CLINICAL STUDY

A simple diagnostic test using GH-releasing peptide-2 in adult GH deficiency

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Abstract

Objective: The international, first-line diagnostic test for adult GH deficiency is the insulin tolerance test (ITT), which is contraindicated in some patients due to severe adverse events. Alternatives such as GH-releasing hormone combined with arginine or GH-releasing peptides (GHRP) have been proposed. We validated the use of GHRP-2 for diagnosing adult GH deficiency (GHD).

Methods: Seventy-seven healthy subjects and 58 patients with peak GH < 3 µg/l by ITT were enrolled. After overnight fasting, a 100 µg dose of GHRP-2 was administered intravenously; blood samples were taken during the subsequent 2 h and GH measured by immunoradiometric assay.

Results: Serum GH peak occurred within 60 min after GHRP-2 administration in all subjects. GH responses to GHRP-2 were not affected by gender, but were slightly lower in elderly subjects and those with adiposity, although these did not influence diagnosis of GHD. Repeated tests showed favourable reproducibility. Peak GH concentrations after GHRP-2 were significantly ($P < 0.001$) lower in patients (1.36 ± 2.60 µg/l) than the healthy group (84.6 ± 60.9 µg/l) with no difference between hypothalamic and pituitary diseases. Serum GH concentration at the point where sensitivity of response crossed with specificity ranged from 15 to 20 µg/l. A cut-off value of 15 µg/l for diagnosing GHD with GHRP-2 corresponded to the diagnostic value of 3 µg/l in the ITT.

Conclusions: The GHRP-2 provocative test showed favourable reproducibility and was mildly influenced by age and adiposity. Severe GH deficiency could be diagnosed with high reliability using a 15 µg/l (9 µg/l when GH calibrated with recombinant World Health Organization 98/574 standard) cut-off for peak GH concentration.

European Journal of Endocrinology 157 19–27

Introduction

With the increasing worldwide recognition that growth hormone (GH) therapy is required at all stages of life for patients with GH deficiency (GHD), accurate diagnosis is required before commencing long-term treatment. GHD, in both adults and children, is diagnosed biochemically by provocative tests of GH secretory reserve (1); the insulin tolerance test (ITT) was accepted as the international, first-line diagnostic test of choice according to the consensus guidelines for the diagnosis and treatment of adults with GHD (2). The diagnostic criterion for GHD severe enough to warrant therapy was defined as a peak serum GH concentration ($C_{\max}$) less than the arbitrary cut-off of 3 µg/l with the ITT (2).

An advantage of the ITT is that it allows the evaluation of the complete hypothalamic–pituitary–somatotroph axis, making it useful in patients with both hypothalamic and pituitary disease. However, there are

a number of disadvantages to this test. GH secretion is induced by hypoglycaemia, so there is a potential for hypoglycaemia-related adverse reactions (3). It is contraindicated in patients with coronary or cerebrovascular disease, a history of ischaemic heart disease or any convulsive disease and particular attention is needed for the elderly (3). Furthermore, it was reported that the reproducibility of the GH response in an ITT was not sufficient (4–6); a false-negative result, suggestive of insufficient GH secretion, was sometimes obtained even in normal subjects and this was particularly influenced by age and adiposity (7). ITT needs multiple blood sampling and requires close monitoring of patients for several hours in a specialised investigation unit. Thus, there is a need for a simple effective test to establish GHD.

Many other GH provocative tests are employed in clinical practice. The combination of arginine plus GH-releasing hormone (GHRH) has been proposed as a

useful, reproducible, alternative test to the ITT (8, 9). It is not affected by age but by gender and adiposity (9, 10). Patients with primary hypothalamic disease, such as radiation-induced GHD, may exhibit false-positive responses to the arginine plus GHRH test (11, 12).

