Effects of Intramuscular Microsphere-Encapsulated Octreotide on Serum Growth Hormone, Insulin-like Growth Factors (IGFs), Free IGFs, and IGF-Binding Proteins in Acromegalic Patients

A. Kaal, J. Frystyk, C. Skjærbæk, S. Nielsen, J.O.L. Jørgensen, C. Bruns, P. Marbach, I. Lancranjan, J. Weeke, and H. Ørskov

Nine acromegalic patients selected to be well-controlled on subcutaneous octreotide injections three times daily were transferred for more than 1 year to intramuscular injections every month with a new microsphere-encapsulated octreotide formulation (Sandostatin LAR®, Sandoz, Basel, Switzerland). The dosage was titrated to between 20 and 60 mg/mo to approach optimum control in each patient. The study compares the efficacy of subcutaneous octreotide three times daily versus intramuscular microsphere-encapsulated octreotide once monthly. For all patients, average serum growth hormone (GH), insulin-like growth factor binding protein-3 (IGFBP-3), and total insulin-like growth factor-I (IGF-I) were as follows (subcutaneous *v* washout *v* intramuscular after 9 months' treatment): 2.5 ± 0.5 versus 13.0 ± 3.3 versus 2.2 ± 0.8 μg/L (GH), 3,240 ± 312 versus 3,880 ± 547 versus 3,190 ± 226 μg/L (IGFBP-3), and 200 ± 30 versus 364 ± 32 versus 207 ± 36 μg/L (IGF-I; mean ± SEM), ie, no significant differences were found between the two regimens. One patient was a relatively poor responder on both regimens, with average 8-hour serum GH concentrations of approximately 5 μg/L and increased IGF-I. In two patients GH was suppressed below 3 μg/L, with IGF-I just above the normal range. The remaining six patients had GH below 2 μg/L and IGF-I within the normal range for age. Concomitant values for IGFBP-1 were 4.9 ± 0.9 versus 1.6 ± 0.3 versus 7.9 ± 1.9 μg/L (*P* = .043 for subcutaneous *v* intramuscular), and for free IGF-I, 1,150 ± 262 versus 2,290 ± 265 versus 660 ± 153 ng/L (*P* = .009 for subcutaneous *v* intramuscular). For all five parameters mentioned above, washout values were different from those obtained during subcutaneous and intramuscular treatments. Total and free IGF-II in serum were unchanged throughout. The new formulation was well tolerated and greatly preferred by the patients. There was occasional slight local tenderness at the injection site lasting up to a few days. Two patients developed asymptomatic biliary sludge. The previously described acute octreotide-induced stimulation of IGFBP-1 release appears to occur also during long-term treatment with elevation in fasting levels. Another novel observation is the drastically reduced serum free IGF-I in fasting samples, which may be an important new facet in octreotide treatment. For both effects, continuous stable octreotide exposure seems to be more effective than intermittent exposure as occurs with conventional subcutaneous octreotide injections three times per day.

WITHIN 10 YEARS of the introduction of octreotide, many hundred acromegalic patients have enjoyed excellent control of their symptoms with this agent, many have attained shrinkage of their adenomas, and serious adverse effects have been practically nil. The sole disadvantage, as with insulin-dependent diabetes, was the need for multiple daily subcutaneous injections throughout a lifetime or until successful neurosurgery. The first innovative octreotide formulation that sought to overcome this inconvenience was intranasally applicable octreotide powder.[1]

When octreotide became available, it was rightly praised for its prolonged plasma half-life of approximately 2 hours, which made possible the subcutaneous injection treatment.

However, it had been noted early that continuous subcutaneous octreotide infusion provided vastly improved stability of serum octreotide concentrations and of suppressed growth hormone (GH) levels[2] and an increased dose efficacy of twofold to threefold.[3] These observations were confirmed by several groups.[4] It was thus an obvious choice to attempt to mimic this constant delivery by encapsulating octreotide in biodegradable microspheres, exploiting the excellent results obtained with administration of bromocriptine (Parlodel; Sandoz, Basel, Switzerland), in a long-acting repeatable formulation.[5]

From the Institute of Experimental Clinical Research and Medical Department M, University Hospital, Aarhus, Denmark; and Sandoz Pharma Limited, Basel, Switzerland.

Address reprint requests to H. Ørskov, MD, Institute of Experimental Clinical Research, University Hospital, Aarhus, Nørrebrogade, 8000 Aarhus C, Denmark.

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SUBJECTS AND METHODS

Nine acromegalic patients (age range, 29 to 66 years) participated in a multicenter octreotide study.[6] They were included if they had a satisfactory control (mean 12-hour serum GH <5 μg/L) on conventional subcutaneous octreotide injection therapy with dosages of 100 to 200 μg three times daily and achieved a mean serum GH elevation of 100% and above 5 μg/L after 2 days' octreotide withdrawal (clinical data in Table 1). Further, the serum GH level was not to be suppressed during an oral glucose tolerance test. Thus, the patients were admitted to the hospital while still on subcutaneous therapy for a 12-hour serum GH and octreotide profile and were readmitted after 2 days' withdrawal and washout for profiles in the untreated condition, whereafter the first intramuscular injection of Sandostatin LAR® (Sandoz, Basel, Switzerland) was administered the next morning, and patients were evaluated at close intervals for the next 60 days with serum profiles obtained on days 1, 2, 3, 7, 14, 28, 35, 42, and 60. During this period the study was double-blind, with patients having received 10, 20, or 30 mg octreotide. Thereafter, they continued on the original dose if GH suppression and insulin-like growth factor-I (IGF-I) had become satisfactory, or else were transferred to a dose of 30 mg.

