ACROMEGALY

New Insights into Medical Treatment of Acromegaly

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New Insights into Medical Treatment of Acromegaly

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General Introduction

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Introduction

HISTORIC OVERVIEW

Giants and Acromegalics fascinated people, since ancient times. Historical artifacts, paintings, illustrations, photographs or articles have documented many. The earliest medical reports date back to 1516 [1]. In 1864 Verga was the first to describe an acromegalic in medical literature and called it "prosopectasia" [2]. However the article did not really characterize the disease. Pierre Marie was the first to do so and describe the disease and gave it the final name "acromegalie", in 1886 [3]. Although Pierre Marie was aware of the enlarged pituitary gland he did not describe this as cause of the disease. In 1887 Minkowski was the first to suggest a pituitary origin of acromegaly [4]. Later Massalongo also described the pituitary origin and additional the relationship between acromegaly and Gigantism [5]. So at the end of the 19th century the disease and origin were unraveled. A decade later Harvey Cushing was the first to observe partial reversal of clinical symptoms after partial hypophysectomy, and the first form of effective treatment was born [6].

Treatment

Surgery

Surgery was the first treatment option for acromegaly and gigantism. A decade before Harvey Cushing observed the partial reversal of clinical symptoms after partial hypophysectomy, Paul and Caton performed a subtemporal decompression in an acromegaly patient [7]. Most operations at that time were transcranial. However Stumme and Hochenegg were the first to operated trough transsphenoidal route [8]. Initial Harvey Cushing adopted their transsphenoidal approach, but converted later back to the craniotomy and so did the majority of neurosurgeons. In the 1960's craniotomy was perfected when microsurgical techniques were introduced and was the treatment of choice. It was not before the 20th century, that microsurgical techniques and transsphenoidal approach were combined and became common practice. This technique and approach were later perfected into a minimal invasive endoscopic method.

Radiotherapy

In Paris radiation therapy for pituitary tumors started in 1907 [9]. Application of radium sources planted in the sphenoid sinus was applied by the early neurosurgeons. More focused methods of pituitary radiotherapy were developed in the late 20th century.

Medical treatment

In the 1970's the first of a series medical therapies were discovered. Bromocriptine, a dopamine agonist, normalized growth hormone (GH) and insulin-like growth factor 1 (IGF1) in < 20 % of the patients [10-11]. Carbergoline, a more recently developed dopamine agonist, was able to achieve normalization of GH and IGF1 in < 30 % of the patients [11-12]. In the 1980's endogenous somatostatin was identified by, a Nobel Prize winner, Guillemin. Somatostatin was described as a hypothalamic peptide, which inhibited GH secretion of the pituitary [13]. The half-life in serum of endogenous somatostatin is 1-3 minutes. Octreotide, a somatostatin analog (SRIF), has a half-life of 1.5 hours and a 20 fold higher potency to suppress GH secretion than somatostatin [14]. Therefore octreotide had to be administered 3 times a day. To date the regular octreotide is rarely used. Depot formulations with microspheres (octreotide LAR) or the water solution (Lanreotide Autogel) facilitates the use of somatostatin analogs and increased efficacy [15-16]. Long-acting SRIF analogs have efficacy rates of about 50 % in normalizing GH and IGF1 [17]. A decade later a new class of antagonist, where developed named Pegvisomant [11,18]. Pegvisomant (PEG-V), a growth hormone receptor antagonist, is to date the most effective therapy for acromegaly.

TREATMENT MODALITIES TO DATE

Surgery

Over 90 % of the patients that are treated by surgery are treated using a transsphenoidal approach. Transsphenoidal surgery is well-established first line treatment for microadenomas in acromegaly. In patients with a microadenoma, remission can be achieved in about 80% [17] and recurrence is reported in 3-10% of the patients [19-22]. Long-term efficacy largely depends on the experience of the neurosurgeon, while GH levels at diagnosis, tumor size and extrasellar invasion also play a major role in this. When e.g. in hospitals without highly specialized neurosurgeons, remission rates in patients with microadenomas of less than 40 % are not uncommon [23]. Compared to SRIF analogs with an efficacy rate of 44-77% in normalizing IGF1 levels, remission rates for surgery of 40%-80%, are within the same range. However, successful surgery is accompanied by a rapid fall in GH and IGF1 and the costs are relative low when compared to long-term drug therapy (SRIF analogs, PEG-V).

However, after surgery in less than 50 % of patients with macroadenomas remission is reached, even in experienced neurosurgical centers [24]. Generally, reported remission rates are between 20-30 % for macroadenomas [23]. When compared to efficacy rates of medical treatment with SRIF analog and/or PEG-V, surgical efficacy rates in patients with macroadenomas are unacceptably low. There might be one exception, which is

the intrasellar macroadenoma. In this specific case remission rates are observed of approximately the same magnitude as with the microadenomas [23].

Radiotherapy

Conventional radiotherapy (RT) has been used as adjuvant treatment in acromegaly for many years and is still used for that purpose to date. Conventional RT is delivered in fractionated doses over a period of 5 to 6 weeks. The total amount administered is generally 40-50 Gray, at the level of the pituitary adenoma. With conventional RT remission rate of 50-60% after a mean follow up of 10 years are common [25-26]. The efficacy rates depend on the baseline GH and IGF1 levels more, as in most other treatment strategies. GH levels decrease to about 50% of their initials values within the first 2 years, and after that, a gradual further decrease in the next two decades can be expected. Within the first year after RT, control of tumor size can be achieved already and in more than 50% of the cases some decrease is tumor size is observed.

Post-radiotherapy hypopituitarism is reported in 50-80% of subjects after a mean follow up of 10 years [25-27].

Medical treatment

Somatotroph adenomas predominately express somatostatin sub-type 2 receptor (SSTR2) and SSTR5 [15]. The two commercially available long-acting SRIF analogs, octreotide LAR and lanreotide autosolution, predominately bind to the SSTR2, with similar affinity [15]. Octreotide LAR and lanreotide autosolution are SRIF analogs that inhibits GH secretion of the pituitary. Both formulations have more or less the same efficacy rates [15]. Normalisation of IGF1 and GH (< 2.5 µg/l) are observed in about 50 % of the patients, for both adjuvant and primary medical therapy [17]. The efficacy rates of somatostatin might be increased by gross debulking of the macroadenoma as was reported by e.g. Petrossians and co-workers [28]. The studies that have been conducted on the long-term treatment with SRIF analogs included patients who where responsive to SRIF analogs before start of the study [24] which is a clear bias.

In one of the few available prospective studies, SRIF analogs reduced sign and symptoms and induce tumour shrinkage in about 42 % of the patients [29]. In theory, pre-operative tumour shrinkage might improve surgical efficacy rates. Recently, a randomized controlled trial indeed observed improved normalization rate of IGF1, but not of GH, in patients with macroadenomas, who were pre-treated for 6 months with octreotide LAR [30]. The efficacy rates of PEG-V are much higher than for SRIF analog, but it has to be administrated by a subcutaneous injection daily and yearly cost are at least twice as high as for SRIF analogs. After long-term treatment with PEG-V, high efficacy in normalising IGF1 levels in more than 90 % of patients has been reported [31].

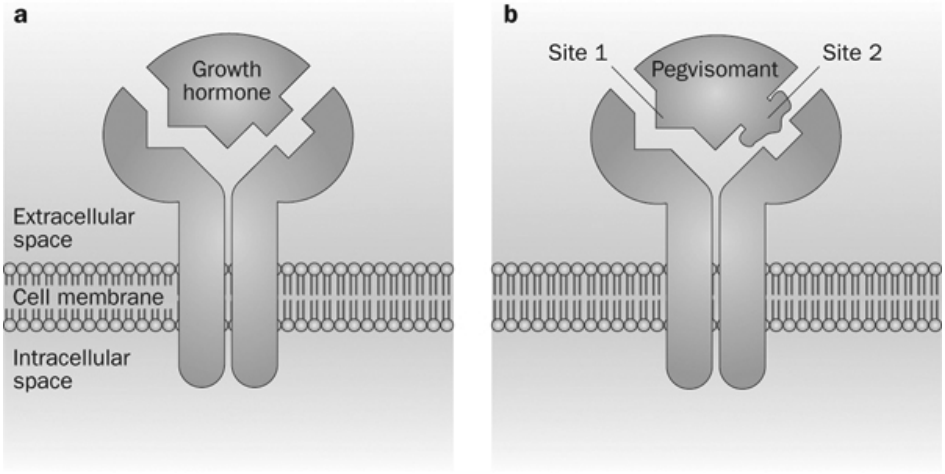

Figure 1: Ligand binding to the growth-hormone receptor. a | A schematic diagram of growth hormone binding to the dimerized growth-hormone receptor, which results in a conformational change of the membrane-bound receptor and activation of the downstream signal-transduction pathway. b | Pegvisomant binding to the dimerized growth-hormone receptor. Binding site 1 of pegvisomant contains eight amino acid substitutions that improve binding of this molecule to the receptor. Binding site 2 contains a single amino-acid substitution (Gly120Arg) that blocks the conformational change of the growth-hormone receptor and thereby inhibits downstream signal transduction.

