Urinary excretion of [D-Ser(t-Bu)^6,Des-Gly^10]GnRH ethylamide (buserelin) during therapy of central precocious puberty: a multicentre study

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Abstract. In order to compare the clinical effects of buserelin on central precocious puberty to its excretion in urine, as a parameter of metabolism or compliance, we have studied 52 patients treated either sc or intranasally. In girls with good control, urinary buserelin excretion represented 0.5 ± 0.1% of the daily intranasal dose vs 12.5 ± 2.3% of the daily sc dose. In boys, it represented 0.8 ± 0.2 and 9.9 ± 2.7%, respectively. In the sc treated group, 3 patients (2 girls and 1 boy) with poor control who exhibited excretion levels similar to those with good control were classified as resistant to therapy. Clinical control was poor in 4 intranasally treated girls: 2 had low excretion values suggesting poor compliance or failure of absorption by the nasal mucosa, and 2 appeared resistant to therapy, as urinary excretion levels of buserelin were similar to those of well-controlled patients. In addition, these data suggest that the small amount of buserelin absorbed by the nasal mucosa, as expressed by urinary excretion, is sufficient to desensitize the pituitary gonadotropes without any significant first-pass effect in the systemic circulation. This may explain the clinical effectiveness of the intranasal route for administration of small hormonal peptides acting on the pituitary gland.

Highly active GnRH agonists are successfully used for the treatment of precocious puberty because of the specificity and long duration of their inhibitory effects (1-3). Long-term administration of these agonists, as continuous infusion or repeated administration, leads to a refractoriness of the pituitary-gonadal axis (desensitization), following a short initial period of stimulation (4). This phenomenon of "desensitization" (5) is due to a decrease of pituitary responsiveness to GnRH, probably in relation to a partial loss of pituitary GnRH receptors, and/or to blockade of post-receptor mechanisms that lead to gonadotropin release (6). The effectiveness of this treatment with GnRH agonists was tested in precocious puberty by several investigators and gave promising results, particularly as to decreasing growth rate (7-9). However, an escape from initial pituitary gonadal suppression or a refractoriness to suppression was observed by several authors in gonadotropin-independent sexual precocity or in some cases of the McCune-Albright syndrome (10,11), the mechanisms of which are unknown. Bad compliance may also be related to this escape phenomenon, in particular when using intranasal administration of the agonist.

In order to monitor therapy with the GnRH agonist buserelin in precocious puberty, measurements of urinary excretion of the immunoreactive agonist were undertaken with the collaboration of several European centres which have investigated

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the long-term effects of this GnRH agonist on central precocious puberty (3, 12, 13) using either the subcutaneous or the intranasal route of administration.

Patients and Methods

A total of 52 patients were recruited in the collaborating centres. In each centre the patients were followed according to a similar protocol which was part of a multicentre study organised by Hoechst Company and their representatives. The protocol was accepted by the Ethical Committee of the Department of Pediatrics and Genetics of the University of Geneva Medical School as well as those of the participating centres. Girls were less than 8 years of age and boys less than 9 at the time of diagnosis. Precocious puberty was considered of central origin at the very beginning of therapy. All patients had had a CT-scan made. Among the 40 girls 3 had hamartomas which were not operated, one girl had hydrocephaly with papilloma of the choroid plexus, 2 presented with the Russell-Silver syndrome, 1 with a partially empty sella syndrome, and 1 with the McCune-Albright syndrome (patient No. 1, Table 2). Two girls (patients No. 2 and 3) were later classified as resistant to the therapy, in addition to patient No. 1 in whom the diagnosis was first central precocious puberty; finally she was classified as resistant to therapy, according to the criteria proposed by Comite et al. (11). Patient No. 2 responded later on to two injections per day. The reasons why patient No. 3 did not respond are unknown, in that she could not be classified as a case of McCune-Albright syndrome. Patient No. 4 developed an ovarian cyst as observed at the ultrasonography (Table 2). Among the 12 boys, 2 had hamartomas and one (patient No. 7) was later classified as having gonadotropin-independent precocious puberty, resistant to therapy, owing to Leydig cell hyperplasia.