Depending on serum GH and IGF-1 responses, dosages were individually titrated up or down during the following months to approach normal serum GH and IGF-I concentrations using a

Table 1. Clinical Data of the Nine Acromegalic Patients

Patient No.	Sex/Age (yr)	Year of Diagnosis	Year of Surgery	Serum GH, SC, Washout, Last IM Injection (μg/L)	Octreotide Last SC Dose Three Times Daily (μg)	IM Octreotide Initial/Final Dose (mg)	Weight Change (kg)*	Octreotide Antibodies,† SC, Washout, IM
1	F/61	1985	1985	5.27	200	30/60	+1.3	9
			1988	11.59				22
				5.68				0
2	F/61	1986	none	2.46	100	10/30	+2.4	0
				7.27				0
				1.26				0
3	M/47	1979	1979	1.22	200	20/30	+2.7	39
			1983	36.96				51
			1985	0.79				10
4	M/41	1982	none	3.90	200	30/60	+0.6	7
				10.61				8
				2.01				0
5	F/66	1991	1991	2.60	100	20/40	−1.5	0
				9.45				0
				1.76				0
6	F/37	1979	1979	1.74	200	10/60	+3.4	0
			1988	16.80				0
				2.90				0
7	F/60	1966	1967	1.50	100	20/20	+3.1	0
				12.82				0
				2.38				0
8	M/29	1991	1991	0.74	100	10/20	+6.3	0
				5.94				0
				0.58				0
9	F/44	1990	1990	2.56	100	30/30	+3.8	23
				4.53				58
				0.79				2

Abbreviations: SC, subcutaneous; IM, intramuscular.

*Weight change during 1 year's IM octreotide treatment.

†The antibody presence is determined as percent bound ^{125}I-octreotide after incubation. The varying results in the four positive patients (no. 1, 3, 4, and 9) on three occasions (during SC treatment and washout, and after ninth IM injection) are undoubtedly due to varying degrees of saturation with cold octreotide. A coprecipitation of 6.1% occurring in normal subjects has been subtracted from all values.

dosage range of 20 to 60 mg/mo. The hourly serum sampling period was reduced from 12 to 8 hours after 60 days, and all means for serum GH and octreotide in this report are calculated on the basis of samples obtained in the interval of 8 to 16 hours. Fasting samples were used for determination of serum IGF-I, IGF-II, IGF binding protein-1 (IGFBP-1), IGFBP-3, free IGF-I, and free IGF-II.

Thyroid function was assessed every 3 months by determination of thyroid-stimulating hormone, thyroxine, triiodothyronine, free thyroxine, and free triiodothyronine in serum. Ultrasonography of the biliary tree was performed with the same intervals, and oral glucose tolerance tests were performed after washout and after 12 months.

The study was approved by the regional ethical committee and the Danish Health Authorities. Patients gave their written informed consent to participate in the study, which was conducted in accordance with the Helsinki Declaration with amendments.

GH levels were measured using a two-site double–monoclonal-antibody immunofluorometric assay (DELFIA, Wallac, Turku, Finland), and IGF-I and IGF-II levels were determined by the same principle using an in-house assay directly in diluted HCl/ethanol extracts of serum.[7] IGFBP-3 was determined by a commercial assay (Diagnostic System Laboratories, Webster, TX), as was IGFBP-1 (Medix Biochemica, Kaūniainen, Finland). Free IGF-I and free IGF-II levels were measured in ultrafiltrates of serum (Amicon YMT 30 membrane, Amicon Division, W.R. Grace and Co., Beverly, MA) after reestablishing in vivo physical and chemical conditions by incubation and centrifugation at 37°C and airing with CO_2,[8] using the assays applied in measurements of totally extractable IGF-I and IGF-II in serum[7] with a sensitivity of approximately 2.5 and 10 ng/L. Serum octreotide was determined by specific radioimmunoassay[9]; reagents were obtained from Anawa Laboratorien, Wangen, Switzerland.

Paired Student's *t* tests were applied when appropriate. A *P* value less than 5% was considered significant. All results are the mean ± SEM.

RESULTS

Serum Octreotide, GH, and IGF-I

A detailed account of the pharmacokinetics of the new octreotide formulation is presented elsewhere in this volume for the entire multicenter population.[6] The nine patients participating in Denmark also exhibited very con-

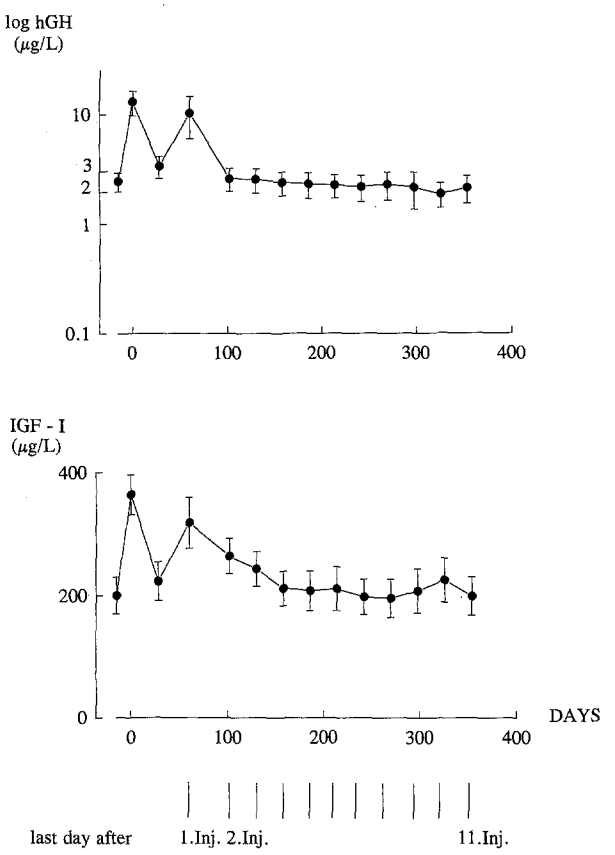


Fig 1. Mean ± SEM of average 8-hour serum GH levels and of two fasting IGF-I samples in nine acromegalic patients. The first point at left was obtained during previous subcutaneous octreotide therapy (100 to 200 μg three times daily); the second corresponds to time point zero after 2 days' withdrawal of the above treatment; the third and fourth, 28 and 60 days after the first intramuscular injection (Inj.) of microsphere-encapsulated octreotide; the fifth, 42 days after the second injection; and thereafter, every 28 days after the following injections.

stant 8- or 12-hour serum octreotide and GH levels (data not shown) after the first 14 days, with a slow build-up thereafter of serum octreotide with time, leading to stable steady-state conditions after the third injection.