PEG-V is a recombinant growth-hormone analog with a Gly120Arg substitution in growth-hormone binding site 2 and eight amino acid substitutions in growth-hormone binding site 1, which result in the lack of functional growth hormone receptor signaling and an enhanced affinity for the growth hormone receptor (Figure 1) [11]. Owing to its pegylation (that is, covalent attachment of polyethylene glycol polymer chains), pegvisomant is a stable, 42–46 kD molecule with decreased immunogenicity and prolonged half-life [18,32].

AIMS OF TREATMENT

The aims of long-term treatment should be morbidity and especially mortality rates are the most important parameters. Mortality rates have been assessed for RT and pituitary surgery. However, no data is available on (primary) medical treatment, yet. However reduction in mortality can be achieved by any effective therapy that normalizes GH and IGF1. Medical treatment modalities are effective therapies therefore it might be expected that in the future data will be available on possible reduction of mortality and morbidity. The most important biochemical predictors of mortality in acromegaly are GH [33-35] and IGF1 [19,36-37]. There is some evidence that the latest GH could be a more important predictor of increased mortality risk than the latest IGF1 [38].

There is an ongoing discussion in literature on what normal GH and IGF1 levels are. Some studies use a single GH of less than 2.5 µg/liter [19]. The consensus guideline states that mean of a 24-hr GH profile should be to less than 2.5 µg/liter or a GH nadir of less than 1.0 µg/liter and an IGF1 level into the age adjusted normal limits [39]. However, using these criteria, Dekkers and co-workers demonstrated that in that case, the standardized mortality rate (SMR) is still increased to 1.3 [40]. The SMR from this meta-analysis is mainly based upon studies conducted in acromegalic patients treated with transsphenoidal surgery. The current effective medical treatment strategies, as PEG-V and/or SRIF analog were not incorporated in this meta-analysis. With these effective medical therapies, it might be expected that mortality rate will indeed completely normalize. Furthermore, most reports on mortality rates in acromegaly used GH values that were assessed by a competitive, less sensitive, RIA. Nowadays, GH is assessed by sensitive sandwich immunoassays. Therefore, it is hard to predict how 'old' GH levels must be translated into 'modern' GH concentrations, assessed by modern assays. The evolution in GH assays does influence the discussion about what level GH should be regarded as safe. The optimal GH nadir is probably below 1.0 µg/l but the optimal threshold in not defined yet. Some authors suggest 0.5 µg/l [41] while others mention 0.4 µg/l [42]. The use of a too high cut-off level for GH as the target level probably explains why the SMR was elevated in the meta-analysis of Dekkers et al. In the recent New Zealand meta-analysis, mortality appeared not to be increased when random GH were <1.0 µg/l, assessed by a sensitive essay [43]. One potential draw-back of using lower target levels for GH together with the availability of effective medical treatment is that it can lead to overtreatment in acromegaly patients.

Quality of life in acromegalic patients

Since it has become possible to normalize biochemical markers in virtually all acromegalic patients, one should endeavor for a new objective. From a patients perspective quality of life (QoL) may be the most important parameter of her or his disease. As previously mentioned, by lowering GH and IGF1 morbidity decreases. Morbidity is part of the QoL, but does not cover the whole concept.

RT decreases the QoL especially joint complaints [37]. Over time this decreased QoL seems even to deteriorate after RT [44]. Improvement in QoL of acromegalic patients will most likely be a topic of increased interest within the next decade.

(PATHO)PHYSIOLOGY

Hypothalamic hormones GH-releasing hormone (GHRH), SRIF analog through the pituitary portal system regulate the pulsatile GH secretion of the anterior pituitary somatotroph cells [45]. Another GH releasing hormone is Ghrelin a gut-derived hormone [46]. Ghrelin acts mainly at hypothalamic level as a GH secretagogue in synergy with GHRH. It acts through the ghrelin secretagogue receptor type Ia (GHS-RIa). These three are the main regulator of GH. GHRH and ghrelin stimulated whereas SRIF analog inhibits GH secretion [46-48]. GH stimulates the IGF1 production in the liver and peripheral tissues [49]. To stabilize IGF1 serum levels, it binds to IGF1 binding protein 3 (IGF1BP3) and acid-labile-subunit (ALS). GH is the main regulator of both IGF1BP3 and ALS. Vice versa IGF1 regulates GH by a negative feedback (Figure 2) [11].

GH is not just a releasing factor for IGF1. In contrast to IGF1, GH has lipolytic and hyperglycemic, as IGF1 has glucose lowering effect. So GH and IGF1 have partly synergistic and antagonistic actions.

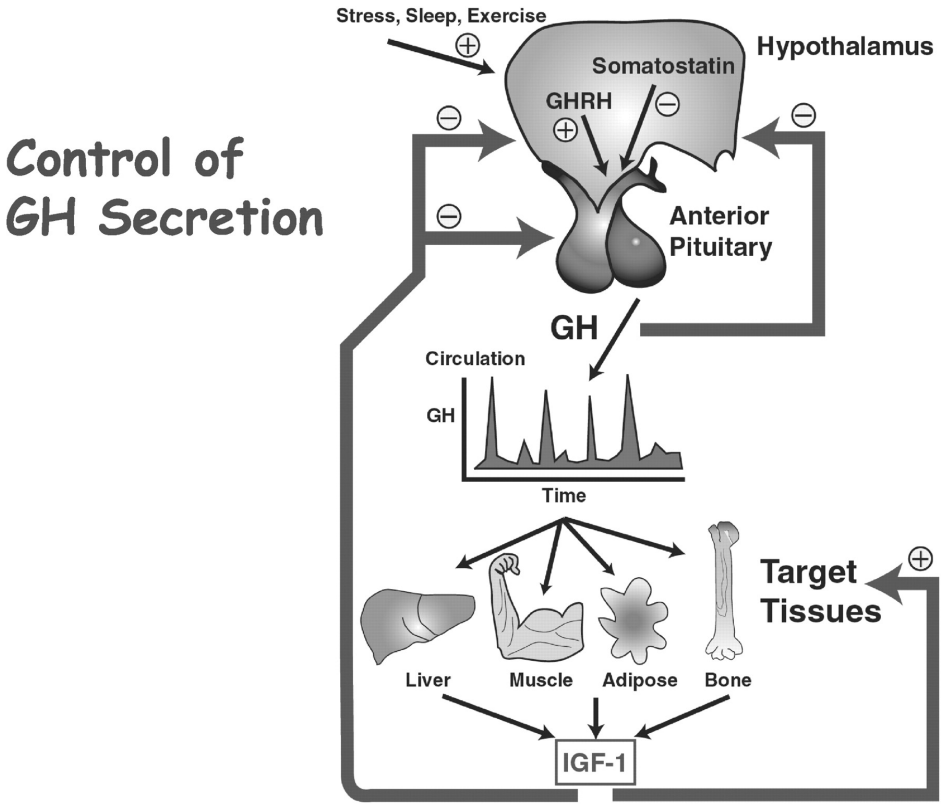

Figure 2: Physiology of the GH secretion by the pituitary.

AIMS AND OUTLINE OF THIS THESIS

The general aim of the research presented in this thesis is to assess the (long-term) efficacy, safety, and cost reduction of combined treatment with somatostatin analogs and a GH receptor antagonist and the tissue specific physiology of the GH/IGF1.

