depot leuprolide injection, indicating that a single sample obtained at any point within this time range is a convenient and accurate tool to assess treatment efficiency.

In the present study we determined whether a single LH determination, 2 h after administration of depot leuprolide, can replace the classical GnRH stimulation test in the monitoring of GDPP therapy. Additionally, we compared the LH response to the pretreatment classical GnRH test with that 2 h after the first depot leuprolide injection.

**Subjects and Methods**

This protocol was approved by the ethical committee of Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo. Written consent was obtained from the patients or their parents. Eighteen girls were recruited for this study. The mean age at onset of treatment of precocious puberty was 5.6 ± 2.6 yr (ranging from 0.9–8.8 yr). All patients had breast and pubic hair development, accelerated growth velocity, advanced skeletal maturation, and pubertal LH responses after classical GnRH stimulation test according to criteria established in our laboratory (8), confirming the diagnosis of GDPP. Bone age was determined by the Greulich and Pyle method (9). Sixteen patients had idiopathic precocious puberty, whereas two had hypothalamic hamartomas (cases 9 and 16). Patients were treated with a standard dose of 3.75 mg depot leuprolide acetate (Lupron, Abbott Laboratories, Chicago, IL) sc, every 28 d; the doses per weight ranged from 89–340 μg/kg.

All girls had a classical GnRH stimulation test performed before treatment by infusing 100 μg GnRH (gonadorelin, Relisorm) iv, and blood samples were drawn at 15 min before and 0, 15, 30, 45, and 60 min after GnRH administration for serum LH measurements. Twelve subjects also had a single LH determination 2 h after the first administration of 3.75 mg depot leuprolide acetate. Clinical parameters monitored at

Abbreviation: GDPP, Gonadotropin-dependent precocious puberty. JCEM is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.
3-month intervals included Tanner staging, growth velocity, and occurrence of menses, with bone age determined every 6 months. A classical GnRH stimulation test was performed in all patients during treatment on the 28th day after depot leuprolide injection. In the following month, after 28 d, another dose of depot leuprolide was administered, and a single LH determination 2 h after the injection was performed in all patients.

Serum LH concentrations were determined by immunofluorometric kits (AutoDELFIA, Wallac Oy, Turku, Finland). The minimal detectable concentration was set at 0.6 IU/liter for LH (8). The inter- and intraassay variabilities were 4% and 6%, respectively.

The criteria for hypothalamic-pituitary-gonadal axis activation obtained from normal prepubertal girls were basal LH greater than 0.6 IU/liter and/or LH peak after classical GnRH test greater than 6.9 IU/liter (8). Adequate hypothalamic-pituitary-gonadal axis suppression during treatment was established by clinical parameters such as regression or arrest of breast development, normalization of growth velocity and rate of bone maturation, and laboratory parameters characterized by prepubertal basal and GnRH-stimulated LH levels.

Statistical analysis

The data are presented as the mean ± sd and range. The correlation between LH peak after classical GnRH stimulation test and single LH determination 2 h after depot leuprolide, at the first administration and during therapy, was performed by Spearman’s method. P < 0.05 was considered statistically significant.

Results

Sixteen of the 18 girls presented adequate control of breast development, growth velocity, and bone maturation (group A). In contrast, two girls presented evidence of pubertal progression and were not included in the statistical analysis (group B).

In group A (n = 16), basal LH levels were at pubertal levels (>0.6 IU/liter) in 13 of the 16 girls (81.25%) before the administration of leuprolide depot. All girls presented LH peak greater than 6.9 IU/liter demonstrating a pubertal response in the classical GnRH stimulation test (Table 1).

Before therapy, the mean ± sd LH peak was 18.4 ± 11.2 IU/liter (ranging from 7–41.5 IU/liter) after a classical GnRH stimulation test, consistent with elevated LH secretion due to GDPP; the LH peak was 22.6 ± 8.3 IU/liter (ranging from 10–35 IU/liter) 2 h after the first depot leuprolide injection. Therefore, an LH level higher than 10 IU/liter 2 h after the first depot leuprolide injection is considered a pubertal response (Table 2). LH values obtained 2 h after the first injection of depot leuprolide presented a positive correlation coefficient with the LH peak after a classical GnRH test (r = 0.6; P = 0.03).

During therapy with depot leuprolide, all girls presented basal LH at prepubertal levels and suppressed LH peak after classical GnRH stimulation test, i.e. LH peak below 6.9 IU/liter according to previously described criteria (8). The mean ± sd LH peak was 1.4 ± 0.6 IU/liter (range, <0.6 to 2.3 IU/liter) after a classical GnRH test and 2.7 ± 1.9 IU/liter (range, 0.7–6.6 IU/liter) 2 h after leuprolide depot, with a positive correlation coefficient (r = 0.62; P = 0.009) between the two LH peaks. The highest LH peaks were 2.3 and 6.6 IU/liter after a classical GnRH stimulation test and 2 h after depot leuprolide, respectively. These values were used as cut-offs for the LH peak after a classical GnRH test and for the LH peak 2 h after leuprolide depot as indicative of adequate hypothalamic-pituitary-gonadal axis suppression (Table 2).

