Plasma GH and Somatomedin C Responses to Single and Repeated Administrations of Human Growth Hormone Releasing Factor (hGRF)

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Abstract

Plasma growth hormone (GH) and somatomedin C responses to single and repeated administrations of synthetic human growth hormone releasing factor (hGRF-44) were studied in 29 patients with GH deficiency. hGRF-44 administered by single iv bolus (10 μ g/kg), repeated iv boluses (50 or 100 μ g, once a day), repeated iv infusions (2.5 μ g/min for 90 min, once a day), and/or repeated im injections (100 μ g, twice a day) for three to six consecutive days.

Ten of 16 patients had plasma GH responses to a single iv bolus injection, i.e., their plasma GH increased above 5 ng/ml or twice the basal level. However none of them showed a plasma somatomedin C increase. To repeated iv bolus injections and repeated iv infusions of hGRF-44, 7 of 17 patients showed plasma GH responses, however in none of 7 patients did somatomedin C increase. Plasma somatomedin C did not increase after repeated im administrations of hGRF-44 for 5 days. Plasma somatomedin C increase was observed in two patients, from 0.32 to 0.54 U/ml and from 0.16 to 0.46 U/ml, in response to repeated iv boluses and repeated iv infusions, respectively.

These results suggest that hGRF-44 stimulates plasma GH secretion in some patients with GH deficiency, however the increases are not enough to stimulate somatomedin generation.

Growth hormone releasing factors (GRFs) were isolated and characterized from pancreatic tumors in patients with acromegaly (Guillemin *et al.*, 1982; Esch *et al.*, 1982; Rivier *et al.*, 1982). More recently human hypothalamic GRF has been isolated and characterized from human pituitary stalk-median eminence extract (Ling *et al.*, 1984). Its structure is identical to that of the 44-amino-acid GH-releasing peptide isolated from the tumor (Guillemin *et al.*, 1982).

Thus we shall only use the term hGRF to designate the human growth hormone releasing factor, tumors or hypothalamic. Using synthetic replicates of these human pancreatic GRFs (hGRFs), it has been demonstrated that some idiopathic GH deficient children have GH responses to a single iv bolus of hGRF-40 (Borges *et al.*, 1983; Grossman *et al.*, 1983). We have also reported that forty percent of patients had GH responses to a single iv bolus administration of hGRF-44 (Takano *et al.*, 1984), repeated administrations of the

peptide by either im, or iv infusion induced GH responses in the patients who did not respond to a single administration, and ultimately seventy percent of the patients had GH responses to hGRF-44 (Hizuka *et al.*, 1984).

In the present study, we have studied the effect of a single iv bolus administration of hGRF-44 and repeated iv boluses, iv infusions or im administrations of hGRF-44 on plasma somatomedin levels in patients with GH deficiency.

Materials and Methods

Human subjects

Twenty-nine patients with pituitary dwarfism aged 6–30 were studied. Twenty-three of them were diagnosed as having idiopathic GH deficiency while the remaining six's GH deficiencies were secondary to hypothalamo-pituitary tumors. The clinical data on the 29 patients are summarized in Table 1. The diagnosis of GH deficiency was established by failure of peak plasma GH to rise above 5 ng/ml after insulin-induced hypoglycemia (ITT) (Roth *et al.*, 1963),