The first part, chapter 2, 3 and 4, of this thesis focuses on the efficacy, safety and costs of combination treatment in previously uncontrolled acromegaly patients. The second part, chapter 5 and 7, will emphasize on QoL in long-term controlled acromegaly patients during long-acting SRIF analog, with or without PEG-V and the tissue specific physiology of the GH/IGF1.

In chapter 6 more physiology of GH is study but at a pituitary level. We studied if an ultrashort feedbackloop for GH on the pituitary is the explanation for the raise in GH serum levels during PEG-V treatment.

Chapter 8 discusses the benefits and side effects of combination treatment and looks to the future of acromegaly treatment and possible changes in the way we treat acromegaly to date.

Finally, chapter 8 covers the general discussion and conclusion.

REFERENCES

1. de Herder WW (2009) Acromegaly and gigantism in the medical literature. Case descriptions in the era before and the early years after the initial publication of Pierre Marie (1886). Pituitary 12 (3):236-244
2. Verga A (1864) [Caso singolare di prosopectasia]. Rendiconti - Reale Instituto 1st Lombardo di Scienze e Letter Classe di Sieze Matermatiche e Naturali 1:111-117
3. Marie P (1886) [Sur deux cas d'acromegalie: hypertrophie singuliere, non congenitale, des extremites superieures, inferieures et cephalique]. Rev Med Liege 6:297-333
4. Minkowski O (1887) Uber einem Fall von Akromegalie. Berliner klinische Wochenschrift 21:371-374
5. Massalongo R (1892) [Sull'acromegalia]. Riforma Med 8:74-77
6. Cushing H (1909) [Partial hypophysectomy for acromegaly: with remarks on the function of the hypophysis]. Ann Surg (50):1002-1017
7. Caton R, Paul FT (1893) Notes on a case of acromegaly treated by operation. Br Med J ii:1421-1423
8. Hochenegg J (1908) Operat geheilte Akromegalie bei hypophysentumour. Langenbecks Archiv für Chirurgie 37:80-85
9. Gramegna A (1909) Un cas d'acromegalie traite par la radiothérapie. Rev Neurol (paris) 17:15-17
10. Wass JA, Thorner MO, Morris DV, Rees LH, Mason AS, Jones AE, Besser GM (1977) Long-term treatment of acromegaly with bromocriptine. Br Med J 1 (6065):875-878
11. Kopchick JJ, Parkinson C, Stevens EC, Trainer PJ (2002) Growth hormone receptor antagonists: discovery, development, and use in patients with acromegaly. Endocrine reviews 23 (5):623-646
12. Abs R, Verhelst J, Maiter D, Van Acker K, Nobels F, Coolens JL, Mahler C, Beckers A (1998) Cabergoline in the treatment of acromegaly: a study in 64 patients. The Journal of clinical endocrinology and metabolism 83 (2):374-378
13. Brazeau P, Vale W, Burgus R, Ling N, Butcher M, Rivier J, Guillemin R (1973) Hypothalamic polypeptide that inhibits the secretion of immunoreactive pituitary growth hormone. Science (New York, NY 179 (68):77-79
14. Bauer W, Briner U, Doepfner W, Haller R, Huguenin R, Marbach P, Petcher TJ, Pless (1982) SMS 201-995: a very potent and selective octapeptide analogue of somatostatin with prolonged action. Life sciences 31 (11):1133-1140
15. Murray RD, Melmed S (2008) A critical analysis of clinically available somatostatin analog formulations for therapy of acromegaly. The Journal of clinical endocrinology and metabolism 93 (8): 2957-2968
16. Freda PU (2002) Somatostatin analogs in acromegaly. The Journal of clinical endocrinology and metabolism 87 (7):3013-3018
17. Holdaway IM (2004) Treatment of acromegaly. Hormone research 62 Suppl 3:79-92
18. Clark R, Olson K, Fuh G, Marian M, Mortensen D, Teshima G, Chang S, Chu H, Mukku V, Canova-Davis E, Somers T, Cronin M, Winkler M, Wells JA (1996) Long-acting growth hormones produced by conjugation with polyethylene glycol. The Journal of biological chemistry 271 (36):21969-21977
19. Swearingen B, Barker FG, 2nd, Katznelson L, Biller BM, Grinspoon S, Klibanski A, Moayeri N, Black PM, Zervas NT (1998) Long-term mortality after transsphenoidal surgery and adjunctive therapy for acromegaly. The Journal of clinical endocrinology and metabolism 83 (10):3419-3426

20. Kreutzer J, Vance ML, Lopes MB, Laws ER, Jr. (2001) Surgical management of GH-secreting pituitary adenomas: an outcome study using modern remission criteria. The Journal of clinical endocrinology and metabolism 86 (9):4072-4077
21. Barker FG, 2nd, Klibanski A, Swearingen B (2003) Transsphenoidal surgery for pituitary tumors in the United States, 1996-2000: mortality, morbidity, and the effects of hospital and surgeon volume. The Journal of clinical endocrinology and metabolism 88 (10):4709-4719
22. Abosch A, Tyrrell JB, Lamborn KR, Hannegan LT, Applebury CB, Wilson CB (1998) Transsphenoidal microsurgery for growth hormone-secreting pituitary adenomas: initial outcome and long-term results. The Journal of clinical endocrinology and metabolism 83 (10):3411-3418
23. Bates PR, Carson MN, Trainer PJ, Wass JA, Group UKNARS (2008) Wide variation in surgical outcomes for acromegaly in the UK. Clinical endocrinology 68 (1):136-142
24. Melmed S (2006) Medical progress: Acromegaly. The New England journal of medicine 355 (24): 2558-2573
25. Minniti G, Jaffrain-Rea ML, Osti M, Esposito V, Santoro A, Solda F, Gargiulo P, Tamburrano G, Enrici RM (2005) The long-term efficacy of conventional radiotherapy in patients with GH-secreting pituitary adenomas. Clinical endocrinology 62 (2):210-216
26. Jenkins PJ, Bates P, Carson MN, Stewart PM, Wass JA (2006) Conventional pituitary irradiation is effective in lowering serum growth hormone and insulin-like growth factor-I in patients with acromegaly. The Journal of clinical endocrinology and metabolism 91 (4):1239-1245
27. Biermasz NR, van Dulken H, Roelfsema F (2000) Long-term follow-up results of postoperative radiotherapy in 36 patients with acromegaly. The Journal of clinical endocrinology and metabolism 85 (7):2476-2482
28. Petrossians P, Borges-Martins L, Espinoza C, Daly A, Betea D, Valdes-Socin H, Stevenaert A, Chanson P, Beckers A (2005) Gross total resection or debulking of pituitary adenomas improves hormonal control of acromegaly by somatostatin analogs. European journal of endocrinology / European Federation of Endocrine Societies 152 (1):61-66
29. Bevan JS (2005) Clinical review: The antitumoral effects of somatostatin analog therapy in acromegaly. The Journal of clinical endocrinology and metabolism 90 (3):1856-1863
30. Carlsen SM, Lund-Johansen M, Schreiner T, Aanderud S, Johannesen O, Svartberg J, Cooper JG, Hald JK, Fougner SL, Bollerslev J (2008) Preoperative octreotide treatment in newly diagnosed acromegalic patients with macroadenomas increases cure short-term postoperative rates: a prospective, randomized trial. The Journal of clinical endocrinology and metabolism 93 (8):2984-2990
31. van der Lely AJ, Hutson RK, Trainer PJ, Besser GM, Barkan AL, Katznelson L, Klibanski A, Herman-Bonert V, Melmed S, Vance ML, Freda PU, Stewart PM, Friend KE, Clemmons DR, Johannsson G, Stavrou S, Cook DM, Phillips LS, Strasburger CJ, Hackett S, Zib KA, Davis RJ, Scarlett JA, Thorner MO (2001) Long-term treatment of acromegaly with pegvisomant, a growth hormone receptor antagonist. Lancet 358 (9295):1754-1759
32. Drake WM, Parkinson C, Besser GM, Trainer PJ (2001) Clinical use of a growth hormone receptor antagonist in the treatment of acromegaly. Trends in endocrinology and metabolism: TEM 12 (9): 408-413
33. Orme SM, McNally RJ, Cartwright RA, Belchetz PE (1998) Mortality and cancer incidence in acromegaly: a retrospective cohort study. United Kingdom Acromegaly Study Group. The Journal of clinical endocrinology and metabolism 83 (8):2730-2734
34. Bates AS, Van't Hoff W, Jones JM, Clayton RN (1993) An audit of outcome of treatment in acromegaly. The Quarterly journal of medicine 86 (5):293-299

