CLINICAL STUDY

Efficacy of 12-month treatment with the GH receptor antagonist pegvisomant in patients with acromegaly resistant to long-term, high-dose somatostatin analog treatment: effect on IGF-I levels, tumor mass, hypertension and glucose tolerance

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Abstract

Objective: We aimed to investigate the efficacy of pegvisomant in patients with acromegaly resistant to long-term (≥ 24 -month), high-dose treatment with octreotide-LAR (40 mg/month) or lanreotide (120 mg/month).

Design: This was an open, prospective study.

Subjects and Methods: We studied 16 patients with acromegaly (nine women; aged 28–61 years). The main outcome measures were IGF-I levels, blood pressure, glucose tolerance and safety (liver function and tumor size). Pegvisomant was given at doses of 10–40 mg s.c. daily. Dose titration was performed every month by IGF-I assay.

Results: Three patients spontaneously stopped pegvisomant treatment after 6–9 months because of poor compliance; from the measurement of serum pegvisomant, another patient was found not to inject herself properly. After 6 months, IGF-I levels decreased by $63 \pm 19\%$ (767.8 ± 152.9 vs 299.8 ± 162.9 $\mu\text{g/l}$, $P < 0.0001$, t -test); serum IGF-I levels normalized in 57%. After 12 months, IGF-I levels normalized in nine (75%) patients and were reduced by over 50% in another three (25%). The mean tumor volume remained stable during the study (1198 ± 1234 vs 1196 ± 1351 mm^3 , $P = 0.37$): it did not change ($\pm 25\%$ vs basal) in nine patients, increased by 39.4% and 40.8% in two and decreased by 30.8–46.5% in four. The total/high-density lipoprotein (HDL):cholesterol ratio (from 4.4 ± 1.0 to 3.7 ± 0.6 , $P = 0.0012$), glucose levels (from 5.6 ± 1.2 to 4.4 ± 1.4 mmol/l , $P = 0.026$), insulin levels (from 12.4 ± 6.7 to 8.1 ± 3.0 mU/l , $P = 0.0023$) and homeostasis model assessment (HOMA) index (from 3.4 ± 2.1 to 1.9 ± 1.0 , $P = 0.0017$) decreased.

Conclusions: Treatment for 12 months with pegvisomant normalized IGF-I levels, and improved cardiovascular risk parameters and insulin sensitivity in patients with acromegaly resistant to long-term, high-dose treatment with somatostatin analogs. The tolerance of treatment was good.

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Introduction

The optimal treatment for acromegaly should remove the tumor while preserving normal residual pituitary function, restore normal growth hormone (GH) and insulin-like growth factor (IGF)-I secretion, and relieve symptoms and complications directly caused by GH excess, ultimately reversing the unfavorable long-term outcome (1). Current treatment options include surgery, radiotherapy and pharmacotherapy with somatostatin

analogs, dopamine agonists and, more recently, a genetically engineered GH-receptor antagonist, pegvisomant.

Trans-sphenoidal adenomectomy remains a cornerstone of treatment for GH-secreting tumors: however, even in the most experienced hands, surgery is successful in the majority of microadenomas and enclosed macroadenomas but fails in larger or invasive tumors that represent the majority of GH-secreting tumors (2–5). Radiotherapy, delivered by external radiation, proton beam, gamma particles and interstitial radiotherapy,

should be reserved for patients in whom surgery is contraindicated or unsuccessful, and in whom medical treatment fails to control persistent hormone excess due to residual tumor (6). The occurrence of severe complications, such as cranial nerve palsies, optic neuritis, impaired memory, lethargy and tissue necrosis, has decreased with modern techniques, but damage of the normal hypothalamic-pituitary region results in hypopituitarism in more than half of the patients within 10 years (1, 6, 7).

Dopamine agonists are less effective than somatostatin analogs in normalizing GH and IGF-I excess: disease control is more likely achieved in patients with mixed prolactin/GH-secreting adenomas than in those with pure GH-secreting adenomas, and in patients with lower GH and IGF-I levels before treatment (1). Currently, somatostatin analogs, especially the long-acting formulations, octreotide-LAR and lanreotide, are the most widely used drugs to control acromegaly. Serum IGF-I levels normalize in two-thirds of selected patients (8–13), and tumor shrinkage (>20%) occurs in most (>70%) *de novo* patients (14–16). Indeed, a recent meta-analysis of the literature reported that, for patients who experienced significant shrinkage, an approximately 50% decrease in pituitary mass is achieved when a somatostatin analog is used exclusively or before surgery or radiotherapy (17). Thus, one-third of patients are not controlled by currently available treatments.