Table 1. Clinical data in 29 patients with GH deficiency

Subj. No.	Sex	Age (y.m.)	Height (cm)	Body weight (kg)	Diagnosis	Other pituitary hormone reserve						
						GnRH		TRH		ITT		GH treat.
						LH	FSH	TSH	PRL	ACTH ^a	ADH	
1	M	6. 4	96. 5	20. 0	Idiopathic	—	—	+ D	+	+	+	under
2	M	7. 7	106. 0	14. 0	"	+	+	+ D	+ E	+	+	"
3	F	8. 1	96. 1	11. 5	"	+	+	+	+	+	+	"
4	M	9. 3	123. 7	33. 0	Cranio-pharyngioma	—	—	+	+	+	—	"
5	M	11. 3	127. 9	37. 0	"	—	—	—	—	—	—	"
6	M	11. 5	110. 7	20. 0	Encephalitis	—	—	+ D	+ E	+	+	"
7	M	12. 5	131. 9	27. 0	Idiopathic	—	—	+	+	+	+	"
8	M	13. 2	144. 6	39. 5	"	—	—	+ D	+	—	+	"
9	M	13. 8	136. 1	32. 0	"	—	—	+	+	+	+	"
10	M	14. 2	140. 2	32. 0	"	+	—	+	+	+	+	"
11	M	14. 10	138. 9	35. 0	"	+	+	+	+	+	+	"
12	M	15. 0	147. 5	38. 0	"	—	—	+ D	+	+	+	"
13	M	15. 4	121. 3	25. 0	"	—	—	+ D	+ E	—	+	"
14	F	15. 8	142. 5	42. 5	Pinealoma	+	+	+	+	+	—	"
15	F	16. 3	133. 4	43. 5	Idiopathic	—	—	+	+	+	+	"
16	M	16. 9	140. 1	31. 0	"	—	—	+ D	+ E	+	+	"
17	M	17. 2	145. 0	56. 0	"	—	—	+ D	+	+	+	"
18	M	17. 2	162. 7	54. 5	"	+	+	+	+	+	—	"
19	F	17. 3	142. 0	43. 0	Pinealoma	—	—	+	+	+	—	"
20	M	17. 5	148. 0	51. 0	Idiopathic	—	—	+	+	+	+	"
21	M	17. 6	149. 7	35. 5	"	+	+	+ D	+ E	+	+	"
22	M	18. 7	152. 3	73. 0	"	+	+	+	+	+	+	"
23	M	19. 4	156. 2	45. 0	"	+	+	+	+	+	+	"
24	M	19. 8	164. 0	64. 0	Pinealoma	—	—	+	+	—	—	"
25	M	19. 9	143. 7	42. 0	Idiopathic	+	+	+	+	+	+	"
26	F	21. 4	148. 5	44. 0	"	—	—	+ D	+	—	+	ceased
27	M	23. 0	160. 6	74. 0	"	+	+	+ D	+	+	+	"
28	M	26. 2	139. 1	36. 5	"	—	—	+	+	+	+	under
29	F	30. 2	146. 3	40. 0	"	—	—	+ D	+ E	+	+	ceased

+, positive; —, no change from the basal level; D, delayed response to TRH; E, elevated basal level; a, cortisol was measured for the evaluation of ACTH reserve

glucagon-propranolol (Parks *et al.*, 1973), and/or L-dopa (Mims *et al.*, 1973) tests. Three patients had ceased hGH treatment more than 6 months earlier when they reached their expected height. The rest of the patients have been treated with hGH for periods ranging from 3 months to 7 years as well as being given appropriate replacement therapy for other deficient pituitary hormones and have showed the good results in height increase. During hGH treatment, increased somatomedin levels were observed and they had no detectable hGH antibody in their plasma.

Informed consent was obtained from each patient and/or the parents, and the study protocol was approved by our Department Review Committee at Tokyo Women's Medical College.

Peptide preparation

Human growth hormone releasing factor (hGRF-44) was synthesized by solid phase methodology as described (Guillemin *et al.*, 1982). One hundred milligrams of the peptide were dissolved in 100 ml distilled water containing 1 mM ascorbic acid and sterilized by filtration through a 0.22 μ m filter (Millipore Corp.). One hundred microliter aliquots of the peptide solution (100 μ g) were prepared in sterile vials, lyophilized and stored at -20°C . A vial was diluted with 2 ml physiological saline immediately before injection.

Test protocol

The patients were fasted and remained recumbent throughout the study. Each subject was fitted with a heparin-locked cannula in a forearm vein for drawing blood and for administration of hGRF-44. The experiment was performed two to four days after the last injection of hGH in these patients. The plasma GH responses in the patients who were studied in experiments c and d have already been reported (Hizuka *et al.*, 1984).