35. Ayuk J, Clayton RN, Holder G, Sheppard MC, Stewart PM, Bates AS (2004) Growth hormone and pituitary radiotherapy, but not serum insulin-like growth factor-I concentrations, predict excess mortality in patients with acromegaly. The Journal of clinical endocrinology and metabolism 89 (4):1613-1617
36. Holdaway IM, Rajasoorya RC, Gamble GD (2004) Factors influencing mortality in acromegaly. The Journal of clinical endocrinology and metabolism 89 (2):667-674
37. Biermasz NR, van Thiel SW, Pereira AM, Hoftijzer HC, van Hemert AM, Smit JW, Romijn JA, Roelfsema F (2004) Decreased quality of life in patients with acromegaly despite long-term cure of growth hormone excess. The Journal of clinical endocrinology and metabolism 89 (11):5369-5376
38. Ayuk J, Sheppard MC (2008) Does acromegaly enhance mortality? Reviews in endocrine & metabolic disorders 9 (1):33-39
39. Giustina A, Barkan A, Casanueva FF, Cavagnini F, Frohman L, Ho K, Veldhuis J, Wass J, Von Werder K, Melmed S (2000) Criteria for cure of acromegaly: a consensus statement. The Journal of clinical endocrinology and metabolism 85 (2):526-529
40. Dekkers OM, Biermasz NR, Pereira AM, Romijn JA, Vandenbroucke JP (2008) Mortality in acromegaly: a metaanalysis. The Journal of clinical endocrinology and metabolism 93 (1):61-67
41. Feelders RA, Bidlingmaier M, Strasburger CJ, Janssen JA, Uitterlinden P, Hofland LJ, Lamberts SW, van der Lely AJ, de Herder WW (2005) Postoperative evaluation of patients with acromegaly: clinical significance and timing of oral glucose tolerance testing and measurement of (free) insulin-like growth factor I, acid-labile subunit, and growth hormone-binding protein levels. The Journal of clinical endocrinology and metabolism 90 (12):6480-6489
42. Melmed S, Casanueva F, Cavagnini F, Chanson P, Frohman LA, Gaillard R, Ghigo E, Ho K, Jaquet P, Kleinberg D, Lamberts S, Laws E, Lombardi G, Sheppard MC, Thorner M, Vance ML, Wass JA, Giustina A (2005) Consensus statement: medical management of acromegaly. European journal of endocrinology / European Federation of Endocrine Societies 153 (6):737-740
43. Holdaway IM, Bolland MJ, Gamble GD (2008) A meta-analysis of the effect of lowering serum levels of GH and IGF-I on mortality in acromegaly. European journal of endocrinology / European Federation of Endocrine Societies 159 (2):89-95
44. van der Klaauw AA, Biermasz NR, Hoftijzer HC, Pereira AM, Romijn JA (2008) Previous radiotherapy negatively influences quality of life during 4 years of follow-up in patients cured from acromegaly. Clinical endocrinology 69 (1):123-128
45. Tannenbaum GS, Epelbaum J, Bowers CY (2003) Interrelationship between the novel peptide ghrelin and somatostatin/growth hormone-releasing hormone in regulation of pulsatile growth hormone secretion. Endocrinology 144 (3):967-974
46. Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K (1999) Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature 402 (6762):656-660
47. Hartman ML, Veldhuis JD, Thorner MO (1993) Normal control of growth hormone secretion. Hormone research 40 (1-3):37-47
48. Bertherat J, Bluet-Pajot MT, Epelbaum J (1995) Neuroendocrine regulation of growth hormone. European journal of endocrinology / European Federation of Endocrine Societies 132 (1):12-24
49. Tannenbaum GS, Guyda HJ, Posner BI (1983) Insulin-like growth factors: a role in growth hormone negative feedback and body weight regulation via brain. Science (New York, NY 220 (4592):77-79

Chapter 2

Long-Term Efficacy and Safety of Combined Treatment of Somatostatin Analogs and Pegvisomant in Acromegaly

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ABSTRACT

Background: We previously reported the efficacy of a combined treatment of active acromegaly with both long-acting somatostatin analogs (SRIF) and pegvisomant (PEG-V).
Objective: Our objective was to assess long-term efficacy and safety in a larger group of acromegalic patients after a period of 138 (35–149) wks [median (range)].
Design: PEG-V was added to high-dose SRIF analogs treatment in 32 subjects (13 females) who had not shown a normalization in serum IGF1 concentrations during SRIF analog monotherapy. PEG-V dosage was increased until IGF1 concentration normalized. The maximal dose was 80 mg twice weekly.
Results: After dose finding, IGF1 remained within the normal range in all subjects with PEG-V administered once (n = 24) or twice (n = 8) weekly, on a total weekly dose of 60 (40–160) mg. Baseline IGF1 levels were positively correlated with the required dosage of PEG-V (r = 0.48; $P = 0.006$). PEG-V-dependent liver enzyme disturbances were observed in 11 (6 diabetic) subjects, of which symptomatic gallstones explained two cases. These liver enzyme disturbances were transient in all subjects without discontinuation or dose adaptation of PEG-V. In our series, diabetic patients had a 5.1 times (odds ratio) (confidence interval, 1.02–25.54; $P < 0.05$) higher risk for developing liver enzyme disturbances. These liver enzyme disturbances seemed to occur earlier. Pituitary adenoma size decreased in four patients. No increase in tumor size was observed in any of the patients.
Conclusion: Long-term combined treatment with long-acting SRIF analogs and (twice) weekly PEG-V for active acromegaly seems to be effective and safe. Patients with acromegaly and diabetes seem to have a higher risk of developing transient liver enzyme disturbances.

INTRODUCTION

The clinical management of acromegaly has changed recently because optimal therapy has proven to reverse the increased mortality associated with acromegaly. Currently, transsphenoidal surgery (TSS) is still the treatment of choice for acromegaly, although in only 60% is an adequate biochemical remission or cure achieved. A chance on remission declines to less than 50% if a macroadenoma is present [1]. Radiotherapy (RT), primary or postsurgically, is limited due to slow onset of effect and has a high occurrence of panhypopituitarism [2-4] and decreased quality of life [5]. Nowadays, medical therapy plays an important role in controlling signs and symptoms of acromegaly, both as primary and secondary medical therapy. Long-acting somatostatin analogs (SRIF) normalize IGF1 levels in two thirds of patients with acromegaly [6-11]. One of its side effects is the suppression of insulin secretion, which could lead to glucose intolerance [12-13].

A more recent medical treatment modality is the GH receptor antagonist pegvisomant (PEG-V). With daily injections of PEG-V, in more than 90% of patients a normalization of IGF1 can be achieved [14-15]. In addition, glucose metabolism is improved [16-17,13,18,15]. On the other hand, PEG-V has to be administered daily, and in some patients it was at least unable to prevent tumor growth [19]. Combination of SRIF analog and PEG-V treatment seems to be an attractive option because tumor suppression is combined with GH receptor blockade. We previously reported that PEG-V, administered weekly in addition to long-acting SRIF analog therapy was able to normalize serum IGF1 levels in 95% of subjects [20]. Here we present the long-term follow-up efficacy and safety data in the same patients plus newly included acromegalic subjects up to 138 (35–149) wks [median (range)].

PATIENTS AND METHODS

Patients

Patients were enrolled from a single center. All subjects [n = 32, 13 females; median age 52 (range 30–81) yrs] had elevated IGF1 levels [56 (32–122) nmol/liter] despite 120 mg lanreotide Autogel (n = 22) or 30 mg octreotide LAR (10) monthly for at least 6 months before the start of this series. Baseline characteristics are presented in Table 1. In our series, patients with a pituitary macroadenoma and compression of the chiasm were not treated with a combination of SRIF analog and PEG-V.