Blocking the activity of the GH receptor by pegvisomant, thereby inhibiting the synthesis of IGF-I, is a new pharmacologic approach to acromegaly. A placebo-controlled, 12-week study found a significant dose-dependent fall in serum IGF-I after pegvisomant compared with placebo, and 89% of the patients treated with 20 mg normalized IGF-I levels (18). In another study, 97% of 186 patients treated for at least 12 months normalized IGF-I levels (19). There are only two reports on the efficacy of pegvisomant, in six and seven patients respectively, with acromegaly resistant to somatostatin analogs (20, 21). Normalized IGF-I levels were found in all patients after 6–12 weeks and 12–24 months respectively. The results, however, were limited to IGF-I levels in both studies.

Here we present our preliminary experience in 16 patients with acromegaly who previously had undergone unsuccessful surgery and/or radiotherapy and were shown to be resistant to long-term, high-dose treatment with octreotide-LAR or lanreotide.

Patients and methods

Inclusion criteria

This open-label, 12-month study included patients 18 years of age or older with previously established diagnosis of acromegaly (GH nadir during oral glucose tolerance test (oGTT) of >1 µg/l). IGF-I levels had to be at

least 1.3 times above the upper limit of normal age- and sex-matched controls, after a 4-month washout of long-acting somatostatin analogs. The patients were required to have had stable hormone replacement therapy for hypopituitarism for at least 6 months prior to study start and stable size of the pituitary tumor (shown by magnetic resonance imaging (MRI)) for the last 12 months. Patients were excluded from the study if they had had treatment with dopamine agonists during the 5 weeks prior to study start, a pituitary adenoma with a distance to the optic chiasm of 3 mm or less, chronic hepatitis, known or suspected drug/alcohol abuse, or any other condition (such as severe hepatic or renal disease, or malnutrition) that could result in abnormal GH or IGF-I concentrations, history of relevant drug or food allergies, or unwillingness or inability to self-administer the study medication. All women of childbearing age were required to use an acceptable form of contraception throughout the study. Women who were pregnant or nursing were excluded from participation. The ethics committee of the University Federico II of Naples approved the protocol, and all patients provided written, informed consent prior to study participation.

Patients

Sixteen patients (nine women, seven men, age range 28–61 years, median 46.5 years) were enrolled. All patients but two had previously undergone surgery and two patients had also received radiotherapy (patient no. 10 for 3 years and no. 16 for 12 years before entering the study; Table 1). All patients had been treated with octreotide-LAR or lanreotide (LAN) at the maximal tolerated dose (LAR 40 mg/month, LAN 120 mg/month) for at least 24 months (24–60 months, median 38 months) without achieving disease control. Patient profiles are shown in Table 1. Hypertension was diagnosed when diastolic blood pressure (DBP) values were over 90 mmHg and was graded as mild at 91–104 mmHg, moderate at 105–114 mmHg, and severe at ≥115 mmHg, in line with WHO criteria (22). Among the 16 patients, four (25%) had hypertension; this was mild in three patients and severe in one. Hypertension was treated with combination therapy of an angiotensin receptor antagonist and a diuretic. Only the patient with severe hypertension was also treated with beta-blockers. Five patients (31.2%) had diabetes mellitus (defined as fasting glucose of >7 mmol/l on two consecutive measurements or 2-h glucose during oGTT of >11.1 mmol/l): during the previous treatment with somatostatin analogs, diabetes was treated with insulin in two patients and insulin sensitizers in the remaining three. However, before starting pegvisomant, the treatment of diabetes was withdrawn, and patients received only a low-calorie, low-carbohydrate diet. One patient (6.2%) had glucose intolerance (defined as 2-h glucose of 7–11.1 during oGTT with an additional value over

Table 1 Patient profiles at study entry and effects of pegvisomant treatment.