Average 8-hour serum patterns of GH and fasting IGF-I are shown in Fig 1. In Fig 2, the individual profiles are seen, including average 8-hour octreotide to illustrate the time course over the first year's treatment while octreotide doses were adjusted. GH suppression waned during the 42 to 60 days after the first injection. There were no significant differences between GH or IGF-I levels attained with the two therapy regimens (2.5 ± 0.5 *v* 2.2 ± 0.8 μg/L for

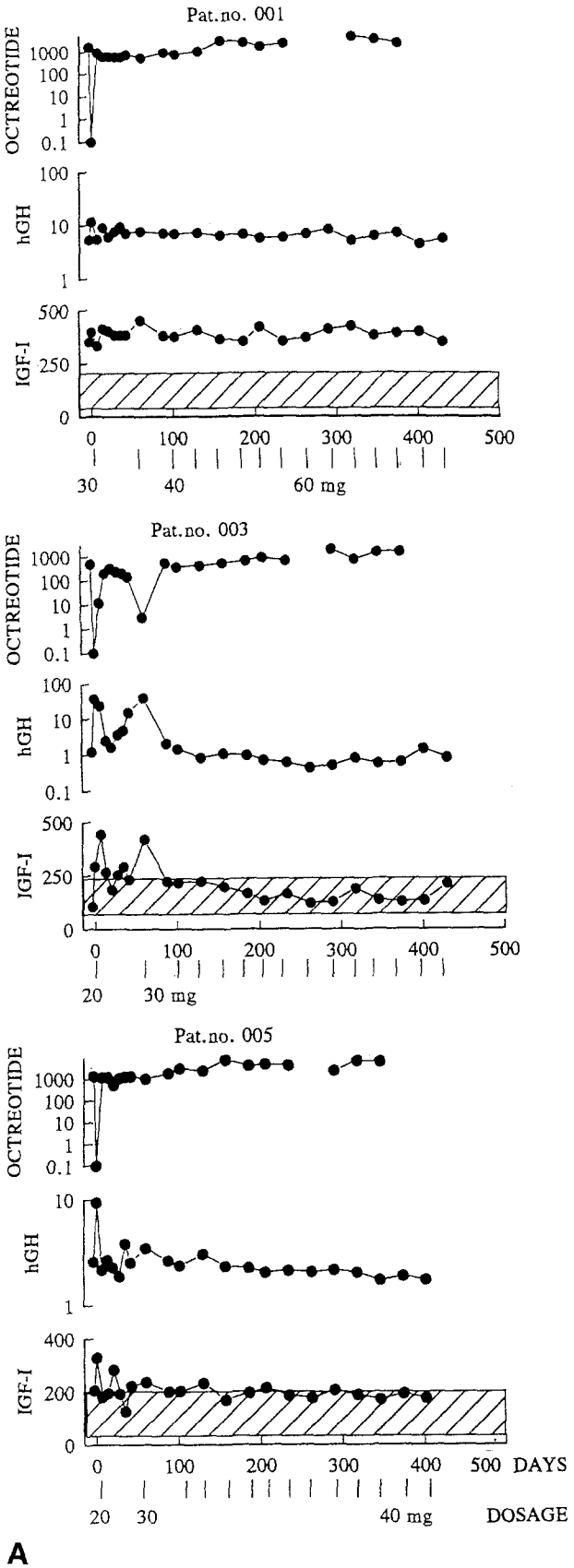
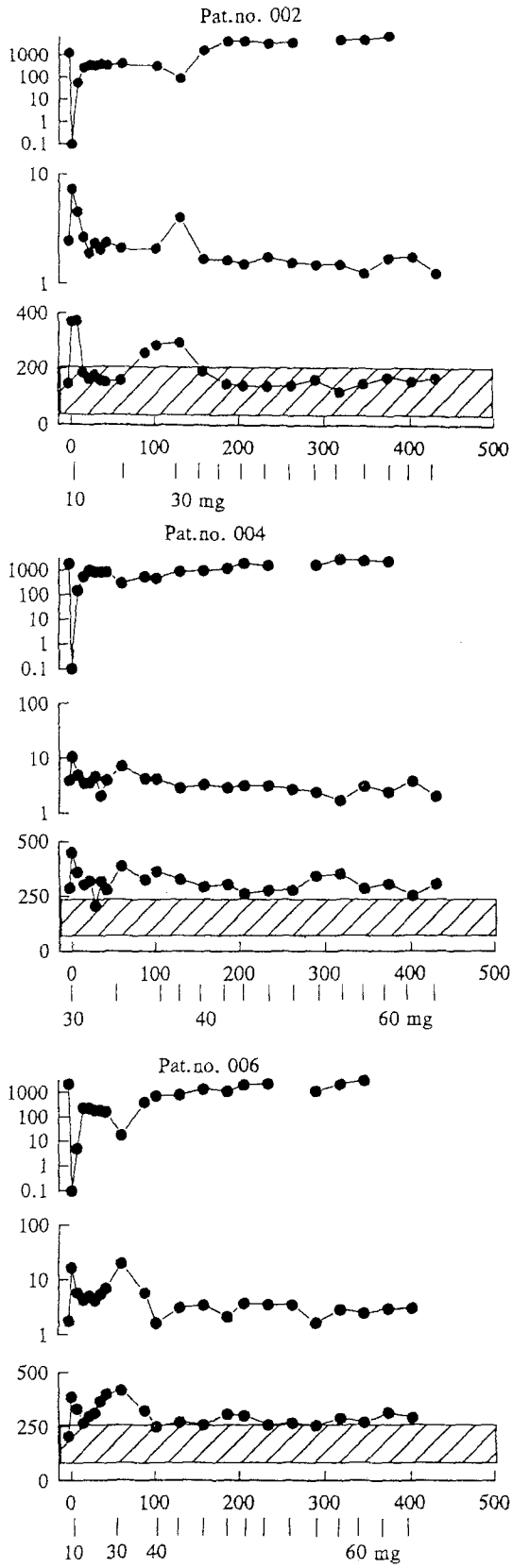
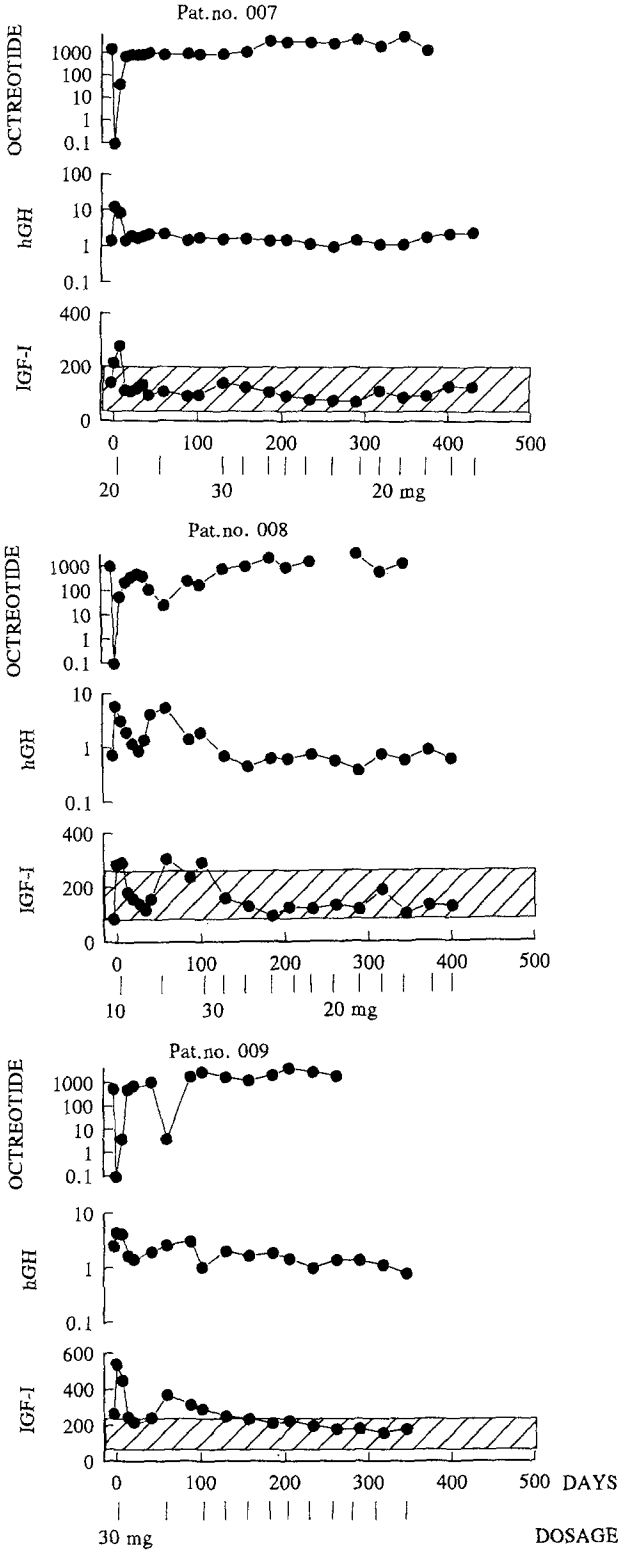