Methods

All patients continued long-acting SRIF analog therapy at monthly intervals and, on top of that, PEG-V was added weekly. Starting dose of PEG-V was 40 mg weekly. PEG-V

Table 1. Baseline characteristics (n=32)

	n (%)[1]
Patients	32 (100)
Sex (female)	13 (41)
Age (yrs), mean (SD,range)	53 (12.8,30-81)
DM	11 (34)
IGF1 at nmol/liter baseline	
Mean (SD)	65.0 (28.9)
Median (range)	60.0 (32.0–122.0)
1–2 x ULN	18 (56)
2–3 x ULN	9 (19)
>3 x ULN	5 (16)
GH (µg/liter) at baseline	
Mean (SD)	10 (14.2)
Median (range)	5.2 (0.4-69.8)
Previous treatment	
Both TSS and RT	8 (25)
TSS	6 (19)
Primary medical therapy	18 (56)
Duration of long-acting SRIF before combination (wks)	
Mean (SD)	114 (82.7)
Median (range)	79.3 (33.1-320.0)
Pituitary insufficiency	
Pan-hypopituitarism	9 (28)
1–2 axis	13 (41)
No hypopituitarism	10 (31)
Long-acting SRIF analogs	
Lanreotide	22 (69)
Octreotide	10 (31)

DM, diabetes mellitus; RT, radiotherapy; SRIF, somatostatin; TSS, transsphenoidal surgery; wks, weeks; x ULN, Times upper limit of normal.

[1] Unless otherwise specified.

dosage was adjusted until IGF1 was within the age-adjusted normal range. If IGF1 fell in the lowest quartile of normal, the PEG-V dosage was reduced. The intervals of dose adjustments were 6 wks until a controlled IGF1 was achieved twice. The subjects then visited our outpatient clinic every 12–16 wks. If a dose of at least 100 mg was required, patients divided the dosage in two (equal) parts and injected twice weekly.

IGF1 and other efficacy and safety data were assessed at these regular visits. Efficacy was assessed at the longest follow-up visit [138 (35–149) wks] for IGF1, GH, glycosylated

hemoglobin (HbA1c), fasting glucose, lipids, and a quality of life questionnaire, Patient-Assessed Acromegaly Symptom Questionnaire [14]. IGF1 and GH concentrations were measured by immunometric assays (Diagnostic Products Corp., Los Angeles, CA). The IGF1 age-adjusted reference ranges were used [21].

Safety assessment included electrocardiogram, serum concentrations of alkaline phosphatase, γ-glutamyltranspeptidase (γ-GT), alanine aminotransferase (ALT), aspartate aminotransaminase (AST), lactate dehydrogenase, total bilirubin, and change in pituitary tumor volume. Tumor volume was assessed at baseline by magnetic resonance imaging, which was repeated every 6 months. Changes in pituitary tumor size were assessed by the same neuroradiologist.

Statistical analysis

Paired nonparametric data were analyzed with the Wilcoxon's signed-rank test. The non-paired data were assessed with the Mann- Whitney U test and cross-tables with the Fisher's exact test. Statistical analyses of the data were performed by Prism version 5.00 for Windows (GraphPad Software, San Diego CA). Statistical significance was accepted at $P < 0.05$ (two-tailed). Data are expressed as median ± SD unless otherwise specified.

RESULTS

Efficacy

Thirty-two patients with acromegaly were enrolled in this series. Normalization of serum IGF1 levels was achieved in all patients (Figure 1). The median IGF1 decreased from 56.00 ± 28.7 nmol/liter at baseline, on SRIF analogs monotherapy, to a lowest level of 17.85 ± 6.16 nmol/liter ($P < 0.0001$) during combined treatment. The PEG-V dose needed for normalization of IGF1 was 60 (40–160) mg weekly [median (range)]. This median dose is identical to the one we reported previously [20]. Eight patients needed PEG-V twice weekly.

The necessary dose of PEG-V for normalization of IGF1 was not significantly different in the primary medical therapy group (60.00 ± 43.27 mg) *vs.* the TSS group (55.00 ± 40.08 mg). A similar observation was made with regard to the diabetes mellitus (DM) group (55.00 ± 58.27 mg) *vs.* non-DM group (60.00 ± 32.70 mg). The necessary dose of PEG-V for normalization of IGF1 was positively correlated with baseline IGF1 levels, corrected for age ($r = 0.48$; $P = 0.006$) (Fig. 2).

Metabolic control in patients with DM improved significantly after normalization of IGF1 during combined treatment. Nine of 10 DM patients had a significant decrease in HbA1c ($P = 0.0156$). Even after normalization of IGF1, and with the same PEG-V dose, a further significant decrease in HbA1c could be observed ($P = 0.0313$) over a period of

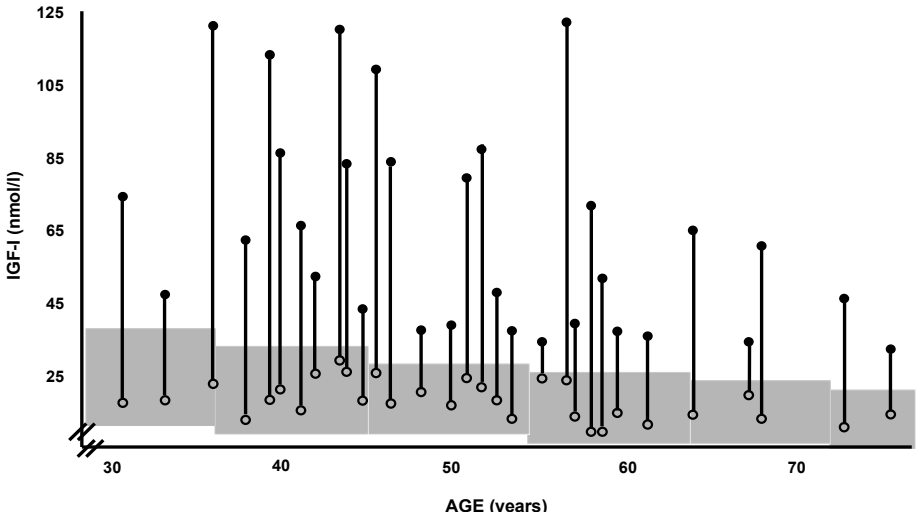

Figure 1: IGF1 concentration in serum of 31 patients with acromegaly , before (●) and after (○) 138 (35-149) weeks of combined therapy.
Shaded area indicates age-dependent normal range for IGF1.

6–18 months. In one patient, HbA1c could not be assessed because of hemoglobinopathy. Patients on insulin therapy (n = 7) had to decrease their insulin dosage, and one patient could stop insulin therapy and continued oral medication for DM. The remaining three patients did not change their insulin dosage, but still their HbA1c levels decreased.

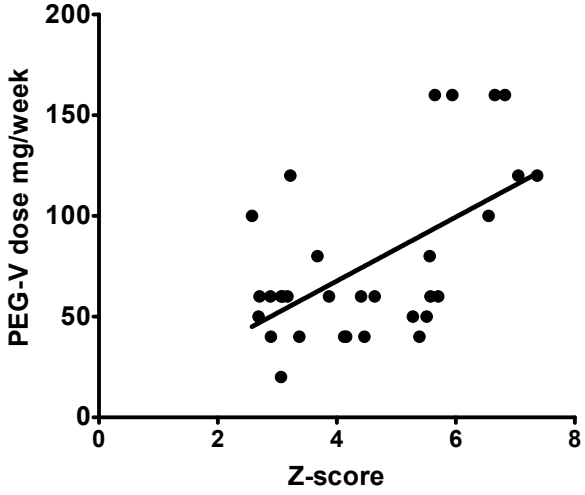

Figure 2: The relation between baseline IGF1 and the necessary dose of pegvisomant (PEG-V) in a linear regression model. IGF1 is corrected for age by Z-score. r =0.48; p=0.006.

Quality of life, compared with baseline, was improved in any assessment during follow-up ($P < 0.05$). IGF1 correlated well with quality of life.