Patient no.		Age (years)	Serum GH levels		Serum IGF-I levels		Tumor description	MRI findings Tumor volume			Pegvisomant treatment			
			Before (μg/l)	After	Before (μg/l)	After		Before (mm ³)	After (mm ³)	Δ Change (%)	Dose (mg/day)	Blood levels (μg/l)	Duration (months)	Side effects
1.	M	28	9.0	17.8	898	267	remnant at r-CS, partial empty sella	364	291.2	−20.0	25	40 960	12	↑ Transaminases ↑ Tumor size
2.	M	29	3.4	14.3	748	222	remnant at l-CS	182	171.6	−5.7	25	40 600	12	
3.	M	32	14.4	66.8	756	279	residual macro I, r-P	936	1318.2	40.8	15	36 560	12	
4.	F	35	5.6	14.4	594	180	negative	49.9	49.9	0.0	10	42 240	12	↑ Tumor size
5.	F	39	71.2	95.7	834	467	residual macro I, r-P	2995.2	2784.6	−7.0	40	45 200	12	
6.	M	42	74.8	145	870	297	residual macro I, S, bilateral P	2386.8	3328	39.4	35	77 280	12	
7.	F	44	4.5	6.3	525	117	remnant at r-CS, partial empty sella	262.1	145.6	−44.4	15	34 930	12	
8.	F	46	18.1	18.1	728	137	remnant at r-CS, partial empty sella	686.4	520	−24.2	15	11 340	12	
9.	F ^E	47	32.9	15.3	828	792	residual macro S, l-P	2652	2652	0.0	40	50	12	
10.	F**	49	48.5	31.5	638	166	remnant at r-CS, partial empty sella	1081.6	748.8	−30.8	25	41 240	12	
11.	M*	52	9.3	7.7	900	195	remnant posterior, partial empty sella	1123.2	1123.2	−33.3	30	49 760	12	
12.	M*	52	21.9	19.9	1023	353	negative	823.7	440.4	−46.5	40	11 780	9	
13.	F	55	17.2	20	729	113	residual macro I, r-P	520	468	−10.0	20	34 400	12	↑ Transaminases
14.	F ^E	56	13.4	13	937	291	remnant at r-CS	218.4	218.4	0.0	10	5680	6	
15.	F	58	8.4	11	674	124	empty sella				25	37 800	12	
16.	M**	61	15	15	935	252	residual macro i, l-P	4160	4160	0.0	25	21 640	6	

*Patients treated with somatostatin analogs only. **Irradiated patients. CS: cavernous sinus; S: suprasellar; l: intrasellar; P: parasellar; r: right; l: left; superscript E: patients excluded from the analysis because the pegvisomant dose was not appropriate. Patient 9 was excluded because did not inject herself appropriately and patient 14 because of stably increased transaminases.

11.1 mmol/l at 0–2 h after oGTT) (23) and did not receive any specific treatment.

Study design

After an initial loading dose of 40 mg pegvisomant, treatment was started with 10 mg/day s.c. The dose was adjusted (± 5 mg) every 6 weeks by IGF-I levels obtained 2 weeks before the time for dose adjustment. The goal was to obtain an IGF-I concentration within the normal range for sex and age; however, the pegvisomant dose was not to be above 40 mg or below 5 mg. In one patient, titration of the dose was not possible due to increase of liver transaminase levels: this patient (no. 14, Table 1) was excluded from the calculation of treatment efficacy. Another patient (no. 9) did not inject herself properly as demonstrated by the blood levels of pegvisomant assayed at study end: this patient was also excluded from the calculation of treatment efficacy. The maximal dose of pegvisomant was 10 mg in two patients, 15 mg in three, 20 mg in one, 25 mg in five, 30 mg in one, 35 mg in one and 40 mg in the remaining three patients (Table 1). Vital signs and adverse events were monitored at each study visit (every 6 weeks) until the 6-month visit and then every 12 weeks. Blood pressure was measured in the right arm, with the subjects in a relaxed sitting position; the average of six measurements (three taken by each of two examiners) with a mercury sphygmomanometer was taken. Moreover, change in ring size of the fourth digit of the right hand (fifth digit if the fourth was too large) was measured by a jeweler's ring-sizing system over the course of the study. Laboratory assessments, including fasting glucose, fasting insulin, GH (calculated as the mean of a 6-h blood sampling, 0800–1400 h, with 30-min sampling), serum triglycerides, total cholesterol, HDL-cholesterol and fibrinogen, were conducted at baseline and at the 6- and 12-month visits. Blood for liver chemistry tests (alanine aminotransferase (ALT), aspartate transaminase (AST) and albumin) and for routine laboratory tests (hematology and serum chemistry) was collected every 4 weeks. The total/ high-density lipoprotein (HDL)-cholesterol ratio, considered an index of severe cardiovascular risk (24), and the estimate of insulin resistance by homeostasis model assessment (HOMA) score, by applying the Matthews *et al.* formula (25) (fasting serum insulin (μ U/ml) $\times$ fasting plasma glucose (mmol/l)/22.5), were also calculated. The conversion factors (mg/dl to mmol/l) for lipids and glucose were as follows: cholesterol 0.02586, triglycerides 0.01129 and glucose 0.05551.