Fig 2. As in Fig 1. (A) and (B) Average 8-hour serum octreotide (ng/L, log scale), GH (μg/L, log scale), and fasting IGF-I (μg/L) including all profiles obtained until now in each of the nine patients. (▨) Range of serum IGF-I in a normal population of the same age decennium. Octreotide dosage is shown below.





GH and 200 ± 30 *v* 207 ± 36 µg/L for IGF-I), whereas both were significantly lower than those obtained after washout (13.0 ± 3.3 µg/L for GH and 364 ± 32 µg/L for IGF-I).

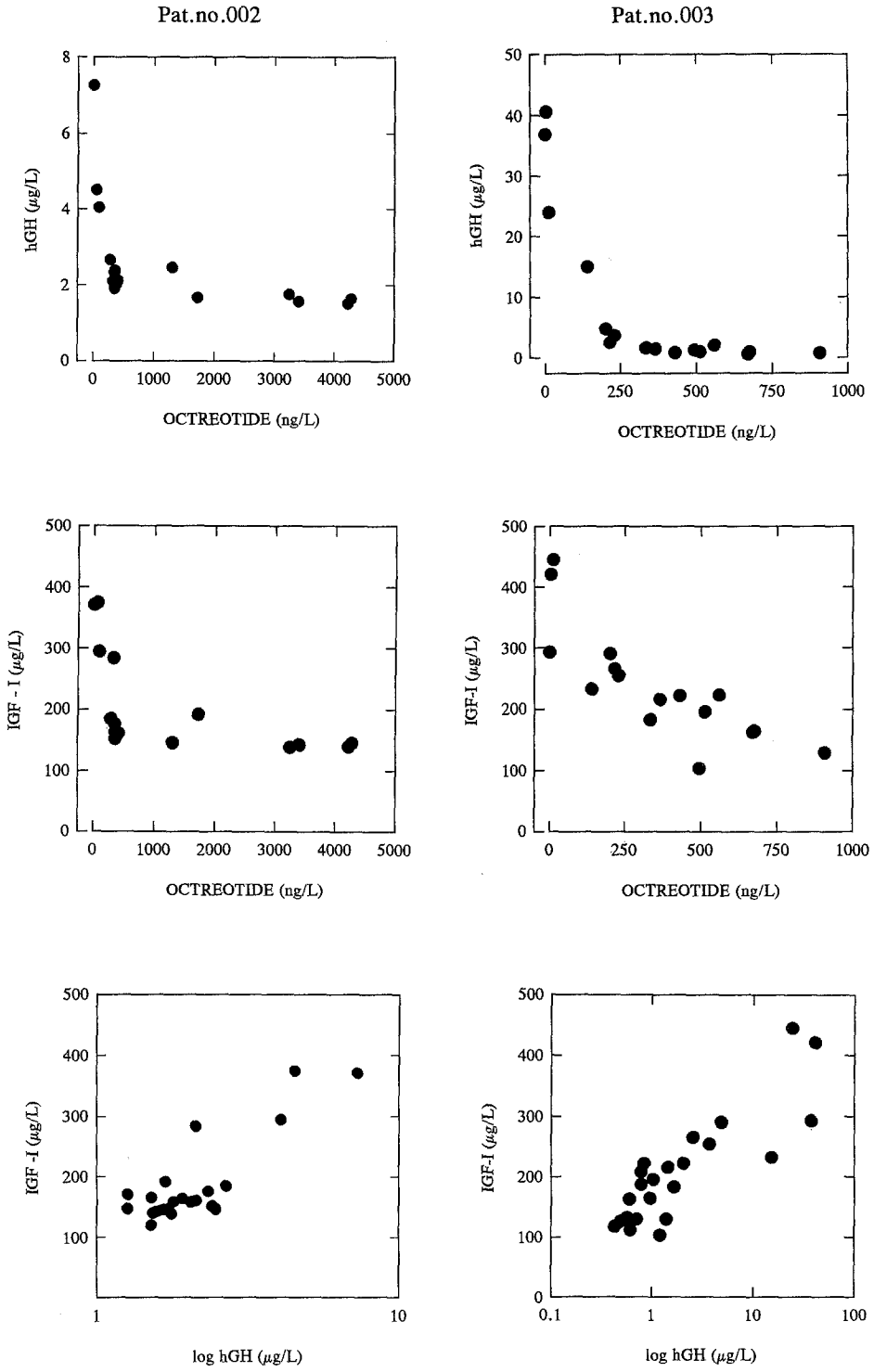


Fig 3. Relationships between serum octreotide and GH, octreotide and IGF-I, and GH and IGF-I in patients no. 2 and 3.

Patient no. 1 was a relatively poor responder to both subcutaneous and intramuscular octreotide therapy, barely fulfilling inclusion criteria. Eight-hour serum GH was just above 5 μg/L and IGF-I was above the normal range for age; no real improvement was attained after an increase of the octreotide dose to 60 mg/28 days. For the remaining eight patients, two achieved GH suppression to below 3 μg/L (no. 4 and 6) and serum IGF-I just above the normal range for age, and GH in the remaining six was suppressed to below 2 μg/L with normal serum IGF-I. Two of them (no. 7 and 8) attained serum IGF-I near the normal lower limit and had their octreotide dose reduced from 30 to 20

