Dose dependency of the serum bio/immuno GH ratio in children during pharmacological secretion tests

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ABSTRACT. Dissociation between GH bioactivity (bio-GH) and GH immunoactivity (immuno-GH) is due to the heterogeneity of the molecule: the measurements do not always provide reliable information on the bio-GH. We studied the ratio of bio-GH and immuno-GH during pharmacological secretion tests in 211 sera to study the concentration-response curve of the assay (C1), 16 samples of normally growing subjects with idiopathic short stature (C2), 13 samples from patients with GH deficiency (GHD1) and 6 samples of 3 patients with GHD and normal provocative tests (GHD2). GH bioactivity was determined by the Nb₂ cell proliferation assay (bio-GH) and immuno-GH by a time-resolved immunofluorometric assay (IFMA) (immuno-GH). A non-linear negative relationship between the serum bio-GH/immuno-GH ratio and serum immuno-GH was observed in C1. In log-log plotting representation, two cut-off lines were drawn: a vertical cut-off line separating above-below cut-off serum peak immuno-GH values in provocative tests, and a diagonal cut-off line separating normal-abnormal serum bio-GH/immuno-GH ratio; four areas were defined. GHD1 had normal ratios, but below cut-off peak immuno-GH responses. P2 and P3 of Group GHD2 had abnormal ratios in samples with low serum immuno-GH but only P2 had autosomal dominant mutation. P1 had the same autosomal dominant isolated GHD as P2 but a low normal ratio. Our data underline the importance of relatively low serum GH concentrations in mediating GH biological actions. An abnormal serum bio-GH/immuno-GH ratio might explain certain cases of GHD and might be useful in detecting abnormal circulating isoforms of GH in patients with growth failure.

INTRODUCTION
The diagnosis of GH deficiency (GHD) in children is primarily based on the combination of auxological parameters of growth failure, abnormally low GH secretion, usually detected by inadequate response to provocative tests, and the absence of other causes of short stature (1). Occasionally, magnetic resonance imaging of the pituitary gland might be of great value. Interassay differences in serum immuno-GH measurements have been published (2); this assay difference leads to large discrepancies in the definition of the cut-off values in response to pharmaco-

logical tests. An alternative approach is to evaluate GH response in terms of GH bioactivity (bio-GH) (3); even if there is no evidence that this approach has a more powerful diagnostic discriminatory potency than immunoassays. The well-known heterogeneity of serum GH opens the possibility of discordance between immuno- and bioassays of serum GH values. Indeed, dissociation between human GH (hGH) bioactivity and immunoactivity is present in physiological conditions (4), as well as during overnight GH secretion (5), indicating that standard hGH measurements do not always provide reliable information on the biological activity of the hormone. In the last few years, a number of serum assays have been developed to measure the bio-GH (6). The Nb₂ rat lymphoma cell bioassay (Nb₂ cell bioassay) of serum GH measures a complex biological response (cell proliferation) which includes receptor events and also makes it possible to assess serum bio-GH quantitatively (7).
The finding of discrepancies between immuno- and bioassays of GH in serum may suggest the presence of mutations in the GH-1 gene that causes the production of GH oligomers with low biological activity (8) or abnormal post-translational processing of GH (9).

The aim of this report is to study the ratio of GH biological and immunological activities in blood samples, drawn during pharmacological tests in children with positive immunoactive GH (immuno-GH) secretory responses and in patients with idiopathic short stature or GHD.

MATERIALS AND METHODS

Subjects and samples

Blood samples were collected from different patients at different times (0, 30, 60, 90 and 120 min) during provocative tests of GH secretion (arginine, insulin or L-Dopa administration). These tests have been described elsewhere (10). Immuno-GH was measured by immunofluorometric assay (IFMA) and bio-GH was assayed by Nb2 cell bioassay.

Control Group 1 (C1)

A total of 211 blood samples were collected from 51 subjects (26 males and 25 females) with serum GH peak values above the cut-off for the IFMA (5 ng/ml). The age of these subjects ranged from 0.50 to 13.1 yr (age mean±SD: 8.56±4.01 yr). Values of serum immuno-GH ranged from 1 to 27.3 ng/ml and those of bio-GH from 1.19 to 34.8 ng/ml.

