

COMBINED TREATMENT WITH RECOMBINANT HUMAN INSULIN-LIKE GROWTH FACTOR-I (rhIGF-I) AND GROWTH HORMONE (rhGH) IN THE DWARF RAT. Deborah Mortensen, Lena Carlsson and Ross Clark, Endocrine Research Department, Genentech, Inc., South San Francisco, CA 94080, USA.

Daily injections of rhGH and infusions of rhIGF-I have additive effects on body growth in GH-deficient rats. We now measure growth responses to a fixed daily dose of rhGH (240 µg/d, sc injection) given once or twice daily, with or without rhIGF-I (120 µg/d, sc infusion) for 8 d in female dw/dw rats (6-8 weeks old, 100 g). Rats were sacrificed on Day 8; organs weighed and serum and tibia taken. Data in the Table are means ± SD (n=5/group).

Group	Weight Gain (g)	Epiphyseal Width (µm)	Serum IGF-I (ng/ml)	Liver (% Bwt)	Spleen (% Bwt)
1) Excipient	4.2 ± 3.2	213 ± 42	142 ± 34	3.7 ± 0.5	0.26 ± 0.01
2) IGF-I	11.2 ± 1.4	235 ± 7	293 ± 65	3.7 ± 0.3	0.35 ± 0.01
3) GH Daily	20.7 ± 1.3	272 ± 21	77 ± 22	4.0 ± 0.2	0.25 ± 0.03
4) GH bid	36.5 ± 7.2	360 ± 24	201 ± 57	4.4 ± 0.2	0.30 ± 0.04
5) 2 + 3	27.7 ± 1.2	299 ± 19	203 ± 61	4.1 ± 0.2	0.36 ± 0.04
6) 2 + 4	44.5 ± 6.7	362 ± 35	339 ± 50	4.6 ± 0.4	0.41 ± 0.04

For weight gain, cartilage and relative liver growth bid rhGH gave larger responses than daily rhGH. Spleen growth and serum IGF-I were optimal with rhIGF-I alone. Combination treatment gave greater weight gain and higher serum IGF-I's but not greater cartilage, liver or spleen growth. We conclude that when rhGH is given in an optimal pattern at high doses additive whole body anabolic effects of rhGH and rhIGF-I are observed.

SKELETAL MATURATION AND GROWTH DURING PUBERTY IN PATIENTS WITH GROWTH HORMONE (GH) DEFICIENCY OR IDIOPATHIC SHORT STATURE TREATED WITH GH. R.L. Hintz, G.P. August, K.M. Attie, A.J. Johanson, J. Baptista, and the Genentech Collaborative Group, Stanford University, Stanford, CA, Children's Hospital National Medical Center, Washington, DC, and Genentech, Inc., So. San Francisco, CA.

Patients with GH deficiency or idiopathic short stature (ISS) demonstrate improved growth rate and predicted adult height with GH therapy prior to puberty. However, some studies have suggested that there is an increased rate of pubertal development and skeletal maturation with GH therapy during puberty. We studied patients with GHID and ISS enrolled in multicenter trials in which rhGH therapy (0.3 mg/kg weekly) was initiated before puberty. Bone ages (BA) were determined centrally by the FELS method. The table below shows the mean and (n) for change in BA, change in height SDS adjusted for bone age (ΔHSDS<sub>BA</sub>), and change in Bayley-Pinneau (BP) predicted adult height, each divided by change in chronological age (CA). Patients were grouped according to pre-pubertal or pubertal bone ages. Bone ages taken at least 1 yr apart were used.

		♂ BA 7-11	♀ BA 5-9	♂ BA 12-15	♀ BA 10-13
GHID	ΔBA/ΔCA (yr)	1.16 (125)	1.31 (50)	0.88 (70)	1.07 (47)
	ΔHSDS <sub>BA</sub> /ΔCA	+0.28 (122)	+0.23 (47)	+0.28 (61)	+0.09 (44)
	ΔBP/ΔCA (cm)	+3.5 (122)	+5.3 (14)	+1.3 (61)	+3.2 (44)
ISS	ΔBA/ΔCA (yr)	0.99 (58)	0.87 (14)	1.18 (22)	0.71 (19)
	ΔHSDS <sub>BA</sub> /ΔCA	+0.31 (58)	+0.36 (14)	+0.14 (21)	+0.36 (19)
	ΔBP/ΔCA (cm)	+2.7 (58)	+2.7 (9)	-0.3 (21)	+4.3 (19)

CONCLUSIONS: The yearly change in height SDS<sub>BA</sub> was positive in all groups. All groups improved BP predicted height with the exception of pubertal male ISS patients, suggesting the importance of early treatment for these patients.