MRI studies

MRI studies were performed on clinical 0.5T, 1T and 1.5T scanners, using T1-weighted gradient recalled-echo (repetition time 200–300 ms; echo time

10–12 ms; flip angle 90°, four signal averages) in the sagittal and coronal planes, as already reported (15). In each measurement, 7–11 slices were obtained, with a slice thickness of 2–3 mm and an in-plane spatial resolution of 0.7–0.97 mm (the matrix was 192–256 $\times$ 256 on a field of view of 24–25 cm on the sagittal plane, and 160–256 $\times$ 256 on a field of view of 18–20 cm in the coronal plane). The acquisitions were repeated before and after the administration of 0.1 mmol gadolinium chelate (diethylenetriamine pentacetate). A pituitary MRI and a visual perimetry were performed at screening (–2 to –4 weeks prior to start with pegvisomant) and at the 6- and 12-month visits. Shrinkage was established on tumor volume calculated by the Di Chiro and Nelson formula (volume = height $\times$ length $\times$ width $\times$ $\pi/6$) and on the maximal tumor diameter. Tumor shrinkage was semiquantitatively evaluated as change of the pre-treatment tumor volume: $\pm 25\%$ absent, ± 25 –50% mild, and $\pm 50\%$ notable (15). The radiologists (F B and F T) were blind to the status of the patients.

Assays

At diagnosis and during therapy with somatostatin analogs, serum GH levels were measured by IRMA (HGH-CTK-IRMA; Sorin, Saluggia, Italy); assay sensitivity was 0.2 μ g/l; the intra- and interassay coefficients of variation (CV) were 4.5% and 7.9% respectively. Before and during pegvisomant treatment, IGF-I assays were performed at the Neuroendocrine Unit Laboratories, Ludwig Maximilians University of Munich. Serum samples were shipped frozen on dry ice. Before analysis, samples were thawed and allowed to reach ambient temperature. IGF-I levels were measured by the automated Advantage chemiluminescent IGF-I assay system (Nichols Diagnostics Institute, Bad Nauheim, Germany). By this method, samples are acidified to separate IGF-I from its binding protein IGFBP-3. To prevent reassociation of IGF-I and IGFBPs, and thus to exclude interference of IGFBP-3, the acidified samples are incubated with an excess of IGF-II. The intra-assay CV were 11.5%, 5.1% and 3.5% at concentrations of 42, 262 and 522 ng/ml respectively. At the same concentrations, between-assay CV were 10.6%, 10.6% and 10.2%. The lower limit of quantification was 17 ng/ml, and the linear range was 17–1000 ng/ml. Assays were performed in a central laboratory (Ludwig Maximilians University of Munich) where the researcher (M B) was blind to patients' response to previous treatments. Results were reported together with the assay-specific sex- and age-matched reference range (26).

In order to monitor endogenous human (h)GH secretion in patients treated with the hGH-analog pegvisomant, a specific assay was designed to be free of interference by the drug. From a panel of monoclonal antibodies (mAbs) raised against hGH, a pair of

antibodies was identified, targeting epitopes in receptor-binding sites 1 and 2 respectively, which have been mutated in the hGH analog. Neither of the mAbs selected showed cross-reaction with pegvisomant, indicating that they target amino-acid residues mutated in pegvisomant. Combining these mAbs (8B11 and 6C1) in a sandwich assay leads to a linear dose-relationship for hGH with a lower detection limit of 0.2 ng/ml and an upper end of the linear working range at 50 ng/ml for 50 μ l samples. WHO IRP 80/505 is used as the calibrator. Intra-assay variabilities were 4.1% and 3.9% at concentrations of 5.2 and 14.6 μ g/l respectively. Inter-assay variabilities at the same concentrations were determined to be 7.3% and 9.2% respectively (27). Serum concentrations of pegvisomant were determined by an immunofluorometric sandwich assay involving two mAbs, 10A7 and 6F1, raised against hGH and retaining high cross-reactivity with pegvisomant (25–50% compared with recombinant human (rh)GH). The assay was calibrated against pegvisomant (Pfizer, New York, NY, USA) diluted in assay buffer. Serum concentrations of pegvisomant in treated patients are at least 500 higher than endogenous hGH concentrations. Therefore, all serum samples were diluted 1:100 in assay buffer prior to analysis to eliminate the interference from the endogenous hGH levels present in the samples. The assay has a linear working range of 0.5–200 ng/ml, corresponding to final concentrations of 50–20 000 ng/ml. Samples above 200 ng/ml have been diluted in assay buffer; samples below 0.5 ng/ml (corresponding to a final concentration of 50 ng/ml) have been reported as '<50 ng/ml'. The within-assay variabilities were 7.5%, 4.6% and 5.2% at concentrations of 160, 650 and 3900 ng/ml respectively. The between-assay variabilities were 13.5%, 6.4% and 8.5% at the same concentrations.