Use of a group of peptides called GH-releasing peptides (GHRP) has also been investigated, either alone or in combination with GHRH (13–15). GHRPs are synthetic secretagogues (16) that elicit a dose-dependent and specific GH release by binding to a specific receptor, for which ghrelin has been shown to be the natural ligand (17). The combined GHRH plus GHRP test has been shown to be well tolerated and sensitive in diagnosing GHD (13–15). The influences of age and adiposity have been investigated (18, 19), but more studies are needed to confirm these findings. GHRP-2 has the structure: D-alanyl-3-(2-naphthyl)-D-alanyl-L-alanyl-L-tryptophyl-D-phenylalanyl-L-lysine dihydrochloride and is one of the most potent GHRPs (20). Pre-clinical studies demonstrated that its GH-stimulating effect was attenuated when the hypothalamic/pituitary gland connection was impaired (21, 22). In the present communication, we report clinical studies in GH-deficient patients, as well as healthy adult subjects, to validate the use of a single dose of GHRP-2 as a diagnostic agent for GHD resulting from hypothalamic or pituitary disease.

Subjects and methods

Subjects

A total of 135 subjects were enrolled in this study, comprising 58 patients previously diagnosed as having severe GHD, from a peak serum GH value of $< 3 \mu\text{g/l}$ in ITT, and 77 healthy adult subjects enrolled as a control group. Patients were excluded from the study for the

following reasons: accompanying hypothyroidism or central diabetes insipidus not treated with adequate substitution therapy, receiving a GH preparation, presence of a chromosome abnormality or a serious complication and pregnancy or possible pregnancy. Efforts were made to recruit patients with hypothalamic and pituitary stalk diseases in the study to validate the efficacy of GHRP-2. Prior to starting the study, approval was obtained from the Institutional Review Board of each medical institution and written informed consent was obtained from each subject.

The 77 healthy subjects included 53 males and 24 females, with an age range from 20 to 76 years (median: 26 years) and a body mass index (BMI) ranging from 13.9 to 36.2 kg/m^2 (median: 22.0 kg/m^2). The subjects included six males, who were classified as overweight (obesity grade 1 by Japanese criteria (23)), with a BMI $\geq 25 \text{ kg/m}^2$ and $< 30 \text{ kg/m}^2$ (median: 25.7 kg/m^2), and six males classified as obese (obesity grade 2 by Japanese criteria, with a BMI $\geq 30 \text{ kg/m}^2$ (median: 33.3 kg/m^2)).

The 58 patients with severe GHD comprised 37 males and 21 females, with an age range from 17 to 64 years (median: 39 years) and a BMI ranging from 19.5 to 30.3 kg/m^2 (median: 24.4 kg/m^2). There were no statistically significant differences in gender or age between the healthy control subjects and the GH-deficient patients; however, the BMI was significantly higher in the patient group ($P=0.001$). Table 1 shows the pertinent clinical data of the patients with GHD; serum IGF-1 s.d. score was calculated according to the recently established normative data from 1110 healthy Japanese adult (24). The most common tumour was germinoma ($n=18$) reflecting the relatively high prevalence of this tumour type in Japan (25). Two out of the thirteen patients with craniopharyngioma and all 18 with germinoma had previous cranial irradiation.

Table 1 Clinical data of the patients with severe GH deficiency.

Etiology	Number	Gender (M/F)	Age median (range)	BMI mean $\pm$ s.d.	IGF-1 s.d. score median (range)	Hormonal replacement ^a			
						1 H	2 H	3 H	DDAVP
Primary pituitary diseases									
Adenoma ^b	10	8/2	47 (31:60)	24.8 $\pm$ 2.7	−2.06 (−7.15:−0.53)	2	2	5	2
Sheehan syndrome	4	0/4	(40:62)	22.7 $\pm$ 3.8	(−6.88:−1.24)	0	3	1	0
Others ^c	5	4/1	41 (31:63)	26.0 $\pm$ 3.5	−4.00 (−6.48:−0.65)	0	1	3	2
Primary hypothalamic diseases									
Craniopharyngioma ^d	13	6/7	31 (17:64)	22.8 $\pm$ 2.4	−3.68 (−8.97:−0.16)	1	3	8	7
Germinoma ^d	18	11/7	32 (22:47)	25.3 $\pm$ 3.1	−3.06 (−11.81:0.23)	2	6	7	14
Pituitary stalk transection	5	5/0	28 (20:41)	23.0 $\pm$ 2.1	−3.74 (−5.33:−2.29)	0	1	4	1
Others ^e	3	3/0	(48:60)	26.8 $\pm$ 1.5	(−3.93:−0.99)	0	3	0	3

^a1 H/2 H/3 H-number of pituitary hormone replacements (thyroid hormone, glucocorticoid, sex steroids).