In group B, basal LH was at pubertal levels (>0.6 IU/liter) in one girl and at prepubertal levels in the other patient before the administration of leuprolide depot (Table 3). Both girls showed LH peak greater than 6.9 IU/liter after a classical GnRH stimulation test, indicating a pubertal response. Before therapy, their LH peaks were 20 and 7.5 IU/liter after a classical GnRH stimulation test, and only one patient had an LH peak of 23 IU/liter 2 h after the first depot leuprolide injection, consistent with elevated LH secretion. During therapy with depot leuprolide, these two girls had basal LH at pubertal levels and LH peaks after a classical GnRH stimulation test of 4.3 and 5.7 IU/liter, respectively. These values were the highest LH concentrations observed among patients from the two groups. In addition, their LH peaks 2 h after leuprolide injection were 11 and 7.5 IU/liter, respectively. Based on pubertal progression and high levels of basal and stimulated LH during treatment, the leuprolide acetate dosage was increased to 7.5 mg, sc, every 28 d in both patients. Three months later, they were retested, and their basal and stimulated LH levels had decreased to prepubertal levels (Table 3).

Discussion

The LH response to a classical GnRH stimulation test is considered the gold standard for biochemical evaluation of hypothalamic-pituitary-gonadal axis activation in children with precocious puberty (10). The new third-generation LH assays allow the diagnosis of GDPP through LH determination under basal conditions. We have previously reported that a basal LH level greater than 0.6 IU/liter diagnosed GDPP in 63% of girls with precocious puberty, and an LH peak greater than 6.9 IU/liter after classical GnRH administration diagnosed GDPP in 92.2% of them (8). In the present study, basal LH was at pubertal levels in 14 of the 18 girls (78%) before the administration of depot leuprolide, and the LH peak after the classical GnRH test was greater than 6.9 IU/liter in all girls.

The design of the present study included a single LH measurement 2 h after the first dose of leuprolide depot to obtain data to compare with the LH peak after a classical GnRH test in girls with GDPP. Before treatment, the lowest LH peak obtained after the first depot leuprolide administration was 10 IU/liter, which is higher than the LH values obtained after classical GnRH stimulation (8). These data could be an alternative to diagnose GDPP in girls with a high clinical suspicion when gonadorelin or nondepot leuprolide is not available.