Statistical analysis

The statistical analysis was performed with the SPSS package (Cary, NC, USA). The effect of pegvisomant was analyzed by the Wilcoxon matched pair test (basal vs 6 or 12 months of treatment according to individual patient follow-up). The percent changes in IGF-I levels, GH levels and tumor volume were correlated with each other by calculating the Spearman correlation coefficient. Data are reported as mean+s.d. unless otherwise specified.

Results

Serum IGF-I levels

Of the initial 16 patients, two were withdrawn from treatment after 6 months because of inability to titrate the dose due to a rise of transaminase levels (no. 14 was positive for hepatitis C virus but had normal liver

function at study entry) or inability to follow the protocol (no. 16); one patient (no. 12), although achieving normal IGF-I levels, was withdrawn from treatment after 9 months for poor compliance with the treatment and, at the completion of study, another patient (no. 9) was found not to inject herself properly by the results of pegvisomant assay (830 and 50 μ g/l, at 3 and 12 months of treatment, Table 1). While patients 12 and 16 were included in the analysis of the results, as they performed the treatment appropriately until withdrawal, the other two patients (nos. 9 and 14) were excluded. After 6-month pegvisomant treatment, IGF-I levels decreased by $67\pm 11\%$. Serum IGF-I levels were in the normal range for age and gender in 8/14 patients (57.1%) and were 1–1.3-fold above the upper normal range in another three patients (20%). After 12 months, serum IGF-I levels were in the normal range in 9/12 patients (75%), and above the normal range but reduced by more than 50% of baseline in the remaining three (25%). Serum GH levels did not significantly increase during treatment (Table 2).

Tumor mass

There was no significant change in residual tumor volume in 9/14 patients (64.3%; Table 1). In two patients, there was an increase of residual tumor mass; by 39% in the former (no. 6, Table 1, Fig. 1) and 41% in the latter (patient no. 3, Table 1, Fig. 2). In neither of these patients was the increase in tumor volume associated with impairment of visual fields or other neurologic symptoms, except for mild headache in the latter patient. However, the former patient had rebound tumor growth after stopping octreotide-LAR therapy (Fig. 1). In both patients 3 and 6, GH levels increased significantly during treatment (from 14.4 to 66.8 and 74.8 to 108 μ g/l respectively). In four patients ((28.6%) nos. 7 and 10–12, Table 1), there was a significant decrease of tumor mass of 30.8–46.5% respectively. One (no. 10, Table 1) of the four patients had undergone radiotherapy 30 months before study entry, while the other two patients did not receive radiotherapy. The percent changes in tumor volume did not correlate with the percent changes either in GH ($r = 0.32$, $P = 0.26$) or in IGF-I levels ($r = 0.2$; $P = 0.49$).

Hemodynamic and cardiovascular risk parameters (Table 2)

At study entry, four patients had hypertension managed with angiotensin receptor inhibitors plus diuretics and/or beta-blockers with partial control of blood pressure. After pegvisomant treatment, there was no change of systolic and diastolic blood pressure, heart rate, fibrinogen, or total cholesterol and triglyceride levels in

Figure 1 Coronal projections of sellar MRI before octreotide-LAR (A), after 24 months of octreotide-LAR at the dose of 40 mg/month (B), and before (C) and after 6 (D) and 12 months of pegvisomant treatment (E) in patient no. 6. Tumor increased by 39% compared with panel C. Tumor size increased by 56% after 4 months of octreotide-LAR withdrawal before starting pegvisomant treatment.

the entire series; HDL-cholesterol levels significantly increased, so that the total/HDL-cholesterol ratio significantly decreased. When hypertensive patients ($n = 4$) were considered separately from normotensive patients ($n = 10$), there was a significant decrease of diastolic blood pressure during treatment in those with hypertension (from 103.8 ± 11.1 to 87.5 ± 6.4 mmHg; $P = 0.04$); only one of the four patients remained hypertensive while doses of hypertensive drugs remained unchanged.