^bIncluded eight non-functioning adenoma, one Cushing's disease and one prolactinoma.

^cIncluded two idiopathic, two trauma and one Rathke's cleft cyst.

^dTwo craniopharyngioma and all 18 germinoma patients received cranial irradiation.

^eIncluded two Langerhans cell histiocytosis and one hypertrophic pachymeningitis.

GH provocative tests

ITT All 58 patients with severe GHD were diagnosed by ITT. After overnight fasting, regular insulin (0.05–0.1 U/kg) was administered via an antecubital vein, blood samples were drawn before and 30, 45, 60, 90 and 120 min after insulin injection and serum GH concentrations were determined. All patients reached the required levels of hypoglycaemia (<2.2 nmol/l) and had clinical signs of hypoglycaemia.

GHRP-2 test A single dose of GHRP-2 (KP-102, Kaken Pharmaceuticals, Tokyo, Japan) was given to each of the subjects under fasting conditions. The GHRP-2 was administered intravenously at a dose of 100 µg. In order to investigate the reproducibility of GH secretion induced by GHRP-2, three doses (25, 50 and 100 µg) were re-administered to 21 healthy subjects with 4-week washout periods between testing.

Blood samples were taken before and 15, 30, 45, 60, 90 and 120 min after GHRP-2 administration and the serum GH concentrations were determined. For evaluation of safety of GHRP-2, blood pressure and heart rate were measured and laboratory tests of haematology and blood chemistry were performed before and after administration.

GH determination

GH concentrations were measured using an IRMA kit (Daiichi Radio Isotope Research Institute, Tokyo, Japan) calibrated with GH standard WHO IRP 66/217. The peak values of serum GH concentration ($C_{\max}$) were standardised with the formula established by the Foundation for Growth Science in Japan to adjust for the inter-assay kit variations (26). However, recent changes have resulted in the use of a new standard, WHO 98/574, in Japan, whereby values are to be revised by multiplying by 0.6 (27). Values below the detection limit (<0.05 µg/l) were handled as 0.05 µg/l.

Data analysis

The data were expressed as mean $\pm$ s.d. if normally distributed or as median and ranges if the data were skewed. Differences between groups were examined by χ^2 or *t*-tests. Kruskal–Wallis one-way ANOVA was used to compare normally distributed multiple independent groups, whereas ANOVA on ranks was used if the data were skewed. A *P* value <0.05 was taken as significant. The receiver-operating characteristics (ROC) and sensitivity–specificity curves were investigated to establish the standard $C_{\max}$ value for diagnosis of GH deficiency with GHRP-2. For evaluation of two-variable conformance, the figure obtained by plotting the difference between $C_{\max}$ values (logarithm) of serum GH obtained

with GHRP-2 and insulin against each mean $C_{\max}$ value (logarithm) (Bland–Altman plot; (28)) was used for the comparison of response intensity between GHRP-2 and insulin.

Results

Figure 1 shows the serum GH $C_{\max}$ value in each subject after the administration of GHRP-2. In the control group, the $C_{\max}$ was 87.4 ± 62.9 µg/l (range: 15.9–345.1 µg/l; median: 69.9 µg/l). In the patient group, the $C_{\max}$ for serum GH was 1.36 ± 2.60 µg/l (range: 0.05–14.8 µg/l; median: 0.38 µg/l). The $C_{\max}$ value was significantly higher in the control group than in the patient group ($P < 0.001$) and was not more than 15 µg/l in any of the 58 patients, but was not <15 µg/l in any of the 77 subjects in the control group.