Administration of buserelin

The GnRH agonist buserelin was administered sc or intranasally (in). Subcutaneous injections were given at a mean dose of 23.1 ± 1.4 μg/kg (± SEM) once a day or of 20.5 ± 0.5 μg/kg twice a day both for girls and boys. Intranasal administration of 300 μg was given three or four times a day (900 to 1200 μg/day) and the dose was calculated according to body weight. Seventeen girls received one sc injection per day and 10, two injections; 13 girls were treated in a mean daily dose of 36.0 ± 0.8 μg/kg; 4 boys received one injection daily (22.9 ± 1.6 μg/kg) and 4 were treated twice daily (2 × 21.3 ± 1.1 μg/kg); 4 boys received a mean in daily dose of 30.7 ± 1.7 μg/kg.

Clinical evaluation

Criteria for "good clinical control" were based on clinical evaluation made by each investigator (decrease in breast volume and uterine size on ultrasonic examination of the pelvis, or decrease in testicular volume). Clinical control was considered good (regression of pubertal signs) in 45 patients (34 girls and 11 boys) and poor (no regression) in 7 patients (6 girls and 1 boy). Any escape phenomenon, either escape from previous good control of the disease or primary failure to respond, was also recorded. Biological "good control" was noted when plasma estradiol-17β was less than 92 pmol/l in girls, and plasma testosterone less than 0.90 nmol/l in boys, and when the GnRH stimulation test performed in each centre during therapy elicited no rise of FSH and LH. A total of 94 urine samples were collected in two daily samples, from 8.00 to 20.00 h and from 20.00 to 8.00 h the next morning. Time of administration of buserelin was recorded for all patients. In case of one injection per day, the urine samples were analysed according to the time of injection, at 12 h after the injection and after the following 13-24 h period.

Radioimmunological measurements

Immunoreactive buserelin and its C-terminal metabolites in urine were measured using a recently described method (14). Assaying immunoreactive "urinary buserelin", this method measures both intact peptide and its metabolites in constant proportions despite different concentrations or urinary buserelin being excrated (15-17). Antiserum R102, kindly given to us by Dr H. M. Fraser (MRC Reproductive Biology Unit, Edinburgh, UK), was raised in a rabbit (18). Highly purified preparations of buserelin kindly supplied by Hoechst Laboratories (Frankfurt, FRG) were used as standard. For this study, radioiodinated buserelin was used as tracer. Urinary buserelin and its metabolites were extracted according to the method described by Bouguignon et al. (19), using Spherosil XOA 400 (Rhône-Poulenc, France) (600 mg) for adsorption and elution by 8 ml of methanol. This extraction procedure allows to recover between 35 to 50% of added buserelin. The lower limit of detection in this assay was 0.81 fmol buserelin/tube. The intra-assay coefficient of variation was 4.0% and the inter-assay coefficient of variation 18.7%. In order to assess the daily differences in the excreted levels, 2 patients had 12-h urine collections during five 24-h periods and two others during four 24-h periods. Urine collections were made two weeks, one, two, three and six months after the beginning of therapy in 4 patients. All 4 patients were treated with buserelin in. The variations in buserelin urinary excretion between the successive periods in the same individual were from 14 to 29%. Results in urinary excretion of buserelin and its metabolites were expressed either by 12 h periods (nmol/12 h) or by urinary buserelin/urinary creatinine ratios (nmol/μmol). The conversion factor for urinary buserelin is 0.81 nmol/l = 1 μg/l. Samples with inadequate creatinine excretion for height were withdrawn from the calculations (20). Plasma estradiol-17β or testosterone concentrations were measured in each participating centre by conventional commercial radioimmunoassays.

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It was observed in all centres that prepubertal levels were < 25 ng/l (92 pmol/l) and <0.25 μg/l (0.9 pmol/l) for plasma estradiol and testosterone, respectively.