Glucose tolerance and diabetes (Table 2)

After pegvisomant treatment, in the entire series of patients, there was a significant decrease in glucose and insulin levels and HOMA index, without any significant change in glycosylated hemoglobin levels. In the six patients with diabetes or glucose intolerance, insulin levels (from 17.8 ± 7.6 to 8.9 ± 3.6 mU/l; $P = 0.048$) and the HOMA index (from 5.3 ± 2.4 to 2.0 ± 0.9 ; $P = 0.019$) significantly decreased, while

Figure 2 Coronal projections of sellar MRI before lanreotide (A), after 24 months of lanreotide at the dose of 120 mg/month (B), and before (C) and after 6 (D) and 12 months (E) of pegvisomant treatment in patient no. 3. Tumor increased by 41% compared with panel C. Tumor size increased by 25% after 4 months of lanreotide withdrawal before starting pegvisomant treatment.

glucose levels also decreased (from 6.9 ± 1.6 to 5.0 ± 0.1 mmol/l; $P = 0.08$).

Changes in symptom score (Table 3)

None of the symptoms recorded significantly improved after treatment, although joint pain, asthenia, paresthesia and snoring showed a favorable trend.

Tolerability of the treatment

At the beginning of treatment, two patients had noted macroglossia that subsided with treatment. Two patients (nos. 2 and 14) had increases in liver transaminases, by 1.5- and 2-fold above the upper normal range after 2 months. Liver transaminases spontaneously normalized in one patient (no. 2) despite treatment continuation and increase of the dose. In the other

Table 2 Effect of pegvisomant treatment on clinical, biochemical and endocrine findings. Data are shown as mean±s.d. and *P* values refer to Wilcoxon matched *t*-test. The two patients noncompliant with treatment (nos 9 and 14 described in Table 1) were excluded from the calculation.

	Before	After	<i>P</i>
Weight (kg)	83.5±15.0	85.7±13.6	0.055
Serum GH levels (μg/l)	22.9±24.0	34.5±40.4	0.29
Serum IGF-I levels (μg/l)	775.1±141.4	237.8±106.7	<0.0001
Tumor volume (mm ³)	1198±1234	1196±1351	0.37
Ring size (mm)	12.7±2.2	12.2±2.3	0.78
Systolic blood pressure (mmHg)	133.9±16.2	129.6±10.1	0.13
Diastolic blood pressure (mmHg)	87.1±13.6	86.2±7.1	0.70
Heart rate (bpm)	72.8±7.6	76.8±7.9	0.14
Total cholesterol levels (mmol/l)	5.3±1.0	5.5±0.8	0.43
HDL-cholesterol levels (mmol/l)	1.2±0.3	1.5±0.2	0.0017
Total/HDL-cholesterol ratio	4.5±1.0	3.7±0.6	0.0012
Triglyceride levels (mmol/l)	1.5±0.9	1.5±0.7	0.86
Glucose levels (mmol/l)	5.6±1.2	4.4±1.4	0.0012
HbA1c levels (%)	5.3±0.7	5.3±0.5	0.24
Insulin levels (mU/l)	12.4±6.7	8.1±3.0	0.0023
HOMA index	3.4±2.1	1.9±1.0	0.0017
Fibrinogen levels (mg/dl)	342.1±75.2	361.6±63.6	0.58
AST levels (U/l)	19.4±8.6	22.9±15.1	0.64
ALT levels (U/l)	18.6±14.0	40.1±61.2	0.017
Albumin levels (g/dl)	3.8±0.4	4.4±0.3	0.0002

patient (no. 14), the dose of pegvisomant was firstly increased from 10 to 15 mg/day but was reduced again to 10 mg/day because of a further and significant increase of transaminases levels accompanied by severe gastrointestinal discomfort. Only in this patient was the increase in transaminases levels accompanied by an increase in gamma-glutamyl transpeptidase (γ-GT) levels. Liver transaminases remained at the baseline level during the subsequent period of treatment that was stopped after the 6-month follow-up. Both patients were positive for hepatitis C virus in the absence of overt hepatic disease at study entry. While AST levels did not change during the treatment, ALT levels significantly increased in the entire series (Table 2).