The influences of age, gender and adiposity on GH response to GHRP-2 were analysed for all 77 control subjects and the results are shown in Table 2. There were no significant differences in GH $C_{\max}$ between males and females in the age range 20–59 years, and between pre- and post-menopausal females in the age range 40–59 years. A small differences in the $C_{\max}$ of serum GH was observed between males and females aged over 60 years ($P = 0.030$). Among the three age groups assessed, significant differences were observed; the GH $C_{\max}$ was higher in the age group of 20–39 years than in the age group of 40–59 years ($P < 0.001$) and over 60 years ($P < 0.001$). A significant difference was also observed between overweight ($\text{BMI} \geq 25$ kg/m² and <30 kg/m²; $P < 0.001$) or obese ($\text{BMI} \geq 30$ kg/m²; $P = 0.019$) subjects and lean ($\text{BMI} < 25$ kg/m²) subjects in the age group 20–39 years. In the age group >40 years, BMI affected the $C_{\max}$ of serum GH even within lean ($\text{BMI} < 25$ kg/m²)

Figure 1 Individual peak GH concentrations after GHRP-2 administration in control subjects and patients with severe GHD defined from an ITT; the horizontal dotted line shows the value of 15 µg/l.

Table 2 Effects of age, gender and menopause, and age and body mass index (BMI), on peak serum growth hormone (GH) concentration ($C_{\max}$) following GH-releasing peptides-2 administration to healthy control subjects.

	<i>n</i>	$C_{\max}$ (μg/l)	Median (range)	
<i>Age, gender and menopause</i>				
20–39 years	45	114.3±67.5	104.9 (24.2–345.1)	
Male	37	117.0±72.2	104.9 (24.2–345.1)	
Female	8	101.5±40.6	97.8 (51.6–167.9)	
40–59 years	16	50.3±22.0	47.5 (20.9–85.6)	<i>P</i> <0.001 vs 20–39 years
Male	8	49.2±20.4	52.1 (20.9–78.8)	<i>P</i> <0.001 vs 20–39 years male
Female	8	51.5±24.9	43.2 (22.8–85.6)	<i>P</i> =0.010 vs 20–39 years female
Pre-menopause	4	60.2±29.6	(27.4–85.6)	
Post-menopause	4	42.9±19.1	(22.8–68.8)	
60–76 years	16	48.7±30.6	38.1 (15.9–130.5)	<i>P</i> <0.001 vs 20–39 years
Male	8	64.8±33.4	63.8 (21.4–130.5)	<i>P</i> =0.005 vs 20–39 years male
Female	8	32.6±17.3	26.4 (15.9–69.3)	<i>P</i> =0.002 vs 20–39 years female <i>P</i> =0.030 vs 60–76 years male
<i>Age and BMI</i>				
20–39 years				
≤21.9 kg/m ²	22	121.2±59.3	115.4 (29.9–329.4)	
22–24.9 kg/m ²	14	138.1±81.0	124.5 (48.6–345.1)	
≥25 kg/m ²	9	60.2±27.8	52.3 (24.2–101.2)	<i>P</i> <0.001 vs ≤21.9 kg/m ² , 22–24.9 kg/m ²
≥30 kg/m ²	6	58.1±27.0	48.2 (28.1–101.2)	<i>P</i> =0.019 vs ≤21.9 kg/m ² , 22–24.9 kg/m ²
40–76 years				
≤21.9 kg/m ²	10	67.4±28.0	64.9 (29.2–130.5)	
22–24.9 kg/m ²	19	41.9±21.2	37.0 (15.9–85.6)	<i>P</i> =0.0106 vs ≤21.9 kg/m ²
≥25 kg/m ²	3	37.9±27.2	(21.4–69.3)	

subjects. Analysis of the area under the curve (AUC) values for serum GH concentration against time after administration (data not shown) showed similar trends to the analysis of $C_{\max}$ values.

In 21 male control subjects, aged 20–29 years, various doses of GHRP-2 (25, 50 and 100 μg) were administered twice to investigate the reproducibility of GH response. The correlation between peak GH values from the first and second injections are shown in Fig. 2. Marked inter-individual variations of GH responses to GHRP-2 appeared to blunt the dose-related increase in GH secretion. However, the Spearman correlation coefficient for $C_{\max}$ was 0.89, indicating favourable reproducibility.

Figure 3 shows a scatter diagram in which the 58 patients with a peak serum GH value < 3 μg/l in the ITT are plotted on the horizontal axis, in the increasing order of $C_{\max}$, with the corresponding serum GH $C_{\max}$ values after administration of GHRP-2. Most of the patients, whose $C_{\max}$ value was less than the detection limit in the ITT showed almost no response to GHRP-2 and seven of the patients, whose $C_{\max}$ was higher than 0.5 μg/l in the ITT showed a $C_{\max}$ value higher than 3 μg/l in response to GHRP-2.