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PLASMA IGF-I AND IGF-BP3 LEVELS AS DIAGNOSIS CRITERIA OF GH DEFICIENCY.

Idiopathic GH deficiency (GHD) is a clinically and biologically heterogeneous condition, making it difficult to diagnose. We have shown that the magnetic resonance imaging (MRI) aspect (Argyropoulou, J Pediatr 1992, p.886) and the growth response during the first 2 yrs of hGH therapy are good criteria of GHD. Forty nine patients with height of <2SD and a GH peak response after 2 pharmacological stimulation tests of <10 ng/ml were classified according to the accuracy of the diagnosis of GHD: Group I (n=22) certain GHD with pituitary stalk interruption or familial form; Group II (n=14) without these criteria; Group III (n=13) transient GHD with GH peak >10 ng/ml after a 3rd provocative test performed after a 15-d interruption of hGH therapy. In groups II and III pituitary height on MRI was normal in 9, <2SD in 6 and not evaluated in 12 patients. Other causes of short stature were excluded. IGF-I was measured in the plasma without extraction by the non-equilibrium technique described by Furlanetto and al. and IGF-BP3 by a RIA kit (DSL, Webster, Texas). The results were compared to those found in prepubertal idiopathic short children: lowest limits were 0.2u/ml and 2 µg/ml for IGF-I and IGF-BP3 respectively (m±SE: 0.8±0.1 u/ml and 410.1 µg/ml).

Groups	Age, yr	GH peak, ng/ml (% <8)	IGF-I, u/ml (% <0.2)	BP3, µg/ml (% <2)
I	4.8 ± 0.9	4.1 ± 0.7 (86)	0.1 ± 0.03 (91)	1.35 ± 0.2 (91)
II	9.8 ± 1.3	7.7 ± 0.5 (69)	0.8 ± 0.17 (18)	3.95 ± 0.5 (10)
III	11.7 ± 1.1	8.4 ± 1.2 (43)	0.7 ± 0.11 (15)	3.80 ± 0.5 (14)

GH peak, IGF-I and IGF-BP3 were lower (p<0.001) in I than in II and III. In Group I, these 3 parameters were not modified by associated hypothyroidism deficiency (10 cases) or spontaneous hypoglycemia (9 cases). Conclusion: Almost all patients with certain GHD had low values of IGF-I and IGF-BP3. Conversely those with transient form and/or normal pituitary anatomy had normal values. Pituitary stalk interruption, height increase >1SD during the 1st year of hGH therapy, IGF-I<0.2u/ml, and IGF-BP3<2µg/ml are markers of the diagnosis of GHD. When these criteria are absent or discordant further evaluation is necessary.

(Supported by a grant from Assistance Publique-Hôpitaux de Paris.)

ASYMMETRIC DEVELOPMENT IN PATIENTS WITH GONOSOMAL MOSAICISM.

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Two cases of gonosomal mosaicism (XO/XX and XO/XY) are described presenting with asymmetric body development before and during growth hormone (GH) treatment. A girl, 11 years of age (bone age 10 yr / Greulich-Pyle) with short stature (height SDS -3.7 / SDS 0.47 for UTS) and mild clinical symptoms of Turner's syndrome developed different mammary size while treated with GH. Her karyotype was 45 XO/46 XX with a recovery of 45 XO cell lines in 20% of peripheral blood cells. Neither her clinical aspect nor endocrine data revealed any sign of pubertal development at this time. Six months following GH-therapy with 4IU/m<sup>2</sup>/d her growth velocity increased from 3.1 to 10.1 cm/yr. Simultaneously an unilateral breast development was noticed being B2 at the left and B1 (Tanner stage) at the right side, whereas pubic and axillary hair development was infantile at a bone age of 11 years (GP). The second patient, a boy presented at the age of 10 years (bone age 10 yr / GP) with growth retardation (height SDS - 2.1), but without any diagnostic reference to growth hormone deficiency. The main clinical feature was a mild body asymmetry with a hemihypotrophy of the left side. The uncommon clinical aspect led to a genetic analysis, showing a karyotype of 45XO/46XY with the normal male cell line found in 60 % of peripheral blood cells. At the age of 14 years (bone age 14 yr / GP) the patient developed spontaneous puberty. The physical asymmetry remained constant. Because of low growth velocity and predicted adult height far below target height GH-therapy was initiated. The beneficial effect of GH-treatment on final height in individuals with gonosomal mosaicism is doubted. Its deteriorating effect in patients with predisposition to hemihypertrophy/hemihypotrophy may be dependant on growth potential in tissues of different sides as a function of percentage distribution of gonosomal abnormal cells.