- a: A single iv bolus injection of hGRF-44: Sixteen patients received an iv bolus injection of 10 μ g per kg BW of hGRF-44.
- b: Repeated iv bolus injections of hGRF-44: Seven patients received an iv bolus injection of 50 μ g (patient No. 2 and 3) or 100 μ g (patient No. 9, 15, 16, 25, and 29) of hGRF-44 for three to five consecutive days.
- c: Repeated iv infusions of hGRF-44: Ten patients were infused at a dosage of 2.5 μ g/

min for 90 min for three to six consecutive days.

- d: Repeated im administrations of hGRF-44: Six patients were injected with 100 μ g of hGRF-44 intramuscularly twice a day at 0800 and 2000 hours for five consecutive days.

Blood samples were drawn at 0, 15, 30, 45, 60 and 90 min after iv injection for plasma GH determination. For somatomedin C determination, blood samples were drawn before and at 24 hours after hGRF-44 administration. The samples were kept in ice and centrifuged immediately at 4°C . The plasma was separated and stored frozen at -20°C .

Radioimmunoassay for hGH and somatomedin C

Plasma hGH was measured with a commercial radioimmunoassay kit (Eiken Chemical Co., Ltd., Tokyo, Japan). The inter- and intra-assay coefficients of variation were 6.6 % and 8.0 %, respectively. The minimal detectable plasma GH level was 1.0 ng/ml. Plasma somatomedin-C was measured by somatomedin-C radioimmunoassay kit (Nichols Institute, San Juan Capistrano, CA). The intra- and inter-assay coefficients of variation were 4.1 % and 12.5 %, respectively. The normal adult range (from 2SD below to 2SD above the mean) is 0.38–1.59 U/ml.

Student's *t*-test and paired *t*-test were used for statistical analysis.

Results

a: A single iv bolus injection of hGRF-44

The plasma GH levels and somatomedin C levels after 10 μ g hGRF-44/kg are shown in Table 2. Six patients (No. 9, 12, 13, 17, 26 and 28) did not respond, i.e., their plasma GH levels never rose above 5 ng/ml or twice the basal level. In five patients (No. 1, 6, 7, 10 and 16), the maximum GH rose to between 6.3 and 10.8 ng/ml and in the rest of five patients (No. 11, 19, 21, 24 and 27), the maximal GH rose to twice the basal level.

The basal plasma somatomedin C in these 16 patients ranged from 0.12 to 0.30

U/ml with a mean of 0.17 ± 0.01 (SEM) U/ml. None of them showed a plasma somatomedin C increase at 24 hours after a single 10 $\mu\text{g/kg}$ iv bolus of hGRF-44 and their plasma somatomedin C concentration varied from 0.11 to 0.33 U/ml with a mean of 0.17 ± 0.01 U/ml.

b: Repetitive iv bolus injections of hGRF-44

Two patients (No. 2 and 3) received 50 μg and others received 100 μg hGRF-44,

which corresponded to 2.3 to 4.3 $\mu\text{g/kg}$ BW, for 3 to 5 consecutive days. The plasma GH and somatomedin C levels are shown in Table 3. In three patients (No. 2, 16 and 29), greater GH responses to hGRF-44 were observed by repeated iv injections of hGRF-44 on the days following the first day of injection, i.e., the peak plasma GH increased from 4.6 to 10.1, from 6.6 to 9.6 and from 4.4 to 6.9 ng/ml on the first day versus the day when the greatest response was obtained, respectively. The rest of the 4 patients did not show any significant GH rise following repeated iv bolus injections.

In one patient (No. 15), the plasma somatomedin C was 1.2 U/ml before hGRF injection. In the rest of the patients, somatomedin C levels ranged between 0.12 and 0.35 U/ml before hGRF injection. In only one patient (No. 25), a slightly progressive increase in somatomedin C from 0.32 to 0.54 was observed at the 4th day, even though he did not show any significant GH rise during the consecutive iv injections. However the remaining patients did not show any significant somatomedin rise in spite of a GH increase in 3 of them.

c: Repetitive iv infusions of hGRP-44

The plasma GH and somatomedin C levels to consecutive iv infusions of hGRF-44 (2.5 $\mu\text{g/min}$, 90 min) for 4 to 6 days are shown in Table 4. The details of GH responses in these patients were reported

Table 2. Plasma GH and Somatomedin C levels to 10 μg hGRF-44/kg BW administration