Safety

Transient elevated liver enzyme tests (TLET) were observed in 11 patients (34%) (Table 2). Of these 11 subjects with TLET, six patients (55%) also suffered from DM. In our study, acromegaly patients with DM had a 5.1 times (odds ratio) (confidence interval, 1.02–25.54; $P < 0.05$) higher risk for developing TLET than nondiabetic subjects. We already reported on one diabetic patient who developed a transient drug-induced hepatitis due to which pegvisomant medication was stopped after 25 wks [22].

Table 2. Clinical and biochemical characteristics of 11 patients with liver enzyme disturbances during combined therapy of SRIF analogs and PEG-V (twice) weekly

Sex	Age (yrs)	Time between start of PEG-V and LFT (weeks)	Weekly PEG-V dose at onset of LFT elevations	Peak LFT ALT AST γGT ----(xULN)----			DM	Follow up In all patients PEG-V was continued
M	61	24	60	3.0	1.8	1.5	+	LFT normalized < 12 weeks
M	38	20	60	1.8	1.3	1.1	-	LFT normalized < 18 weeks
F	66	12	40	2.0	1.2	2.0	+	LFT normalized < 18 weeks
M	48	46	160	2.4	1.1	1.2	+	LFT normalized < 18 weeks
M	61	24	60	3.0	1.8	1.5	+	LFT normalized < 12 weeks
F	57	24	60	5.1	2.6	3.0	-	LFT normalized < 12 weeks
M	53	17	60	31.6	14.2	18.4	+	MRCP showed gallstones, LFT declined rapidly and normalized < 8 weeks
M	30	86	60	8.8	4.7	5.7	-	Bile duct obstruction. LFT normalized after cholecystectomy < 1 week
M	44	48	60	4.8	2.9	5.1	-	LFT normalized < 18 weeks
F	45	92	40	2.3	1.2	1.0	-	LFT normalized < 3 weeks
M	47	25	60	31.9	7.1	7.6	+	PEG-V withdrawn, LFT normalized
M	62	18	40	2.8	2.0	10.4	+	LFT normalized < 12 weeks
M	38	20	60	1.8	1.3	1.1	-	LFT normalized < 18 weeks
M	48	46	160	2.4	1.1	1.2	+	LFT normalized < 18 weeks
M	61	24	60	3.0	1.8	1.5	+	LFT normalized < 12 weeks
M	44	48	60	4.8	2.9	5.1	-	LFT normalized < 18 weeks
M	30	50	200	1.5	0.4	0.8	+	LFT normalized < 6 weeks
M	46	8	80	4.3	3.3	2.2	-	LFT normalized < 12 weeks
M	47	25	60	31.9	7.1	7.6	+	PEG-V withdrawn, LFT normalized

F, Female; M, male; LFT, liver function tests; xULN, times upper limit of normal; DM, diabetes mellitus; MRCP=Magnetic resonance cholangiopancreatography.

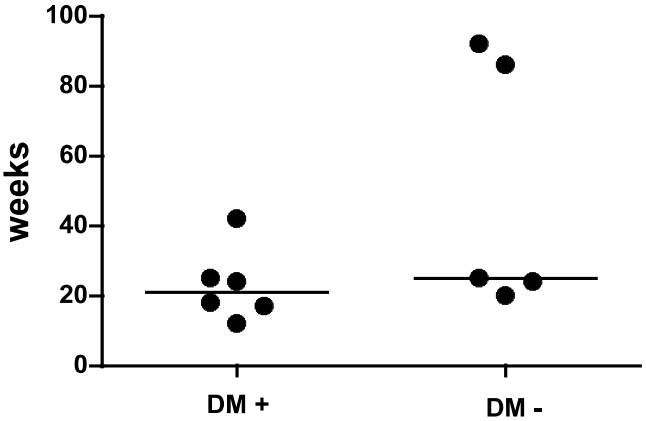

Figure 3: The interval between start of therapy and onset of transaminases abnormalities divided in acromegaly patients with (+) and without (-) DM. The individual subjects are shown as closed circles, and the line represents the median.

Data of this patient are included in the safety data and baseline analyses. Subjects with DM also tended to have an earlier onset of TLET (21.0 ± 10.5 wks) than subjects without (25.0 ± 36.3 wks), although this was not significant (P = 0.178) (Fig. 3). The duration of the TLET was the same in both DM (12.0 ± 6.1 wks) and non-DM (18.0 ± 11.5 wks). In two patients, gallstones, possibly related to long-term use of SRIF analogs, could explain the TLET. Patient 8 (Table 2) was diagnosed as having cholecystitis and underwent cholecystectomy, after which TLET normalized rapidly. In patient 7, a magnetic resonance imaging scan of the liver revealed gallstones, and the TLET spontaneously disappeared without intervention. We concluded that in this patient, passage of a gallstone caused the TLET.

In 28 subjects, no increase in size of the pituitary tumor was observed. In four subjects (13%), a regression in tumor size by more than 25% occurred. None of these four patients had received RT before enrollment in this series, whereas three subjects (9%) were on primary medical therapy and had been treated for 43, 45, and 73 wks with monotherapy SRIF analogs and for 74, 148, and 143 wks with combination therapy. The other subject had TSS 2 yr before enrollment and had been treated for 71 wks with monotherapy SRIF analog and 143 wks with combination therapy.

DISCUSSION

We report here that long-term combined therapy of acromegalic patients with long-acting SRIF analogs and PEG-V (twice) weekly is highly effective and safe. In all patients, IGF1 could be brought back within the age-adjusted reference range. Compared with the reported remission rates of long-acting SRIF analogs of about two thirds of patients,

the combination therapy seems superior. It is comparable with the efficacy of PEG-V daily administrations as monotherapy for acromegaly, which is considered to be more than 90% [14-15]. We observed that the (twice) weekly dose of PEG-V that is necessary for IGF1 normalization is equal to the one, we reported earlier (median, 60 mg; range, 40–160 mg) [20]. Probably mainly because of the reduced levels of endogenous GH due to the SRIF analog therapy, less PEG-V is needed to normalize serum IGF1 levels, because it meets less GH to compete for GH receptor binding. The necessary dose of PEG-V for normalization of IGF1 was associated with baseline IGF1 levels on monotherapy SRIF analogs. In previous studies with PEG-V monotherapy, such a relationship was not observed. The dose of PEG-V necessary for normalization of IGF1 did not differ between patients with primary medically treated patients and patients who previously underwent TSS (with or without RT). This is in contrast to studies on SRIF analogs monotherapy, showing that previous tumor debulking leads to a higher percentage of patients achieving biochemical remission with SRIF analogs [23].

In 11 patients (34%), we observed TLET. Two patients had TLET due to gallstones probably related to the use of long-acting SRIF analogs [20]. Liver enzyme disturbances during daily PEG-V treatment, AST, and ALT are usually more elevated than γ-GT and bilirubin [24]. The more prominent elevation in γ-GT and bilirubin led to the conclusion that in two patients, the TLET was caused by cholelithiasis. The majority of patients who developed TLET had DM. These patients have an odds ratio of 5.1 to develop TLET during combination treatment and also tended to develop TLET earlier compared with patients without DM, although this was not significant. Another study assessed TLET in 12 patients with daily PEG-V of whom two subjects were cotreated with monthly long-acting octreotide (30 mg) [24]. The liver enzyme disturbances were not dose related and occurred after 15.7 ± 10.7 wks (mean ± SD). However, no association of a phenotypic predictor was found. The mechanism of PEG-V-induced TLET remains unclear. The reason why the DM acromegalic patients in our series develop TLET earlier and more frequently also remains the subject of conjecture. We could not find another predictor that could explain the TLET. Although in our patients we did observe elevated liver enzyme tests, we consider the combined treatment as a safe treatment option of acromegalic patients that do not respond enough to monotherapy with SRIF analogs, because these mild disturbances of liver enzymes are transient without discontinuation or dose adaptation of PEG-V. This is in contrast to some other studies in which PEG-V is withdrawn. A frequent assessment of liver enzymes, as already indicated by the package insert of PEG-V, seems mandatory, however, especially in patients with DM.

In our series, we did not observe tumor growth in any of the patients. Four subjects even showed a regression in tumor size by more than 25%. It is noteworthy that none of these subjects were previously treated with RT.