GHD patients were divided by underlying primary causes according to the presumed hypothalamic or pituitary origin. Presumed hypothalamic causes were craniopharyngioma, germinoma, pituitary stalk transection and other hypothalamic tumour such as Langerhans cell histiocytosis; presumed pituitary causes included pituitary adenoma, Sheehan syndrome and other causes. Figure 4 summarises the results of $C_{\max}$ in control subjects and in patients with presumed primary hypothalamic and pituitary diseases according to the age

group of 17–39 years or ≥40 years. Both hypothalamic and pituitary diseases showed equally attenuated GH responses to GHRP-2 irrespective of the age.

Table 3 shows the results for peak serum GH in patients with GHD and in controls stratified by age and BMI because $C_{\max}$ was dependent on both parameters in healthy subjects. There was no overlap between peak GH values in the patient group and the corresponding control group. Table 4 shows the time of peak serum GH

Figure 2 Correlation between peak GH values after the first and second injections of GHRP-2, at doses of 25, 50 and 100 μg, in healthy control subjects.

Figure 3 Comparison of peak GH values during an ITT (open symbols) and after GHRP-2 administration (closed symbols), in patients with severe GHD; horizontal dotted lines show 15 and 3 µg/l.

Figure 4 Box and whisker plots representing peak GH responses ($C_{\max}$) to GHRP-2 in control subjects (C) and GHD patients with presumed primary hypothalamic (H) and pituitary (P) diseases; the horizontal line in the box indicates the median, the lower and upper boundaries of the box indicate the 25th and 75th percentiles and error bars above and below the box indicate the 97.5th and 2.5th percentiles.

concentration ($T_{\max}$) after the administration of GHRP-2. The peak value was seen within 15 min after administration in 74% (43/58) of the GH-deficient patients and within 30 min in 88% (68/77) of the control subjects. The peak value was seen within 60 min after the administration in all 132 subjects, except for three of the control group who showed GH peak at 60 min after GHRP-2 administration. When the serum GH concentration at 30 min ($C_{30 \text{ min}}$) was compared with the peak serum GH concentration ($C_{\max}$) during the GHRP-2 test, both values were almost of the same magnitude (Fig. 5) with no overlap for $C_{30 \text{ min}}$ between the patient and control group.

In order to set a cut-off value for diagnosis of GHD with GHRP-2, the sensitivity and specificity on changing the assumed cut-off value were evaluated as an ROC curve and as a sensitivity–specificity curve. The AUC of the ROC curve was close to 1 (Fig. 6), confirming that GHRP-2 showed high precision as a diagnostic agent for GH secretory capacity. In the sensitivity–specificity curve (Fig. 6), the serum GH concentration at the point where the sensitivity crossed with the specificity, i.e. the borderline value showing both high sensitivity and high specificity, ranged from 15 to 20 µg/l, if all patients were included. This was consistent with the results of the Bland–Altman analysis (data not shown) performed to compare the GH secretory capacity of GHRP-2 with that of insulin. The mean difference in $C_{\max}$ of serum GH between GHRP-2 and insulin was 0.702; in retro-conversion, this means that the GH secretion capacity of GHRP-2 was 5.03 times higher than that of insulin. Based on the above results, it was considered reasonable to set the cut-off value for diagnosis of severe GHD with GHRP-2 at 15 µg/l, corresponding to the cut-off value of 3 µg/l for the diagnosis by ITT.

The adverse reactions noted after the administration of GHRP-2 are shown in Table 5. Adverse reactions were reported by 31 of the 58 subjects (53.4%, 45 episodes) in the patient group and 23 of the 77 subjects (29.9%, 34 episodes) in the control group, i.e. in 54 of the 135 subjects (40.0%, 79 episodes) in total. The major adverse reactions were hot flush, borborygmus and white blood cell count increase; all adverse events were mild, except one episode of sweating (moderate) in a healthy control subject, and all were transient and resolved without any treatment.

Discussion

The present findings show that a single dose of GHRP-2 elicited reproducible GH secretion in control subjects and that GH response is not influenced by gender but is affected by age and adiposity to some extent. Severe GHD can be diagnosed with high reliability using a cut-off of 15 µg/l for peak serum GH concentration in patients with both hypothalamic and pituitary diseases.