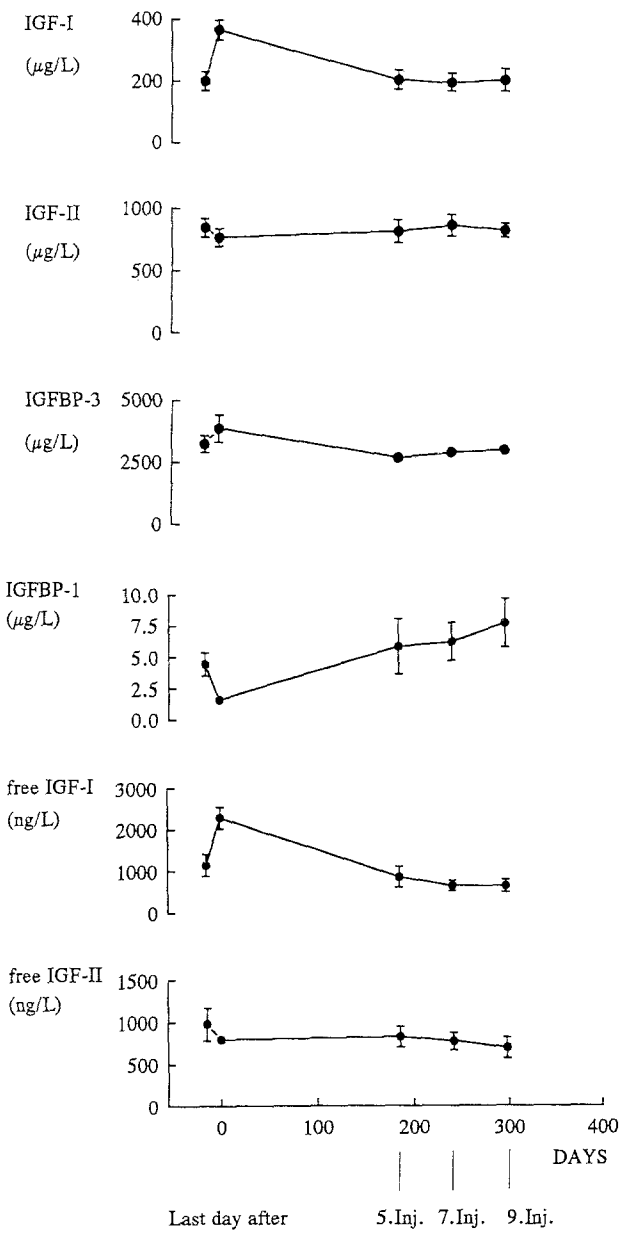


Fig 4. Variations in mean fasting serum total extractable IGF-I, total IGF-II, IGFBP-3, IGFBP-1, free IGF-I, and free IGF-II in samples obtained (left to right) during subcutaneous injections, after withdrawal, and 28 days after the fifth, seventh, and ninth intramuscular injection (Inj.) of microsphere-encapsulated octreotide.

mg/mo. (In patient no. 7, this induced an increase in serum GH from 1.15 to 2.38 μg/L, without significant change in serum IGF-I.)

In Fig 3, typical examples are presented for the relationships between octreotide and GH, octreotide and IGF-I, and GH and IGF-I (patients no. 2 and 3). Little if any additional effect on GH and IGF-I was attained by increasing serum octreotide beyond 2,000 ng/L. This result was similar to that in all nine patients.

Serum IGF-II, IGFBP-3, IGFBP-1, Free IGF-I, and Free IGF-II

Figure 4 shows the average fluctuations in these parameters in fasting samples obtained at selected time intervals: during conventional subcutaneous therapy, after 2 days' withdrawal (washout), and 28 days after the fifth, seventh, and ninth injection. IGFBP-3 follows the pattern already shown for GH and IGF-I. Neither total IGF-II nor free IGF-II displays significant fluctuations during the study. In contrast, fasting serum IGFBP-1 is significantly lower after washout than during either treatment regimen; and notably, the value after the ninth intramuscular octreotide injection is significantly higher than during subcutaneous injection therapy (7.9 ± 1.9 *v* 4.9 ± 0.9 μg/L, $P = .043$). Fasting serum free IGF-I is significantly lower after the ninth (and seventh) intramuscular injection than during the subcutaneous injection regimen (660 ± 153 *v* 1,150 ± 262 ng/L, $P = .009$)—corresponding to 29% of the washout value of 2,290 ± 265 ng/L ($P < .0001$). Figure 5 depicts the very similar alterations in serum free IGF-I in all patients. Compared with the normal range of free IGF-I for the age decennium, all patients attained circulating concentrations below the upper limit during intramuscular octreotide therapy, except for patient no. 1. However, her value declined from 3,017 ng/L (washout) to 1,785, 1,246, and 1,176 ng/L (fifth, seventh, and ninth injection) as compared with the upper limit for 60- to 70-year-old normal subjects of 880 ng/L. During subcutaneous therapy, five patients (no. 1, 4, 5, 7, and 9) were above normal limits; at washout all patients were above normal limits except no. 8, and after the ninth injection all but no. 1 and 9 were below the upper-normal limit and two patients (no. 2 and 8) were below the normal range.

Serum IGF-I and free IGF-I in the nine patients were positively related at all specified time intervals. Figure 6 illustrates the relationship at washout and after the seventh injection.

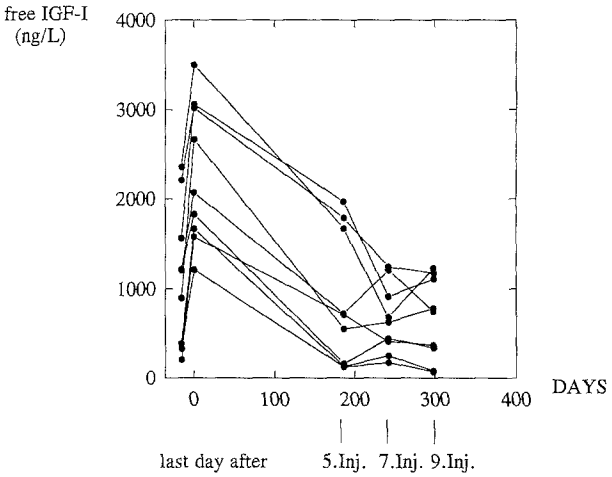


Fig 5. As in Fig 4. Variations in fasting serum free IGF-I in each of the nine patients.