From our clinical experience, one starts with a weekly dose of 40 mg PEG-V, which can be increased by 20 mg every 6 wks until normalization of IGF1 has been achieved. In our hands, we need two to three steps of dose titration to normalize IGF1 in the majority of patients, achieving disease control within 3 months after starting combination therapy.

In conclusion, although more research is necessary in larger cohorts of patients, our series indicates that long-term combined treatment of active acromegaly with both long-acting SRIF analogs and (twice) weekly injections of PEG-V seems to be effective and safe, at least for those subjects in whom serum IGF1 remains elevated during SRIF analogs monotherapy. Transient and mild elevations in liver functions were observed in one third of patients, and especially diabetic patients seemed to be more prone to this side effect.

REFERENCES

1. Swearingen B, Barker FG, 2nd, Katznelson L, Biller BM, Grinspoon S, Klibanski A, Moayeri N, Black PM, Zervas NT (1998) Long-term mortality after transsphenoidal surgery and adjunctive therapy for acromegaly. The Journal of clinical endocrinology and metabolism 83 (10):3419-3426
2. Barkan AL (2003) Radiotherapy in acromegaly: the argument against. Clinical endocrinology 58 (2):132-135
3. Minniti G, Jaffrain-Rea ML, Osti M, Esposito V, Santoro A, Solda F, Gargiulo P, Tamburrano G, Enrici RM (2005) The long-term efficacy of conventional radiotherapy in patients with GH-secreting pituitary adenomas. Clinical endocrinology 62 (2):210-216
4. Roberts BK, Ouyang DL, Lad SP, Chang SD, Harsh GRt, Adler JR, Jr., Soltys SG, Gibbs IC, Remedios L, Katznelson L (2007) Efficacy and safety of CyberKnife radiosurgery for acromegaly. Pituitary 10 (1):19-25
5. Biermasz NR, Pereira AM, Smit JW, Romijn JA, Roelfsema F (2005) Morbidity after long-term remission for acromegaly: persisting joint-related complaints cause reduced quality of life. The Journal of clinical endocrinology and metabolism 90 (5):2731-2739
6. Ayuk J, Clayton RN, Holder G, Sheppard MC, Stewart PM, Bates AS (2004) Growth hormone and pituitary radiotherapy, but not serum insulin-like growth factor-I concentrations, predict excess mortality in patients with acromegaly. The Journal of clinical endocrinology and metabolism 89 (4):1613-1617
7. Bronstein MD (2006) Acromegaly: molecular expression of somatostatin receptor subtypes and treatment outcome. Frontiers of hormone research 35:129-134
8. Cozzi R, Attanasio R, Montini M, Pagani G, Lasio G, Lodrini S, Barausse M, Albizzi M, Dallabonzana D, Pedroncelli AM (2003) Four-year treatment with octreotide-long-acting repeatable in 110 acromegalic patients: predictive value of short-term results? The Journal of clinical endocrinology and metabolism 88 (7):3090-3098
9. Jallad RS, Musolino NR, Salgado LR, Bronstein MD (2005) Treatment of acromegaly with octreotide-LAR: extensive experience in a Brazilian institution. Clinical endocrinology 63 (2):168-175
10. Gilbert J, Ketchen M, Kane P, Mason T, Baister E, Monaghan M, Barr S, Harris PE (2003) The treatment of de novo acromegalic patients with octreotide-LAR: efficacy, tolerability and cardiovascular effects. Pituitary 6 (1):11-18
11. Sheppard MC (2003) Primary medical therapy for acromegaly. Clinical endocrinology 58 (4):387-399
12. Koop BL, Harris AG, Ezzat S (1994) Effect of octreotide on glucose tolerance in acromegaly. European journal of endocrinology / European Federation of Endocrine Societies 130 (6):581-586
13. Parkinson C, Drake WM, Roberts ME, Meeran K, Besser GM, Trainer PJ (2002) A comparison of the effects of pegvisomant and octreotide on glucose, insulin, gastrin, cholecystokinin, and pancreatic polypeptide responses to oral glucose and a standard mixed meal. The Journal of clinical endocrinology and metabolism 87 (4):1797-1804
14. Trainer PJ, Drake WM, Katznelson L, Freda PU, Herman-Bonert V, van der Lely AJ, Dimaraki EV, Stewart PM, Friend KE, Vance ML, Besser GM, Scarlett JA, Thorner MO, Parkinson C, Klibanski A, Powell JS, Barkan AL, Sheppard MC, Malsonado M, Rose DR, Clemmons DR, Johannsson G, Bengtsson BA, Stavrou S, Kleinberg DL, Cook DM, Phillips LS, Bidlingmaier M, Strasburger CJ, Hackett S, Zib K, Bennett WF, Davis RJ (2000) Treatment of acromegaly with the growth hormone-receptor antagonist pegvisomant. The New England journal of medicine 342 (16):1171-1177

15. van der Lely AJ, Hutson RK, Trainer PJ, Besser GM, Barkan AL, Katznelson L, Klibanski A, Herman-Bonert V, Melmed S, Vance ML, Freda PU, Stewart PM, Friend KE, Clemmons DR, Johannsson G, Stavrou S, Cook DM, Phillips LS, Strasburger CJ, Hackett S, Zib KA, Davis RJ, Scarlett JA, Thorner MO (2001) Long-term treatment of acromegaly with pegvisomant, a growth hormone receptor antagonist. Lancet 358 (9295):1754-1759
16. Colao A, Attanasio R, Pivonello R, Cappabianca P, Cavallo LM, Lasio G, Lodrini A, Lombardi G, Cozzi R (2006) Partial surgical removal of growth hormone-secreting pituitary tumors enhances the response to somatostatin analogs in acromegaly. The Journal of clinical endocrinology and metabolism 91 (1):85-92
17. Lindberg-Larsen R, Moller N, Schmitz O, Nielsen S, Andersen M, Orskov H, Jorgensen JO (2007) The impact of pegvisomant treatment on substrate metabolism and insulin sensitivity in patients with acromegaly. The Journal of clinical endocrinology and metabolism 92 (5):1724-1728
18. Trainer PJ, Drake WM, Perry LA, Taylor NF, Besser GM, Monson JP (2001) Modulation of cortisol metabolism by the growth hormone receptor antagonist pegvisomant in patients with acromegaly. The Journal of clinical endocrinology and metabolism 86 (7):2989-2992
19. van der Lely AJ, Muller A, Janssen JA, Davis RJ, Zib KA, Scarlett JA, Lamberts SW (2001) Control of tumor size and disease activity during cotreatment with octreotide and the growth hormone receptor antagonist pegvisomant in an acromegalic patient. The Journal of clinical endocrinology and metabolism 86 (2):478-481
20. Feenstra J, de Herder WW, ten Have SM, van den Beld AW, Feelders RA, Janssen JA, van der Lely AJ (2005) Combined therapy with somatostatin analogues and weekly pegvisomant in active acromegaly. Lancet 365 (9471):1644-1646
21. Elmlinger MW, Kuhnel W, Weber MM, Ranke MB (2004) Reference ranges for two automated chemiluminescent assays for serum insulin-like growth factor I (IGF-I) and IGF-binding protein 3 (IGFBP-3). Clin Chem Lab Med 42 (6):654-664
22. Feenstra J, van Aken MO, de Herder WW, Feelders RA, van der Lely AJ (2006) Drug-induced hepatitis in an acromegalic patient during combined treatment with pegvisomant and octreotide long-acting repeatable attributed to the use of pegvisomant. European journal of endocrinology / European Federation of Endocrine Societies 154 (6):805-806
23. Petrossians P, Borges-Martins L, Espinoza C, Daly A, Betea D, Valdes-Socin H, Stevenaert A, Chanson P, Beckers A (2005) Gross total resection or debulking of pituitary adenomas improves hormonal control of acromegaly by somatostatin analogs. European journal of endocrinology / European Federation of Endocrine Societies 152 (1):61-66
24. Biering H, Saller B, Bauditz J, Pirlich M, Rudolph B, Johne A, Buchfelder M, Mann K, Droste M, Schreiber I, Lochs H, Strasburger CJ, German pegvisomant i (2006) Elevated transaminases during medical treatment of acromegaly: a review of the German pegvisomant surveillance experience and a report of a patient with histologically proven chronic mild active hepatitis. European journal of endocrinology / European Federation of Endocrine Societies 154 (2):213-220