Group C2

Sixteen blood samples corresponding to peak values of pharmacological tests were collected from 16 subjects (9 males and 7 females) with mild idiopathic short stature or suprasellar lesions. Inclusion criteria were normal growth velocity (±1 height velocity SDS) during the previous year and normal serum immuno-GH response to provocative tests (>5 ng/ml, as determined by IFMA). The chronological age of this group ranged from 2.24 to 13.4 yr (age mean±SD: 8.26±4.13 yr), bone age from 2 to 11.5 yr (mean±SD: 8.31±3.58 yr), height (SDS) from −1.8 to +1.0 (mean SDS: −0.75±1.32) and growth velocity (SDS) from −1.1 to 1.6 (mean SDS: −0.04±0.82). Peak serum of GH measured by IFMA after two provocative tests ranged from 6.97 to 39.4 ng/ml (mean±SD: 19.1±7.44 ng/ml).

Group GHD1

Thirteen blood samples corresponding to peak values of pharmacological tests collected from 13 subjects (8 males and 5 females) with GHD. These patients had clinical features of GHD. Inclusion criteria were low growth velocity (<−1 SDS) and peak serum immuno-GH levels <5 ng/ml. The chronological age ranged from 1 to 15.25 yr (mean±SD: 9.07±4.95 yr), bone age from 0.5 to 12 (mean±SD: 7.12±4.92 yr), height (SDS) from −3.75 to −1 (mean SDS: −2.49±0.80) and growth velocity (SDS) from −3.7 to −0.9 (mean SDS: −2.19±0.82). Peak serum of GH measured by IFMA after 2 provocative tests ranged from 1.25 to 4.6 ng/ml (mean±SD: 2.80±1.02 ng/ml).

Group GHD2

Six blood samples were collected during two pharmacological tests from three patients with the diagnosis of GHD, despite positive responses to pharmacological tests when assayed by either a GH polyclonal immunoassay or IFMA. These patients were selected on the basis of severe short stature (height <=−2.5 SDS), poor growth velocity (<−1 SDS), and serum immuno-GH response to provocative tests >10 ng/ml (2). The clinical data of these patients are reported in Table 1.

However, serum GH concentrations evaluated with the Nb2 cell bioassay, in two pharmacological tests, remained below or just above the cut-off value. P1 and P2 had autosomal dominant GH-1 gene mutation (R183H), whose phenotype descriptions have been published previously (11). Instead, no mutation was detected in P3 after GH-1 gene sequencing. Therefore, the etiology of the GHD remained undetermined.

The study was approved by the patients’ parents and the Internal Review Board of the Garrahian Pediatric Hospital.

GH immunoassays

The time-resolved IFMA of serum GH (Delfia, Wallac Oy, Turku, Finland) has been previously described (2). The monoclonal antibodies used for this IFMA selectively bind the 22-kDa form of hGH (12). The intra- and interassay coefficient of variation (CVs) were 3.3% (no. = 90) and 4.7% (no. = 90), respectively. The Serono GH polyclonal immunoassay (Bio Chem Immuno Systems, Rome, Italy) has been previously described (2). This assay uses a mixture of antibodies for several epitopes of the GH molecule. World Health Organization (WHO) IRP human GH for RIA 66/217 is used for calibration. The intra- and interassay CVs were 5% (no. = 90) and 7% (no. = 90), respectively.

Nb2 cell bioassay

This bioassay was carried out using a Nb2 cell line according to the method of Tanaka et al. (7) with minor modifications (13). The Nb2 rat node lymphoma cell line was routinely cultured in 25 cm² tissue culture flasks in RPMI 1640 medium supplemented with 10% horse serum, 10% fetal calf serum (FCS), L-glutamine (2 mmol/l), gentamicin (50 mg/ml), and 2-mercaptoethanol (10⁻⁴ mol/l) at 37°C in a humidified atmosphere of 5% CO₂ and 95% air. Under these conditions, the cell population doubled in approximately 20 h. Stationary cultures of the Nb2 cells were obtained by transferring cells from the medium supplemented with FCS to RPMI 1640.

Table 1 - Clinical data of group GH deficiency (GHD) (patients P1, P2, P3).

<table>
<thead>
<tr>
<th></th>
<th>W (Kg)</th>
<th>H (cm)</th>
<th>CA (yr)</th>
<th>BA (yr)</th>
<th>GV (cm/yr)</th>
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<tr>
<td>P1</td>
<td>7.4</td>
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<td></td>
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<tr>
<td>P3</td>
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<td>120</td>
<td>9.80</td>
<td>8.0</td>
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</table>

W: weight; H: height; CA: chronological age; BA: bone age; GV: growth velocity.