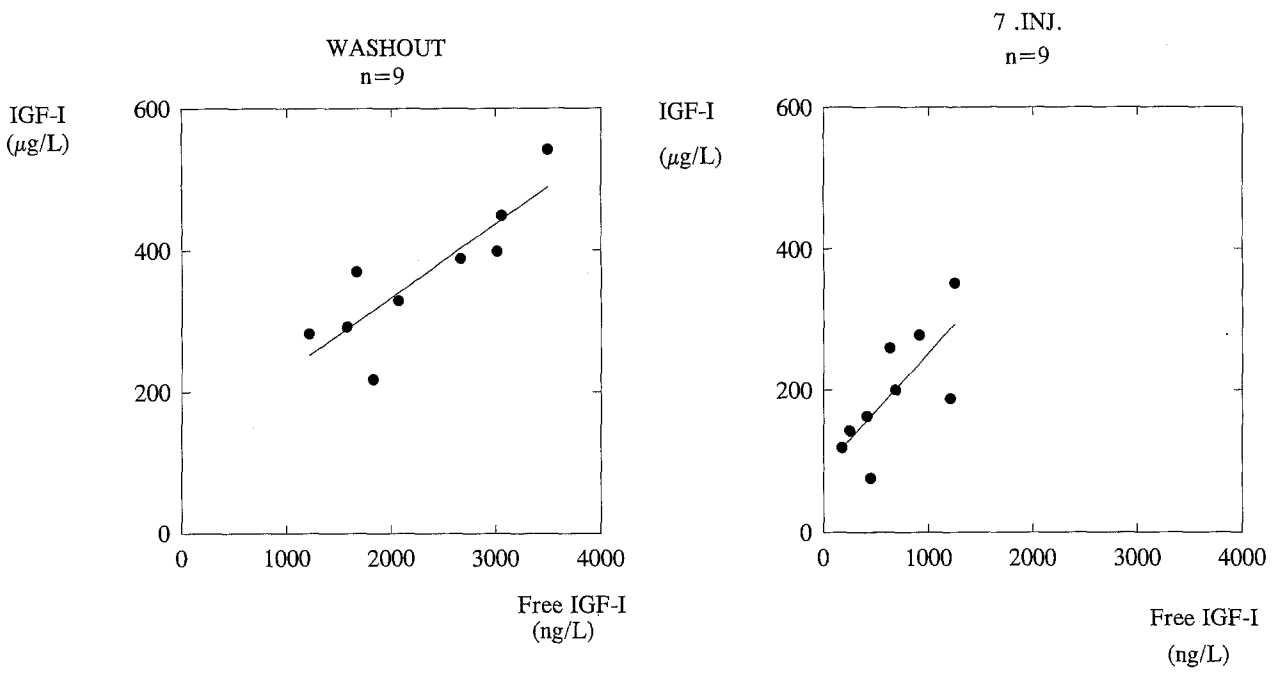


Fig 6. Relationship between total extractable serum IGF-I and serum free IGF-I in nine acromegalic patients at washout (after withdrawal of octreotide for 2 days, $r = .853$, $P = .003$) and 28 days after the seventh intramuscular injection (Inj.) of octreotide ($r = .738$, $P = .023$).

There were no changes in thyroid function (data not shown), no changes in glucose tolerance (data not shown), nor any changes in body weight (average, +2.5 kg during the study) different from those found in nine randomly selected patients who had been successfully adenomectomized (data not shown). Two patients developed asymptomatic biliary sludge in the study.

None of the patients had developed antibodies against octreotide[10] 1 year after the first intramuscular depot injection. But four patients had low-titer antibodies already at entry (Table 1).

Patients complained rarely of local tenderness at the injection site lasting up to a few days. However, all patients were eager to continue on treatment with the new formulation.

DISCUSSION

Microsphere-encapsulated octreotide (Sandostatin LAR®) appears to be a major breakthrough in the medical treatment of acromegaly as judged by the ongoing multicenter trials. Our experience throughout 1 year's therapy in nine patients is totally favorable, and this is also the patients' opinion.

Our data do not provide evidence of a greater suppressive effect on serum GH or total IGF-I, but previous results with subcutaneous pump infusion[2-4] indicate that continuous release is superior to intermittent subcutaneous injections two to four times daily in terms of suppression of serum GH levels and maintenance of stable serum octreotide levels, as well as efficacy of average serum octreotide concentrations.[3] The advantages for the patients of replacing several daily subcutaneous injections with only one intramuscular injection per month are obvious.

It must be noted that we studied only nine patients who were selected to be relatively good responders to subcutaneous octreotide and to have a serum GH level above 5 μg/L in the untreated condition. We cannot therefore conclude that our results are totally applicable in intramuscular octreotide treatment of less responsive acromegalic patients, or patients with lower untreated serum GH levels, who may require different doses of octreotide or exhibit a different relationship between serum octreotide and GH or IGF-I.

The incidence of biliary sludge (two patients) was similar to what would be expected in traditional octreotide treatment.[11] We initially suspected that weight gain was greater than expected, but when comparing body weight changes in nine successfully adenomectomized patients we likewise found widely varying gains and losses 2 years after neurosurgery, at a time when the shift between muscle and fat is probably important and very dependent on patients' awareness of food intake and activity. The only adverse effects we encountered were the well-known gastrointestinal symptoms, which occurred when octreotide therapy was reintroduced after washout, and local tenderness in a few percent of the injections, present for a maximum of a few days.

Thyroid function and glucose tolerance were unaffected during the study, in keeping with previous data on long-term octreotide therapy.[12] In contrast, glucose tolerance is impaired if assessed shortly after a subcutaneous octreotide injection, although 24-hour mean glucose is usually unaffected.[2,3,12,13] In a previous study with continuous subcutane-

ous octreotide infusion, oral and intravenous glucose tolerance were also unchanged,[3] demonstrating again that continuous octreotide release ensures better intermediate metabolism than intermittent injections.

As expected, serum IGFBP-3 concentrations paralleled the variations in serum GH and IGF-I and were also similar in subcutaneous and intramuscular therapy. Serum IGF-II and serum free IGF-II were unaffected by the kind of treatment and by withdrawal of octreotide, indicating that the rather long-term metabolic events occurring in the present study were compensated for by yet-unidentified regulatory mechanisms. This is in contrast to acute fluctuations such as those occurring after short-term starvation and during a glucose tolerance test, where serum free IGF-I and IGF-II variations are closely related and inversely related to variations in serum IGFBP-1.[8]

In our opinion, the most interesting and novel facets of our study are the variations in serum IGFBP-1 and in free serum IGF-I, with these being probably interdependent as in the case of the acute metabolic situations mentioned above. Ezzat et al[14-16] recently demonstrated that octreotide increased serum IGFBP-1 acutely 1 to 2 hours after injection, and that octreotide in vitro enhanced IGFBP-1 mRNA in human hepatoma cells. We found[17,18] that this ability is shared by native somatostatin, as well as lanreotide, another synthetic somatostatin octapeptide analog, and that the effects were independent of the almost-inavoidable concomitant suppression of insulin release. However, the IGFBP-1 increase was abolished with concomitant hyperinsulinemia.[18] This is why we decided to study fasting samples, which showed that long-term octreotide treatment over several months also maintains the stimulatory effect on IGFBP-1 release.