GROWTH AND ACROMEGALOID FEATURES IN A HYPOPHYSECTOMIZED (HY) MALE: THE ROLE OF PRANDIAL HYPERINSULINISM (HI). L.A. Silverman, M.M. Grumbach & F.A. Conte, Dept. of Pediatrics, University of California, San Francisco, San Francisco, Ca. 94143, USA

Normal growth as well as acromegaloid features have been described in patients after HY for craniopharyngioma. This growth has been attributed to multiple factors including prolactin, IGF-I, insulin and anti-GH receptor antibodies. We have followed for 18 y a now 22 year old male who was HY for a craniopharyngioma at age 4. Subsequently he was replaced with thyroid, cortisone DDAVP and low dose halotestin. He grew normally and attained a final height of 179.5 cm (+2 S.D. for T.H.; +0.4 SD for C.A.); weight 96 kg, 30 % above IBW, BMI 30 kg/m<sup>2</sup>. He had coarse features, large hands and feet, and a calcaneal heel pad of 25 mm, consistent with acromegaly. GH response to arginine, L-dopa, GRF and sleep were < 0.5 ng/ml. Circulating Prl < 2 ng/ml, IGF-I 17 ng/dl, IGF-II 104 mcg/l and IGF-BP3 0.4 mg/l were very low. No plasma GH bio-activity was detected. He had a strikingly elevated insulin response to an OGTT (peak > 400 mU/l) basal insulin and glucose tolerance were normal, consistent with insulin resistance. We postulate two mechanisms by which insulin induced the growth promotion and acromegaloid features in the presence of very low GH, IGF-I and Prl: the frequent and strikingly elevated concentrations of plasma insulin in response to feeding 1) act on the IGF-I receptor to produce an IGF-I effect and 2) the hyperinsulinemia increases local IGF-I production in cartilage (Alarid et al Endocrinology 130: 2305, 1992). We suggest the combination of these two effects of HI account for the growth pattern and acromegaloid features of this and similar patients.

INSULIN-LIKE GROWTH FACTOR (IGF) BINDING PROTEINS INHIBIT IGF-I-INDUCED DIFFERENTIATION IN L6E9 RAT SKELETAL MUSCLE CELLS. L.A. Silverman, D. Hsiao and S.M. Rosenthal, Department of Pediatrics, UCSF, San Francisco, CA 94143, USA

The insulin-like growth factors (IGFs) stimulate the differentiation of muscle cells. IGF binding proteins (BPs), which are expressed by skeletal muscle cells, may enhance or inhibit IGF actions. To explore the role of muscle IGF-BPs in IGF-induced myogenesis, we compared the effects of IGF-I and des(1-3)IGF-I (gift of Genentech, Inc.), an IGF-I analog with markedly reduced affinity for IGF-BPs but normal affinity for the IGF-I receptor, on creatine phosphokinase (CK) activity in rat L6E9 skeletal muscle cells. CK activity was determined using ADP and phosphocreatine in a colorimetric assay. Conditioned media were analyzed by <sup>125</sup>I-IGF-I ligand blot. Myoblasts grown in medium supplemented with 20% fetal bovine serum were transferred to medium supplemented with 2X heat inactivated horse serum and studied for up to 5 days in the absence or presence of 3 nM IGF-I or des-IGF-I. In comparison to untreated cells, cells treated with IGF-I demonstrated an 8-9 fold increase in CK activity. In contrast, cells treated with des-IGF-I demonstrated a 19-20 fold increase in CK activity versus untreated cells. In dose-response studies (0.7nM-7nM), des-IGF-I was 3-6 times more potent than IGF-I in inducing muscle CK activity. IGF-BP production was greatest at the time at which the largest difference in CK activity induced by des-IGF-I vs. IGF-I was observed. Since an IGF-I analog with reduced affinity for IGF-BPs is more potent than native IGF-I in stimulating CK activity, these data suggest that IGF-BPs expressed by skeletal muscle cells inhibit differentiation induced by IGF-I.