Discussion

The most important result of the current study is that after 12 months of treatment with the GH-receptor

antagonist, pegvisomant, normal IGF-I levels for age and sex were achieved in 11/14 patients (78.6%), and acromegaly was proven to be resistant to long-term, high-dose treatment with long-acting somatostatin analogs. In another three patients, IGF-I remained higher than normal but decreased by more than 50% of the baseline level. IGF-I normalization was accompanied by improved cardiovascular risk, that is, reduced total/HDL cholesterol ratio, and improved insulin sensitivity and blood pressure control. Additionally, there were no detrimental effects on tumor growth with the exception of two men who both had tumors with proven growth during the period of withdrawal from somatostatin analogs before entering the pegvisomant trial.

Even if data using pegvisomant in the treatment of acromegaly are still scant compared with those available with the somatostatin analogs, it is evident that this drug lowers serum IGF-I levels in patients with active acromegaly (27). Pegvisomant administration at daily doses of 10, 15 or 20 mg for 12 weeks induced a dose-dependent reduction of serum IGF-I, free IGF-I, insulin-like growth factor-binding protein (IGFBP)-3 and ALS in 112 patients with active acromegaly based on pretreatment IGF-I levels 1.3 times above the upper limit of an age-related reference range (18). Serum IGF-I levels within the age-adjusted reference range were achieved in 54% of the patients treated with 10 mg/day, 81% in those treated with 15 mg/day and 89% in those treated at 20 mg/day (18). Besides, a significant reduction of symptoms and signs was recorded, including a reduction of ring size (18). Long-term follow-up of the patients included in previous clinical studies has indicated normalization of serum IGF-I levels in 97% of 186 patients treated for up to 18 months with doses up to 40 mg

Table 3 Effect of pegvisomant treatment on symptom score*. Data are shown as mean±s.d. and *P* values refer to Wilcoxon matched *t*-test. The two patients noncompliant with treatment (nos 9 and 14 described in Table 1) were excluded from the calculation.

	Before	After	<i>P</i>
Headache	2.6±1.9	3.0±2.4	0.59
Sweating	2.0±2.1	1.6±2.2	0.36
Joint pain	3.9±2.7	2.8±2.6	0.08
Asthenia	3.8±2.3	2.6±2.6	0.09
Soft tissue swelling	2.1±1.9	2.1±1.8	1.0
Paresthesia	3.0±2.7	1.6±2.5	0.08
Snoring	2.4±2.3	1.5±1.9	0.08

*Disease signs and symptoms were scored on an 8-point scale from 0 = absent to 8 = severe, incapacitating.

(19). More recently, pegvisomant in weekly administrations was also shown to be very effective combined with somatostatin analogs in controlling patients with active acromegaly (28).

However, all these studies were not designed to investigate the response to pegvisomant in patients with proven resistance to long-term, high-dose therapy with somatostatin analogs; these are the patients allowed to receive pegvisomant treatment in Europe according to the product label.

The only two studies (20, 21) reporting the efficacy of pegvisomant in patients with acromegaly resistant to somatostatin analogs included six and seven patients only; data were limited to IGF-I levels only; and, in one study (20), results were available only after a very short period (6–12 weeks) of treatment. All the 13 patients achieved normalization of IGF-I levels, and no side effects were noted. Among our 16 patients, of whom 12 were treated for 12 months with pegvisomant, 11/14 in whom the doses could be titrated according to the protocol (75%) achieved normal IGF-I levels for the age-adjusted range, consistent with previous data (18). Moreover, IGF-I fall was accompanied by improvement of cardiovascular risk markers, such as total/HDL-cholesterol ratio, insulin sensitivity and blood pressure. The decrease in serum IGF-I levels during pegvisomant treatment has been reported to be accompanied by a dose-dependent increase of serum GH levels (18). GH levels rose after 2 weeks of treatment and remained stable thereafter (18). The GH increase during therapy has raised concerns about potential tumor regrowth. Increase in tumor volume occurred in two of 131 patients during the long-term follow-up, although in both of these cases no clear association between tumor growth and treatment was found (18). GH levels during pegvisomant treatment did not increase significantly in our series of patients. The mechanism for the increase in tumor mass observed in two of our patients remains to be fully clarified: an increase in tumor size after stopping somatostatin analogs is, however, not unexpected and has previously been reported (29–31). In patient no. 6 (shown in Fig. 1), tumor shrinkage was observed during octreotide-LAR, and rebound growth occurred after octreotide-LAR withdrawal; after 6 months of pegvisomant treatment, when IGF-I levels were still not normalized, tumor size was similar to that measured before octreotide-LAR. Even in patient no. 3 (shown in Fig. 2), tumor regrowth was observed after surgery, tumor size was partially reduced during lanreotide therapy, and post-pegvisomant tumor size was not different from that before lanreotide therapy. Thus, in both our patients, tumor regrowth could be due to withdrawal from somatostatin analog treatment. Neither of the two patients showing tumor growth during pegvisomant had received irradiation. It should be noted, however, that neither the percent changes of GH levels nor those of IGF-I levels were