It is tempting to speculate that the observed tonic increase in serum IGFBP-1 is responsible for the concomitant octreotide-induced reduction in serum free IGF-1, and that these effects may be important extra bonuses in octreotide therapy as already foreseen on a theoretical basis.[14-18] This may explain the clinical impression that octreotide therapy sometimes achieves better control of acromegalic symptoms than expected from the attained reductions in serum GH and total IGF-I. The observed effects on serum IGFBP-1 and free IGF-I were significantly greater than with traditional subcutaneous octreotide administrations.

Finally, the stimulatory effect of somatostatin analogs on IGFBP-1 has previously been shown to be dose-dependent,[17] so this may also be the case for the suppressive action on serum free IGF-I. If these effects are as important as we hypothesize, they should probably be considered when trying to establish optimum doses of intramuscular octreotide in each individual patient. Considerable suppression of serum free IGF-I and an increase in IGFBP-1 may be achieved by administration of large doses even in patients whose adenoma is quite octreotide-resistant. This is indicated by our findings in patient no. 1, who closely approached the upper-normal limit for free IGF-I despite grossly elevated total serum IGF-I and an average serum GH just above 5 μg/L.

In conclusion, the sustained slow release obtained with microsphere-encapsulated octreotide induces very constant suppression of serum GH and IGF-I, at least as great as with subcutaneous injections of octreotide three times daily. These actions persist, so that monthly injections ensure constant serum octreotide and GH levels in responsive patients. The adverse effects are minimal and thus comparable to those in traditional octreotide treatment. The sustained elevation of IGFBP-1 was important, as was the (related?) drastic reduction in serum free IGF-I, which may imply an additional benefit at the cellular level during octreotide therapy.

REFERENCES

1. Weeke J, Christensen SE, Ørskov H, et al: A randomised comparison of intranasal and injectable octreotide administration in patients with acromegaly. J Clin Endocrinol Metab 75:163-169, 1992

2. Christensen SE, Weeke J, Ørskov H, et al: Continuous subcutaneous pump infusion of somatostatin analogue SMS 201-995 versus subcutaneous injection schedule in acromegalic patients. Clin Endocrinol (Oxf) 27:297-306, 1987

3. Christensen SE, Weeke J, Ørskov H, et al: Long-term efficacy and tolerability of octreotide treatment in acromegaly. Metabolism 41:44-50, 1992 (suppl 2)

4. James RA, White MC, Chatterjee S, et al: A comparison of octreotide delivered by subcutaneous infusion with intermittent injection in the treatment of acromegaly. Eur J Clin Invest 22:554-561, 1992

5. Pagani MD, Tengattini F, Montini M, et al: Efficacy of a new long-acting injectable form of bromocriptine in hyperprolactinaemic patients. Clin Endocrinol (Oxf) 36:369-374, 1992

6. Lancranjan I, Bruns C, Jaquet P, et al: Sandostatin LAR®: Pharmacokinetics, pharmacodynamics, efficacy, and tolerability in acromegalic patients. Metabolism 43:18-26, 1994 (suppl 1)

7. Frystyk J, Skjærbæk C, Dinesen B, et al: Non-competitive time-resolved immunofluorometric assays for determination of human insulin-like growth factor I and II. Growth Regul (in press)

8. Frystyk J, Skjærbæk C, Dinesen B, et al: Free insulin-like growth factors (IGF-I and IGF-II) in human serum. FEBS Lett 348:185-191, 1994

9. Marbach P, Neufeld M, Pless J: Clinical application of somatostatin analogs. Adv Exp Med Biol 188:339-353, 1985

10. Ørskov H, Christensen SE, Weeke J, et al: Effects of antibodies against octreotide in two patients with acromegaly. Clin Endocrinol (Oxf) 34:395-398, 1991

11. Dowling RH, Hussaini SH, Murphy GM, et al: Gallstones during octreotide therapy. Metabolism 41:22-33, 1992 (suppl 2)

12. Christensen SE, Weeke J, Kaal A, et al: SMS 201-995 and thyroid function in acromegaly: Acute, intermediate and long-term effects. Horm Metab Res 24:237-239, 1992

13. Ho KY, Weissberger AJ, Marbach P, et al: Therapeutic efficacy of the somatostatin analog SMS 201-995 (octreotide) in acromegaly. Effects of dose and frequency and long-term safety. Ann Intern Med 112:173-181, 1990

14. Ezzat S, Ren SB, Braunstein GD, et al: Octreotide stimulates insulin-like growth factor binding protein-1 (IGFBP-1) levels in acromegaly. J Clin Endocrinol Metab 73:441-443, 1991

15. Ezzat S, Ren SB, Braunstein GD, et al: Octreotide stimulates insulin-like growth factor binding protein-1: A potential pituitary-independent mechanism of drug action. J Clin Endocrinol 75:1459-1463, 1992

16. Ren SB, Ezzat S, Melmed S, et al: Somatostatin analog induces insulin-like growth factor binding protein-1 (IGFBP-1) expression in human hepatoma cells. Endocrinology 131:2479-2481, 1992

17. Wolthers T, Grøfte T, Flyvbjerg A, et al: Dose-dependent stimulation of insulin-like growth factor binding protein-1 by lanreotide, a somatostatin analog. J Clin Endocrinol Metab 78:141-144, 1994

18. Ørskov H, Wolthers T, Grøfte T, et al: Somatostatin-stimulated insulin-like growth factor binding protein-1 release is abolished by hyperinsulinemia. Clin Endocrinol Metab 78:138-140, 1994