Subject No.	Plasma GH ng/ml		Somatomedin C U/ml	
	0 min	Peak	0 min	Peak
1	1.9	6.3	0.15	0.15
6	1.3	8.3	0.30	0.18
7	1.5	7.3	0.18	0.17
9	2.0	2.9	0.14	0.14
10	4.2	10.8	0.29	0.33
11	1.6	4.7	0.14	0.14
12	2.4	4.3	0.19	0.16
13	1.6	2.3	0.15	0.14
16	2.2	8.7	0.12	0.12
17	2.0	2.9	0.17	0.15
19	1.4	4.7	0.12	0.19
21	1.0	4.6	0.17	0.21
24	1.8	4.0	0.18	0.20
26	1.6	3.1	0.12	0.11
27	1.3	2.9	0.14	0.17
28	2.1	1.9	0.13	0.12
M	1.9	5.0	0.17	0.17
SD	0.7	2.6	0.15	0.05
SEM	0.2	0.7	0.01	0.01

Table 3. Plasma GH and somatomedin C levels during repeated iv injections of hGRF-44

Subj. No.	Plasma GH concentration, ng/ml										Somatomedin-C, U/ml				
	1D		2D		3D		4D		5D		1D	2D	3D	4D	5D
	0 min	Peak	0 min	Peak	0 min	Peak	0 min	Peak	0 min	Peak					
2	1.4	4.6	2.1	10.1	2.6	6.7	2.0	9.3			0.12	0.11	<0.1	<0.1	
3	1.4	1.4	1.3	1.4	2.6	3.1					0.35	0.33	0.35	0.32	
9	1.8	2.8	1.8	2.4	1.7	2.7					0.19	0.17	0.15	0.14	
15	2.0	2.2	3.7	4.0	3.4	3.8	3.0	4.1	3.0	3.3	1.2	0.6	0.45	0.29	0.18
16	1.7	6.6	2.1	9.6	2.3	8.2					0.16	0.16	0.16	0.12	
25	1.8	3.0	2.0	3.4	1.7	3.3					0.32	0.37	0.39	0.54	
29	3.0	4.4	3.4	5.6	3.1	5.2	2.7	6.9	3.2	4.7	0.17	0.19	0.16	0.19	0.23

Table 4. Plasma GH and Somatomedin C levels in response to repeated iv infusions of hGRF-44

Subject No.	Peak GH level during infusion						Somatomedin C					
	ng/ml						U/ml					
	1D	2D	3D	4D	5D	6D	1D	2D	3D	4D	5D	6D
5	3.3	3.6	2.7	2.7			0.13	0.16	0.14	0.15		
8	4.4	5.8	7.5	6.0			0.15	0.13	0.11	0.16		
11	3.4	5.4	7.5	5.7			0.40	0.24	0.21	0.26		
18	3.3	5.0	6.0	3.0	9.8	5.1	0.69		0.64	0.66	0.61	0.53
20	3.3	3.2	3.0	3.3			0.16	0.46	0.21	0.33		
21	3.8	10.2	12.5	8.2	8.5	8.9	0.21	0.16	0.17	0.13	0.15	0.18
22	2.8	3.1	3.4	3.8			0.61	0.49	0.42	0.34		
23	2.6	4.6	4.9				0.41	0.30	0.25			
25	3.1	3.1	3.0	3.7			0.63	0.62	0.42	0.35		
28	3.0	3.8	3.7	4.3			0.16	0.12	0.13	0.15		

earlier (Hizuka *et al.*, 1984). In four patients (No. 8, 11, 18 and 21), greater GH responses to hGRF-44 were induced by repeated iv infusions of hGRF-44 on the days following the first day of infusion. In the remaining six patients, plasma GH responses did not change significantly during repeated iv infusions of hGRF-44.