Long-acting GnRH analogs represent the standard treatment for GDPP. A fixed dose of 3.75 mg every 28 d ranging from 89–340 μg/kg was used initially in all patients studied. In the majority of girls (16 of 18), this dose resulted in adequate clinical and hormonal control. In the two girls who showed evidence of pubertal progression and pubertal LH levels in basal and stimulated conditions during treatment, the leuprolide acetate dosage was increased to 7.5 mg monthly, achieving adequate clinical and hormonal control. The usually recommended dose for the 1-month depot leuprolide is lower in the European Union (80–120 μg/kg) than in the United States (200–300 µg/kg). However, the lower
<table>
<thead>
<tr>
<th>Case no.</th>
<th>CA (yr)</th>
<th>BA (yr)</th>
<th>Dose (µg/kg)</th>
<th>Classical GnRH test (100 µg, iv)</th>
<th>2 h after depot leuprolide LH (IU/liter)</th>
<th>CA (yr)</th>
<th>Dose (µg/kg)</th>
<th>Classical GnRH test (100 µg, iv)</th>
<th>2 h after depot leuprolide LH (IU/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Basal LH (IU/liter)</td>
<td>Peak LH (IU/liter)</td>
<td></td>
<td></td>
<td>Basal LH (IU/liter)</td>
<td>Peak LH (IU/liter)</td>
</tr>
<tr>
<td>1</td>
<td>8.8</td>
<td>12</td>
<td>120</td>
<td>1.1 7</td>
<td>10</td>
<td>11</td>
<td>100</td>
<td>&lt;0.6</td>
<td>&lt;0.6</td>
</tr>
<tr>
<td>2</td>
<td>7.2</td>
<td>8.8</td>
<td>89</td>
<td>&lt;0.6 8.8</td>
<td></td>
<td>9.7</td>
<td>73</td>
<td>&lt;0.6</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>6.7</td>
<td>13</td>
<td>118</td>
<td>3.3 7</td>
<td></td>
<td>7.3</td>
<td>81</td>
<td>&lt;0.6</td>
<td>1.1</td>
</tr>
<tr>
<td>4</td>
<td>5.2</td>
<td>11</td>
<td>129</td>
<td>1.8 25.5</td>
<td>25.6</td>
<td>9</td>
<td>83</td>
<td>&lt;0.6</td>
<td>0.8</td>
</tr>
<tr>
<td>5</td>
<td>7.8</td>
<td>11</td>
<td>114</td>
<td>1 12</td>
<td>20</td>
<td>9</td>
<td>86</td>
<td>&lt;0.6</td>
<td>1.6</td>
</tr>
<tr>
<td>6</td>
<td>2.6</td>
<td>5</td>
<td>220</td>
<td>1.1 41.5</td>
<td>35.3</td>
<td>7.5</td>
<td>89</td>
<td>&lt;0.6</td>
<td>1.9</td>
</tr>
<tr>
<td>7</td>
<td>6.4</td>
<td>8.8</td>
<td>119</td>
<td>1.3 23</td>
<td>15</td>
<td>8</td>
<td>90</td>
<td>&lt;0.6</td>
<td>&lt;0.6</td>
</tr>
<tr>
<td>8</td>
<td>3.1</td>
<td>7.8</td>
<td>178</td>
<td>1 12.2</td>
<td>14</td>
<td>6.2</td>
<td>102</td>
<td>&lt;0.6</td>
<td>1.9</td>
</tr>
<tr>
<td>9</td>
<td>1.8</td>
<td>6.8</td>
<td>223</td>
<td>1.1 23.4</td>
<td>27</td>
<td>6.1</td>
<td>113</td>
<td>&lt;0.6</td>
<td>&lt;0.6</td>
</tr>
<tr>
<td>10</td>
<td>6.1</td>
<td>7.8</td>
<td>147</td>
<td>&lt;0.6 7.7</td>
<td>12</td>
<td>7.25</td>
<td>129</td>
<td>&lt;0.6</td>
<td>2</td>
</tr>
<tr>
<td>11</td>
<td>7.0</td>
<td>11</td>
<td>171</td>
<td>2.6 16</td>
<td>24</td>
<td>8</td>
<td>150</td>
<td>&lt;0.6</td>
<td>1.4</td>
</tr>
<tr>
<td>12</td>
<td>2.7</td>
<td>6.8</td>
<td>204</td>
<td>2.8 41</td>
<td>28</td>
<td>4.9</td>
<td>156</td>
<td>&lt;0.6</td>
<td>1.7</td>
</tr>
<tr>
<td>13</td>
<td>8.1</td>
<td>11</td>
<td>111</td>
<td>0.7 7.5</td>
<td>31</td>
<td>8.6</td>
<td>100</td>
<td>&lt;0.6</td>
<td>1.4</td>
</tr>
<tr>
<td>14</td>
<td>8.4</td>
<td>11</td>
<td>106</td>
<td>2.3 15</td>
<td></td>
<td>9.75</td>
<td>81</td>
<td>&lt;0.6</td>
<td>2.3</td>
</tr>
<tr>
<td>15</td>
<td>7.7</td>
<td>11</td>
<td>131</td>
<td>&lt;0.6 25</td>
<td></td>
<td>8.25</td>
<td>122</td>
<td>&lt;0.6</td>
<td>1</td>
</tr>
<tr>
<td>16</td>
<td>0.9</td>
<td>2</td>
<td>340</td>
<td>1.4 23.3</td>
<td>29.7</td>
<td>5</td>
<td>207</td>
<td>&lt;0.6</td>
<td>2</td>
</tr>
</tbody>
</table>

Mean ± SD
5.6 ± 2.6
9.0 ± 2.9
157.5 ± 64
1.4 ± 0.9
18.4 ± 11.2
22.6 ± 8.3
7.8 ± 1.7
111 ± 35
0.6 ± 0
1.4 ± 0.6
2.7 ± 1.9

Range
9.9–8.8
2.0–13.0
89–340
<0.6 to 3.3
7.0–41.5
10–35.3
4.9–11
73–214
<0.6 to <0.6
<0.6 to 2.3
0.7–6.6

CA, Chronological age; BA, bone age.
* Leuprolide depot dosage at the first administration.
TABLE 3. Hormonal data for two girls with GDPP before and during treatment with 3.75 mg depot leuprolide monthly, presenting evidence of pubertal progression (group B)