Results

A highly significant correlation in urinary buserelin (and its metabolites) excretion per 12 h versus buserelin-creatinine ratio was found (r= 0.857 for the subcutaneous group and 0.604 for the intranasal group, p<0.001). A good correlation between the urinary excretion of buserelin and the dose administered was found both in girls and boys (r = 0.712 and 0.415, p<0.001 and <0.01, respectively), but not in the sc treated groups. In girls, urinary buserelin excretion represented 0.5±0.1% (mean ± SEM) of the daily in dose, and 12.5±2.3% of the daily sc dose of buserelin. In boys, it represented 0.8±0.2% of the daily in dose and 9.9±2.7% of the sc dose, respectively. No difference as to doses of buserelin per kg was observed between girls and boys or between the in and sc routes.

Table 1 gives mean values of dose of buserelin, urinary excretion of immunoreactive buserelin, and urinary buserelin-creatinine ratio measured after sc administration once or twice a day in the female patients with good therapeutical control. Mean plasma estradiol-17β levels in these well-controlled girls was 32.7±3.3 mol/l. Girls with poor clinical control showed excretion levels similar to those with good control (Table 2): one of them (patient No. 4) developed an ovarian cyst during this period with a high level of plasma estradiol-17β, another one (patient No. 2) was on only one injection per day. Urinary excretion was similar to that of the girls with good control and estradiol-17β level was in the pubertal range. Two injections a day were prescribed, resulting in good control.

Data of the 9 girls with good control treated in are presented in Table 1. Mean plasma estradiol-17β was 26.4±5.1 pmol/l. Patients No. 5 and 6 with poor control had very low excretion values of buserelin and buserelin-creatinine ratios owing to poor compliance (Table 2). When compliance was re-established in these two patients, the excretion of urinary buserelin rose to the values observed in well-controlled patients and estradiol levels were in the prepubertal range. In addition, 2 patients (patients No. 1 & 3) were in poor control with high excretion of urinary buserelin. One of them (patient No. 1) presented a McCune-Albright syndrome and was later classified according the criteria of Comite et al. (11). The second one (patient No. 3) showed a resistance of unknown origin, and was resistant to further sc therapy.

Data of the 7 sc treated boys with good control are presented in Table 1. Mean plasma testosterone concentration was 0.62 ± 0.2 nmol/l. One boy (patient No. 7) was resistant to the treatment, as observed clinically by the absence of decrease in testicular volume and by a plasma testosterone concentration above 10.4 nmol/l (Table 2). Urinary excre-

Table 1
Dose of buserelin, buserelin excretion, and buserelin-creatinine (B/Cr) ratios (Mean ± SEM) in patients with good clinical control.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number of patients</th>
<th>Dose of buserelin (µg/kg)</th>
<th>Buserelin excretion (nmol/12 h)</th>
<th>B/Cr excretion (nmol/µmol)</th>
<th>% buserelin excreted per 24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>/sample per 24 h</td>
<td></td>
<td>0-12 h</td>
<td>13-24 h</td>
<td>0-12 h</td>
</tr>
<tr>
<td>Girls</td>
<td>1 × sc</td>
<td>16/24</td>
<td>23.1±1.4</td>
<td>67.2±9.6</td>
<td>2.8±0.8</td>
</tr>
<tr>
<td></td>
<td>2 × sc</td>
<td>9/15</td>
<td>2×20.5±0.5</td>
<td>52.5±15.5</td>
<td>62.0±11.5</td>
</tr>
<tr>
<td></td>
<td>in</td>
<td>9/28</td>
<td>36.0±0.8</td>
<td>2.4±0.7</td>
<td>1.7±0.3</td>
</tr>
<tr>
<td>Boys</td>
<td>1 × sc</td>
<td>4/7</td>
<td>22.9±1.6</td>
<td>48.5±9.9</td>
<td>11.6±12.8</td>
</tr>
<tr>
<td></td>
<td>2 × sc</td>
<td>3/3</td>
<td>2×21.3±1.1</td>
<td>27.4±4.1</td>
<td>23.2±8.6</td>
</tr>
<tr>
<td></td>
<td>in</td>
<td>4/6</td>
<td>30.7±1.7</td>
<td>3.5±0.7</td>
<td>2.2±0.6</td>
</tr>
</tbody>
</table>

sc: subcutaneous administration
in: intranasal administration
Table 2.
Dose of buserelin, buserelin excretion, and buserelin-creatinine ratio (B/Cr) in patients with poor clinical control.