In five patients, basal plasma somatomedin C levels were above 0.4 U/ml. Most of the patients did not show a somatomedin C increase during repeated iv infusions of hGRF-44. In only one patient (No. 20), a slight increase in somatomedin C from 0.16 to 0.46, 0.21 and 0.33 U/ml before and on three consecutive days of hGRF infusion was observed in spite of unchanged GH responses to hGRF-44.

d: Repeated im injections of hGRF-44

Plasma somatomedin levels before and 5 consecutive days after im administration of hGRF-44 are shown in Table 5. Plasma somatomedin C varied between 0.16 and 1.00 U/ml with a mean of 0.50 ± 0.15 U/ml before hGRF administration. In three patients, basal levels were above 0.4 U/ml. After 5 days of hGRF-44 administration, none of them showed a significant somatomedin C rise. The mean plasma somatomedin C level dropped to 0.27 ± 0.07 U/ml,

Table 5. Somatomedin C concentration before and 5 consecutive days after im administration of hGRF-44

Subject No.	Somatomedin C		
	U/ml		
	Before	5 days	after
4	n.t.		0.19
10	0.60		0.36
11	0.18		0.10
12	0.16		0.24
14	1.00		0.56
19	0.54		0.19
M	0.50		0.27
SD	0.35		0.16
SEM	0.15		0.07

n.t. = not tested

although it was not statistically significant.

Discussion

After a single iv bolus injection of 10 μ g hGRF-44/kg BW, 10 of 16 patients showed a plasma GH increase, i.e., their maximal GH levels reached above 5 ng/ml or their maximal GH levels showed twice the basal level. This is consistent with our earlier report using 2 μ g hGRF-44/kg BW (Takano *et al.*, 1984). However, one of the patients showed any significant increase in

plasma somatomedin C, in spite of plasma GH rise. These findings are different from those reported by Borges *et al.* (1983). They observed an increase in somatomedin C in 6 of 10 adult patients with GH deficiency after a single iv bolus administration of 10 μ g hGRF-40/kg and their plasma GH responded to 0.8–7.3 ng/ml. Recently, Schriock *et al.* (1984) reported that there was no increase in somatomedin C when determined 24 hours after 5 μ g hGRF-44 per kg body weight administration to 12 patients with GH deficiency. Their results are consistent with ours and the majority of our patients were also children, therefore the difference in age might play a role in the discrepancy in the findings.

To repetitive iv bolus injections and repeated iv infusions, 7 of 17 patients showed plasma GH responses above 5 ng/ml, ranging from 6.9 to 12.5 ng/ml. However, there was no increase in somatomedin C in these 7 patients. In two of the patients (No. 20 and 25) who did not show any GH increase, there were somatomedin increases from 0.16 to 0.46 and from 0.32 to 0.54 U/ml, respectively. Plasma somatomedin C did not increase after repeated im administrations of hGRF-44 for 5 days. We studied the effect of repeated sc administrations of hGRF-44 (200 μ g, twice a day) to 11 patients with idiopathic GH deficiency for five consecutive days (Takano *et al.*, 1985). However there was no effect on somatomedin C production. Thus, hGRF-44 stimulates plasma GH secretion in some patients with GH deficiency, however the increases are not enough to stimulate somatomedin generation.

Nine of the patients with GH deficiency had normal somatomedin C levels before hGRF-44 administration. Four of them had been studied in more than two types of our studies and showed low levels of somatomedin C on another occasions. It is well known that somatomedins circulate in blood with carrier proteins and the carrier proteins are also regulated by hGH (Moses *et al.*,

1976; Takano *et al.*, 1979; Hintz and Liu 1980). Therefore, increased somatomedin levels due to repeated administrations of hGH in some patients did not return to basal levels at 72 hours after the last hGH injection (Takano *et al.*, 1977; Furlanetto *et al.*, 1977). These seem to be the reason that some of our patients had normal levels of somatomedin C before hGRF-44 administration, even though they ceased to receive hGH injections at least 2 days before. In all these patients, plasma somatomedin C levels decreased after repeated administrations of hGRF-44, indicating these kinds of administration were not enough to maintain or to stimulate somatomedin production.

Recently, Borges *et al.* (1984) reported that 0.33 μ g/kg hGRF-40 every 3 hours for 5 days increased somatomedin C significantly from 0.24 ± 0.07 to 0.78 ± 0.32 U/ml. The normalization of the circulating somatomedin C concentration is important as an index of response for induction of linear growth. In order to stimulate enough somatomedin generation, a strong and long acting analog of hGRF might be desirable.

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