<table>
<thead>
<tr>
<th>Case no.</th>
<th>CA (yr)</th>
<th>BA (yr)</th>
<th>Dose (µg/kg)</th>
<th>Classical GnRH test (100 µg, iv)</th>
<th>Basal LH (IU/liter)</th>
<th>Peak LH (IU/liter)</th>
<th>Classical GnRH test (100 µg, iv)</th>
<th>Basal LH (IU/liter)</th>
<th>Peak LH (IU/liter)</th>
<th>2 h after depot leuprolide (3.75 mg)</th>
<th>Basal LH (IU/liter)</th>
<th>Peak LH (IU/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>7.2</td>
<td>11</td>
<td>106</td>
<td>2 h after depot leuprolide (111)</td>
<td>8.5</td>
<td>0.9</td>
<td>2 h after depot leuprolide (111)</td>
<td>8.7</td>
<td>0.6</td>
<td>23</td>
<td>8.2</td>
<td>1.1</td>
</tr>
<tr>
<td>Re-test</td>
<td>18</td>
<td>7.7</td>
<td>111</td>
<td>&lt;0.6</td>
<td>8.5</td>
<td>0.6</td>
<td>2 h after depot leuprolide (111)</td>
<td>8.5</td>
<td>&lt;0.6</td>
<td>23</td>
<td>8.2</td>
<td>1.1</td>
</tr>
</tbody>
</table>

CA, Chronological age; BA, bone age.

a Leuprolide depot dosage at the first administration.

b With 7.5 mg depot leuprolide.

doses usually result in the same efficacy as the larger ones, as demonstrated by 1-month treatment with 3.75 mg depot leuprolide in 49 patients (11) and more recently with 3-month 11.25 mg depot leuprolide in 44 patients with GDPP (12).

The criteria for adequate LH suppression during GnRH analog therapy are controversial. In our view, the main laboratory aim is to achieve basal and stimulated LH levels similar to those observed in normal prepubertal children. Assessment of the adequacy of GnRH analog therapy involves evaluation of clinical parameters and gonadotropin secretion (7, 10). The advantage of laboratorial parameters over clinical ones during GDPP therapy with GnRH analog is that they allow a prompt correction of the depot leuprolide dose, avoiding pubertal advancement. The LH response to a GnRH test is the most conclusive way to determine adequate hypothalamic-pituitary-gonadal axis suppression. It has been demonstrated that a complete standard test may not always be necessary. Several studies have sought alternative means of evaluating LH suppression in children receiving GnRH analog therapy. A single LH sample 40 min after sc GnRH (13), overnight LH values (14), 24-h urinary gonadotropin excretion (4), and a single plasma estradiol measurement 12 h after an im injection of a GnRH agonist (15) are some procedures that simplify this evaluation. However, a single LH measurement in blood collected 2 h after the depot leuprolide injection used for treatment itself is a more simple, comfortable, and inexpensive way to monitor therapy in girls with GDPP. There is no consensus regarding the criteria for LH suppression after classical GnRH stimulation test in patients using a GnRH analog (13). Using specific assays for intact LH, such as the immunofluorometric assay, LH suppression has been defined as an LH peak after iv GnRH stimulation of less than 5 IU/liter (16), less than 2 IU/liter (13, 17), or less than 1.75 IU/liter (4). In our series, we defined the cut-off LH peak based on 16 clinically well controlled girls with GDPP. We determined adequate suppression of LH levels during depot leuprolide treatment as LH less than 2.3 IU/liter after classical GnRH stimulation and LH less than 6.6 IU/liter 2 h after depot leuprolide injection. Although the two girls who showed evidence of pubertal progression presented basal LH at pubertal levels, we cannot conclude that basal LH measurement alone is enough to evaluate hypothalamic-pituitary-gonadal axis suppression, because in normal pubertal children basal LH levels may still be in the prepubertal range (8, 18). However, it is noteworthy that basal pubertal LH levels (>0.6 IU/liter) can eliminate the need for a classical GnRH test (8).

In conclusion, we established the LH cut-off values for an adequate depot leuprolide therapy as an LH peak less than 2.3 IU/liter after classical GnRH test or less than 6.6 IU/liter 2 h after depot leuprolide. The latter measurement may replace the classical GnRH test as a reliable and convenient tool for monitoring therapy in girls with GDPP.

Acknowledgments

We gratefully acknowledge the collaboration of the children in this study as well as laboratory staff for hormonal measurements, and Ms. Sonia Strong for the English review.

Received September 3, 2003. Accepted May 27, 2004.

Address all correspondence and requests for reprints to: Dr. Vinicius N. Brito, Hospital das Clinicas Faculdade de Medicina da Universidade de Sao Paulo, Disciplina de Endocrinologia e Metabologia, Avenue Dr. Eneas de Carvalho Aguiar, 155 2° andar Bloco 6, 05403900, Sao Paulo SP, Brazil. E-mail: vinbrito@uol.com.br or beremen@usp.br.

References


JCEM is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.