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Dose of buserelin (µg/kg)</th>
<th>Buserelin excretion (nmol/12 h)</th>
<th>B/Cr excretion (nmol/µmol)</th>
<th>Plasma sex steroids</th>
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<tr>
<td></td>
<td></td>
<td>0-12 h</td>
<td>13-24 h</td>
<td>0-12 h</td>
</tr>
<tr>
<td>1</td>
<td>35 in</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>2</td>
<td>1×20 sc</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>19 in</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>2×20 sc</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>5</td>
<td>25 in</td>
<td>0.006</td>
<td>0.006</td>
<td>0.002</td>
</tr>
<tr>
<td>6</td>
<td>30 in</td>
<td>0.001</td>
<td>0.060</td>
<td>0.001</td>
</tr>
<tr>
<td>7</td>
<td>31 in</td>
<td>0.003</td>
<td>0.075</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>2×20 sc</td>
<td>14.9</td>
<td>30.5</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>2×20 Sc</td>
<td>19.4</td>
<td>24.9</td>
<td>3.6</td>
</tr>
</tbody>
</table>

sc: subcutaneous administration  in: intranasal administration
1: McCune-Albright syndrome
2: Resistance? Two injections/day prescribed
3: Resistance of unknown origin?
4: Resistance? Leydig cell hyperplasia?

Discussion

There were no differences as to recruitment, diagnostic procedure or management of the patients among the centres, so the clinical and biochemical observations were fairly homogenous in this multicentre study. The data presented confirm previous investigations suggesting that measurement of buserelin and immunoreactive buserelin metabolites in the urine may be of help in studying the pharmacodynamics of this GnRH agonist (14-17, 21). The level of urinary immunoreactive buserelin can be used as a pharmacokinetic parameter of patient compliance and, in patients with precocious puberty, appears to be useful for comparing the clinical effects of the intranasal therapy when using suppressive buserelin concentrations. The present study confirms the good correlation previously observed between the urinary excretion of buserelin and the intranasal dose (14). In the case of intranasal administration, measurement of urinary excretion of buserelin was useful for establishing the diagnosis of resistant forms as observed in patients with the McCune-Albright syndrome (with low gonadotropin levels) or with gonadotropin-independent Leydig cell hyperplasia (10,11), or for establishing an escape phenomenon after initial suppression. Measurement of urinary excretion of buserelin is useful for differentiating resistance from
poor compliance or low absorption of buserelin by the nasal mucosa. It has been shown that experimental rhinitis does not affect the absorption of buserelin (22), and clinical observations suggest that a common cold or allergic rhinitis does not affect absorption.

As already observed (14), there is a marked difference in absorption and excretion of buserelin given sc and in, using comparable daily doses (23 to 42 μg/kg). This is so because the absorption of oligopeptides such as buserelin by the nasal mucosa is limited (23,24). However, the good clinical control of most of these intranasally treated patients suggests that a limited amount of the agonist reaches the pituitary gland without any significant first-pass effect in the systemic circulation. Anatomically, there is no direct vascular connection between the nasal mucosa and the pituitary gland. One could imagine a simple diffusion of the peptide through the lamina cribrosa and some small recesses of the meningeal tissue and then, through the vascular endothelium of the hypothalamic-pituitary portal system. One could also imagine that the peptide could diffuse to the olfactory lobes or to the terminal nerve, a cranial nerve which is part of the accessory olfactory system and embryologically projects from the nose to the septal-preoptic nuclei in the brain and contains GnRH immunofluorescence (25). Whether buserelin can induce desensitization of these neurones remains to be proven. Whatever the mechanism is, the small amount of peptide absorbed is sufficient for a therapeutic effect and gives validity to the use of the intranasal route for administration of small hormonal peptides acting on the pituitary gonadotropes.

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