Table 3 Peak GH concentrations (C_{max}) in response to GH-releasing peptides-2 in patients with GH deficiency compared with control subjects, stratified by age and body mass index.

Age (years)	BMI (kg/m ²)	<i>n</i>	GHD patient C_{max} (μg/l)		<i>n</i>	Control subject C_{max} (μg/l)	
			Mean ± s.d.	Median (range)		Mean ± s.d.	Median (range)
17–39	≤21.9	10	1.7 ± 2.9	0.35 (0.05–9.1)	22	121.2 ± 59.3	115.4 (29.9–329.4)
	22–24.9	12	1.6 ± 4.2	0.17 (0.05–14.8)	14	138.1 ± 81.0	124.5 (48.6–345.1)
	≥25	9	1.1 ± 2.2	0.19 (0.05–6.8)	9	60.2 ± 27.8	52.3 (24.2–101.2)
40+	≤21.9	5	0.63 ± 0.63	0.48 (0.05–1.7)	10	67.4 ± 28.0	64.9 (29.2–130.5)
	22–24.9	4	2.0 ± 1.8	(0.48–4.2)	19	41.9 ± 21.2	37.0 (15.9–85.6)
	≥25	18	1.2 ± 1.9	0.43 (0.05–7.6)	3	37.9 ± 27.2	(21.4–69.3)

The GHRP-2 test met the criteria reported to be necessary for the validation of a GH provocative test (29), which are: i) to be potent and reproducible, with reproducibility assessed in normal subjects, ii) the influence of gender, age and adiposity should be validated in control subjects, iii) effectiveness needs to be assessed in controls and patients, evaluated by ROC curve analysis, and iv) the cut-off point for the test should be established by ROC curve analysis and is the value that provides the best pairing of sensitivity and specificity.

The magnitude of GH response to GHRP-2 in control adult subjects was reproducible and consistent with previous studies (30, 31). Rapid GH response to GHRP-2 may reduce the number of blood samples and time required for patients in the investigation unit. In the majority of control subjects, the peak occurred at 30 min and for most GH-deficient patients, it was earlier, at 15 min, but the GH values at 30 min were almost of the same magnitude as the peak values. Therefore, a single 30 min sample would provide similar diagnostic information on peak GH as that reported for the GHRH plus GHRP test (14, 15, 32).

Gender had no significant influence on GH response to GHRP-2, but age- and adiposity-affected response in the present study. Previous studies using GHRP-6, hexarelin or ghrelin had shown that gender did not influence the GH response in control subjects (33, 34). However, in older subjects aged 49–76 years, males were reported to respond better to GHRP-2 than females (35), which is in agreement with our findings in control subjects > 60 years.

With regard to age, our findings that GH response to GHRP-2 was reduced in control subjects over 40 years when compared with younger adults are in agreement with previous studies using hexarelin or ghrelin (33, 34). However, GHRP-6 alone or combined with GHRH

was not affected by age (13, 18, 36). We could not explain why GHRP-6 was apparently not affected by age but GHRP-2 is.

Recent studies have shown that normative values of GH response to GHRH plus GHRP-6 should be defined according to the BMI (18, 19). In our study, GH response to GHRP-2 was found to be affected by an increase in BMI. GH response was reduced but remained substantially preserved in control subjects of BMI over 25 kg/m². Further investigation is needed to define normative data.

In our analysis of the sensitivity–specificity curves, comparing healthy subjects with GH-deficient patients, the serum GH concentration at the point where sensitivity crossed with specificity, i.e. the value showing high sensitivity and high specificity, ranged from 15 to 20 μg/l. The intended use of GHRP-2 is not mass screening but exact diagnosis of subjects highly likely to have the disease. Therefore, it was considered reasonable to set the cut-off value for diagnosis of severe GHD with GHRP-2 at the more stringent level of 15 μg/l and this cut-off value was provided 100% sensitivity and 100% specificity. The value was also consistent with results from tests using combined GHRP-6 and GHRH in lean subjects. GHRP-6 shows a weaker GH stimulating effect than GHRP-2 and requires augmentation with GHRH (37). GHRP and GHRH act through different receptors and have a synergistic effect on GH secretion. Combined administration of GHRH and GHRP-2 has been suggested as a diagnostic test of GH deficiency (16). However, our results may indicate that GHRP-2 alone is sufficient to evoke GH secretion.