correlated with the percent changes of tumor volume. Therefore, changes of GH levels are unlikely to predict tumor increase. Since pegvisomant is a modified GH analog, it is essential that GH levels are subjected to a specific assay that excludes the interference of the treatment itself. Recently, Besser *et al.* (32) reported that significant tumor regrowth occurs in under 10% of patients cured surgically, and in under 1% of patients receiving radiotherapy. Similarly, 2.2% of somatostatin analog-treated tumors continue to grow. Therefore, 1–3% of GH-secreting pituitary adenomas grow independently of the treatment approach to them.

Few data are available on the effects of pegvisomant on intermediary metabolism. In a recent study (33), patients with elevated or normal IGF-I levels on octreotide-LAR were switched to treatment with pegvisomant for 28 weeks; both groups had improved glucose and hemoglobin (Hb)A1c levels, and the most marked effects were observed in patients with diabetes. In another study, insulin sensitivity measured by the homeostatic model assessment equation was reported to be improved (34). Another recent study also reported a better glucose profile in patients receiving combined treatment with somatostatin analogs and pegvisomant than when receiving somatostatin analogs only (35). The results of the current study confirm the ability of pegvisomant to improve glucose homeostasis and insulin sensitivity. Pegvisomant treatment normalized glucose levels in all six patients who had abnormalities of glucose tolerance at study entry and in whom the diabetes had been difficult to manage during somatostatin analog therapy. We also observed an increase in HDL-cholesterol levels and reduced total/HDL-cholesterol ratio, considered to be an independent risk factor for cardiovascular diseases (24). These data are partially in agreement with a previous study (36) reporting an improvement of lipoprotein metabolism in patients with acromegaly treated with pegvisomant, although previous data noted an increased level of low-density lipoprotein (LDL) and total cholesterol. Even if the effects on lipid profile and glucose tolerance should be investigated in a larger series of patients, the reduction in the total/HDL-cholesterol ratio could be of clinical relevance in patients with acromegaly who are at risk of developing cardiovascular disease, mainly diastolic heart failure (37). The increase in HDL levels is not, however, an unexpected finding since it was also shown with somatostatin analogs or surgery (38, 39).

In the current group of patients, there was a trend to improvement in all symptoms except headache. The lack of statistically significant changes may be due to the small cohort; alternatively, it is possible that the previous long-term treatment with somatostatin analogs, even if unsuccessful, reduced soft tissue swelling to a threshold limit and patients could not appreciate further improvements.

As far as tolerability of pegvisomant is concerned, less than 2% of patients with increased transaminase levels

have been reported in the literature (18). We found this complication in 2/15 patients (13.3%); however, it is important to point out that both our patients with elevated liver transaminases after pegvisomant treatment were positive for hepatitis C virus, although having normal baseline transaminases. But it is well known that liver transaminases may fluctuate in such patients. These two patients had been treated for a long time with somatostatin analogs and also had gallbladder abnormalities. In one of these patients, liver transaminases decreased despite increasing the dose of pegvisomant; thus, we can conclude that only in one of our 16 patients did the increase in liver enzymes prevent dose titration and thus the ability to adhere to the protocol. We also found a significant increase of albumin levels after treatment, indicating a preserved secretory liver function. In any case, liver function should be monitored, as recommended in the package insert of the registered indications.

In conclusion, this study reports that 12-month pegvisomant treatment in patients with persistent acromegaly after surgery and resistance to long-term, high-dose treatment with long-acting somatostatin analogs normalizes IGF-I and controls hemodynamic and metabolic complications in the majority of patients, with overall good tolerance of the drug.

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