The sites of action of GHRP are considered to be both pituitary and hypothalamus. Popovic *et al.* (38) have shown that about 16% of patients, who have had cranial radiotherapy may have hypothalamic–pituitary dysfunction, evident from a poor GH response to I-TT, and

Table 4 Time to peak serum GH concentration after GHRP-2 stimulation in control subjects and patients with GH deficiency.

	<i>n</i>	Times (min)			
		15	30	45	60
Controls	77	13	55	6	3
Patients	58	43	14	1	0

Figure 5 Relationship between serum GH concentration at 30 min ($C_{30 \text{ min}}$) and peak concentration (C_{max}) after GHRP-2 administration in patients with GHD (closed symbols) and control subjects (open symbols).

exhibit false-positive responses to the combined administration of GHRH plus GHRP-6. The same phenomena were also observed in the case of combined arginine plus GHRH (11, 12). The comparative analysis of hypothalamic and pituitary diseases in the present study indicated that GHRP-2 could equally diagnose GHD of both types, similar to the ITT. However, more patients with mild GHD due to hypothalamic lesion are needed to clarify the sensitivity and specificity of GHRP-2 tests.

The adverse reactions observed in the GHRP-2 provocative test were flushing, borborygmus and white blood cell count increase. All were transient and disappeared without treatment, indicating good safety profile of GHRP-2. Flushing and sweating were observed frequently in other studies with GHRP (15, 30, 34). The endogenous GH secretagogue, ghrelin, was reported to

Figure 6 Plot of the variation in sensitivity (open symbols) and specificity (closed symbols) for GH peak following GHRP-2 administration; insert indicates the ROC curve of GH responses to GHRP-2.

Table 5 Adverse reactions following GHRP-2 administration to healthy control subjects and patients with GH deficiency.

	Controls (n=77)	Patients (n=58)	Total (n=135)
Hot flush	14	10	24
Borborygmus	8	11	19
Increased WBC	5	1	6
Sweating	5	1	6
Nausea	0	2	2
Drowsiness	0	3	3
Taste perversion	2	0	2
Dry mouth	0	2	2
Dizziness	0	1	1
Others	0	14	14
Total adverse reactions (n (%))	23 (29.9)	31 (53.4)	54 (40.0)

enhance digestive tract movement (39) and GHRP-2 has similar effects to ghrelin in increasing appetite in humans (40); the borborygmus probably relates to the physiological effects on gastric motility.

In Japan, the guidelines for diagnosis of GH deficiency, issued by the Hypothalamic–Pituitary Dysfunction Study Group of the Ministry of Health, Labour and Welfare, states that at least two kinds of provocative tests are necessary to diagnose GHD. Diagnostic tests are performed using stimulating agents selected from among insulin, arginine, glucagon and L-DOPA for the diagnosis of adult GH deficiency. When the peak serum GH concentration is $< 3 \mu\text{g/l}$ in at least two different stimulation tests, severe GH deficiency is diagnosed. In the paediatric field, GH stimulating agents include clonidine in addition to those used in adults; severe GH deficiency is diagnosed when the peak serum GH concentration is $< 5 \mu\text{g/l}$ in at least two tests. GHRH has also been used as a diagnostic agent for GH deficiency, but the test is strongly influenced by the GH secretion-suppressing system (somatostatin). It is thought that many false-negative results are seen, that the GH response decreases with aging and the reproducibility of GH secretion is insufficient, with large inter-individual differences. GHRH test is, therefore, not included in the GH provocative tests specified in the Japanese guidelines. From the present result, we decided to include the GHRP-2 test as an alternative for diagnosing severe GHD in adults.

In conclusion, the provocative test with a single dose of GHRP-2 showed favourable reproducibility and was not influenced by gender. The decreased response with aging or adiposity did not affect the discrimination between healthy control subjects and GH-deficient patients, and the diagnostic capacity of GHRP-2 for severe GHD was very high irrespective of the hypothalamic or pituitary causes of diseases. From the results of this study, we would recommend a GHRP-2 dose of $100 \mu\text{g}$ intravenously and measurement of GH response at 30 min. Maximum specificity and sensitivity was achieved using a diagnostic cut-off value of $15 \mu\text{g/l}$ ($9 \mu\text{g/l}$ when

calibrated with GH standard WHO 98/574 as reference (27)). Subsequent studies are needed to directly compare the utility of GHRP-2 and the combined GHRH plus arginine or GHRH plus GHRP as an alternative to the ITT. We believe that GHRP-2 is a useful and safe alternative diagnostic agent for severe GHD.

Acknowledgements

We would like to thank Dr Peter Bates, Cambridge Medical Writing Services, CB10 1SH, UK for his help in the preparation of the manuscript. We are also very grateful for the help of the following investigators involved in the study: K Kobayashi, T Tajima, K Sato, K Fujieda, J Nakae, N Shinohara, Department of Pediatrics, Hokkaido University School of Medicine; T Suda, N Tamazawa, T Nigawara, A Sakisaka, Third Department of Internal Medicine, Hirosaki University School of Medicine & Hospital; M Mori, M Yamada, T Sato, T Monden, First Department of Internal Medicine, Gunma University Hospital; Y Saito, I Tatsuno, D Uchida, Second Department of Internal Medicine, Chiba University Hospital; H Niimi, T Yasuda, Department of Pediatrics, Chiba University Hospital; H Demura, K Takano, K Yasumoto, Second Department of Internal Medicine, Tokyo Women's Medical University; F Marumo, Y Hirata, M Shichiri, T Imai, Second Department of Internal Medicine, Tokyo Medical and Dental University; I Wakabayashi, T Shibasaki, H Sugihara, J Kamei, Y Shuto, S Ishii, Third Department of Internal Medicine, Nippon Medical School; R Horikawa, M Goto, N Ikema, M Ishikawa, Division of Endocrinology and Metabolism, National Center for Child Health and Development; M Ishibashi, M Honda, Fourth Department of Internal Medicine, Teikyo University School of Medicine University Hospital, Mizonokuchi; Y Miyachi, H Ueshiba, First Department of Internal Medicine, Toho University Omori Hospital; H Nakamura, Y Oki, Second Department of Internal Medicine, Hamamatsu University School of Medicine; H Saito, Y Oiso, Y Miura, First Department of Internal Medicine, Nagoya University Hospital; M Ogawa, T Asai, Department of Pediatrics, Nagoya University Hospital; K Yasuda, T Mune, S Daido, Gifu University Hospital; S Ichiyama, S Kosugi, Department of Clinical Laboratory, Kyoto University Hospital; T Usui, Clinical Research Institute, Kyoto National Hospital; M Nishikawa, N Toyoda, Second Department of Internal Medicine, Kansai Medical University; T Sugimoto, H Kaji, Y Okimura, K Iida, M Murata, G Iguchi, H Abe, Department of Clinical Molecular Medicine, Kobe University Graduate School of Medicine; S Kanzaki, Department of Pediatrics, Okayama University School of Medicine; Y Nishi, Department of Pediatrics, Hiroshima Red Cross Hospital & Atomic-bomb Survivors Hospital; Y Murakami, M Sohmiya, M Nishiki, First Department of Internal Medicine, Shimane University Faculty of Medicine; K Murao, H Hosokawa, S Matsubara, J

Takahara, M Sato, First Department of Internal Medicine, Kagawa University Faculty of Medicine; K Hashimoto, T Takao, T Nishioka, K Asaba, Second Department of Internal Medicine, Kochi Medical School; H Nawata, T Yanase, K Sato, M Nomura, T Okabe, Third Department of Internal Medicine, Kyushu University Faculty of Medicine; S Matsukura, H Katakami, T Yonekawa, Third Department of Internal Medicine, Miyazaki University Faculty of Medicine; T Kohno, Fukuoka Children's Hospital. This work was supported by research grants from Kaken Pharmaceutical Co. Ltd., in part by Health and Labour Grants from the Ministry of Health, Labour and Welfare of Japan and by Grant-in-Aid for Scientific Research (B) and Grant-in-Aid for Exploratory Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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Received 1 February 2007

Accepted 16 April 2007