Chapter 3

Combined treatment for acromegaly with long-acting somatostatin analogs and pegvisomant: long-term safety for up to 4.5 years (median 2.2 years) of follow-up in 86 patients

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ABSTRACT

Background: We previously reported on the efficacy, safety, and quality of life (QoL) of long-acting somatostatin analogs (SRIF) and (twice) weekly pegvisomant (PEG-V) in acromegaly and improvement after the addition of PEG-V to long-acting SRIF analogs.
Objective: To assess the long-term safety in a larger group of acromegalic patients over a larger period of time: 29.2 (1.2–57.4) months (mean (range)).
Design: Pegvisomant was added to SRIF analog monotherapy in 86 subjects (37 females), to normalize serum IGF1 concentrations (*n*=63) or to increase the QoL. The median dosage was 60.0 (20–200) mg weekly.
Results: After a mean treatment period of 29.2 months, 23 patients showed dose-independent PEG-V related transient liver enzyme elevations (TLET). TLET occurred only once during the continuation of combination therapy, but discontinuation and re-challenge induced a second episode of TLET. Ten of these patients with TLEE also suffered from diabetes mellitus (DM). In our present series, DM had a 2.28 odds ratio (CI 1.16–9.22; *p*=0.03) higher risk for developing TLET. During the combined therapy, a clinical significant decrease in tumor size by more than 20% was observed in 14 patients. Two of these patients were previously treated by pituitary surgery, 1 with additional radiotherapy and all other patients received primary medical treatment.
Conclusion: Long-term combined treatment with SRIF analog and twice weekly PEG-V up to more than 4 years seems to be safe. Patients with both acromegaly and DM have a 2.28 higher risk of developing TLET. Clinical significant tumor shrinkage was observed in 14 patients during combined treatment.

INTRODUCTION

Recent studies of new medical treatment strategies with pegvisomant (PEG-V) for acromegaly have reported on high efficacy. PEG-V, alone or in combination with somatostatin analog (SRIF), has an efficacy of >90% to control insulin-like growth factor (IGF1) [1-4]. This is much higher than SRIF analogs, with an efficacy of ±66% [5]. However, PEG-V is at least unable to prevent tumor growth [6,4] in contrast to SRIF analogs. During SRIF analog treatment as primary medical therapy, pituitary tumor shrinkage was observed in 52% of the patients versus 21% for adjuvant therapy [7]. With combination therapy of SRIF analog and PEG-V twice weekly, tumor shrinkage was observed in 14% of the patients [2].

Side effects of SRIF analog therapy and PEG-V are elevated liver enzymes, but they have a different etiology. SRIF analogs increase the risk for cholelithiasis, which can cause cholestatic liver enzyme disturbances. PEG-V induces mainly elevated transaminases. The mechanism behind these PEG-V-induced elevated transaminases is unknown. A long-term report on daily PEG-V treatment reported elevated liver enzymes in 21 out of the 229 patients [6]. When PEG-V is combined with SRIF analog, there seems to be a better control of tumor size, but transient liver enzyme elevations (TLET) seem to occur in about 34% of the patients and diabetic patients seem to be more prone [2]. SRIF analogs alone have the highest efficacy for tumor shrinkage but for the control of IGF1 they are less effective.

Recently, we have reported on the improved quality of life (QoL) after the addition of PEG-V to acromegaly patients with an IGF1 within the age-adjusted normal range during the long-acting SRIF analog therapy [8]. Therefore, combined therapy might be an attractive option for the treatment of acromegaly.

Despite these observations, the Canadian Health Authorities sent out a safety warning about the combination therapy on June 11, 2008, because of the development of TLEE. This warning was based on a post-marketing study in 26 patients, conducted by Pfizer. We now report the safety of combined therapy assessed in 86 acromegalic patients over 29.2 (1.2–57.4) (mean (range)) months.

PATIENTS AND METHODS

Patients

Eighty-six acromegalic patients, 37 female, with a mean age of 54 (range 19–83) years were enrolled in our center, after their informed consent and approval by the local ethics committee. Their medical history revealed transsphenoidal surgery (TSS) in 44 subjects, TSS and radiotherapy in 20, while 42 subjects received primary medical treatment.

Twenty-one subjects also suffered from diabetes mellitus (DM). All patients were on SRIF analog treatment (octreotide LAR n=31, lanreotide Autogel n=55) for at least 6 months before PEG-V was added.

Methods

All patients continued long-acting SRIF analog therapy and on top of that, PEG-V was added twice weekly. Results were derived from two data sets. The first data set contains data from acromegalic patients (n=63) with elevated IGF1 levels at baseline. This group is described in this article as the 'uncontrolled group'. The second data set of patients (n=23), who were titrated up with PEG-V to improve the QoL, are described as 'QoL group'.

Of the uncontrolled group, 19 acromegalics started with a 25 mg, another 13 with 40 mg, and the last 26 patients started with a variable dose of PEG-V weekly guided by their baseline IGF1. This variable start dose was based on our previous observation that baseline IGF1 predicted the PEG-V dose that is necessary to control IGF1 [2]. Intervals of dose adjustments were 6 weeks until a controlled IGF1 was achieved twice. The subjects then visited our outpatient clinic every 16 weeks. The QoL group started with 20 mg PEG-V weekly and the dosage was adjusted on the basis of their QoL. The intervals of dose increments were 8 weeks until either serum IGF1 decreased below 2 S.D. or a worsening in QoL was observed after the initial improvement. Subjects visited our outpatient clinic at least every 16 weeks. The QoL was assessed by the acromegaly QoL questionnaire [9] and the patient-assessed acromegaly symptom questionnaire [3-4]. The dose of PEG-V was administered by the patients themselves. If the once weekly dose exceeded 80 mg, patients divided the dosage into two equal parts and injected twice weekly. At every visit to our outpatient clinic, safety parameters were assessed.

Safety assessment included: EKG, serum concentrations of alanine aminotransferase, aspartate aminotransaminase, alkaline phosphatase, γ-glutamyltranspeptidase, total bilirubin, and lactate dehydrogenase. Yearly, change in pituitary tumor volume was assessed by magnetic resonance imaging (MRI) and by the same neuroradiologist.

Statistical analysis

The non-paired data were assessed with the Mann–Whitney test, and cross-tables and odds ratio with the Fischer's exact test. Statistical analysis of the data was performed by GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego, CA, USA. Statistical significance was accepted at $P<0.05$. Data are expressed as mean ±S.D. unless otherwise specified.

RESULTS

Safety analyses were performed in all 86 patients with a follow-up of 29.2±20.2 months (Figure 1) and a median dose of 60 mg (range; 20–200) mg weekly. The dose of PEG-V in the uncontrolled group was median 60 mg (mean, 77; range, 20–200) weekly. The dose in the QoL group was also 60 mg (53; 20–80) (median (mean; range) weekly.

TLEE were observed in 23 patients (23/86, 27%; Table 1). Relevant TLEE of more than three times the upper limit of normal (ULN) were observed in 13 patients (13/86, 15%). In 2 out of these 13 patients, the TLEE could be explained by gallstones and 1 patient developed a transient drug-induced hepatitis, which have been reported elsewhere [10,2]. Ten out of 23 subjects with TLEE also suffered from DM. The odds ratio for developing TLEE was 2.28 (CI 1.16–9.22; p=0.03) for diabetic subjects. Six patients with TLEE 3x ULN were also diabetic. The risk for developing relevant TLEE for diabetics subjects was 3.31 (odds ratio, CI 1.00–11.37; p=0.07). TLEE occurred after 33±25 (range 8.0–92.0) weeks after the start of PEG-V. In diabetic acromegalic patients, TLEE were observed after 29.6±7.4 weeks versus 34.6±30.2 weeks in non-diabetic subjects, which was not significantly different. The TLEE of 3x ULN in diabetics were observed after 25.3±18.0 weeks versus 29.4±25.8 weeks in non-diabetic subjects, neither significantly different. The duration of the TLEE was 12.1±6.1 weeks, without a significant difference between diabetic 13.0±5.1 weeks versus non-diabetic acromegalics 11.3±7.0 weeks. No correlation was observed between TLEE and PEG-V dose. In the QoL group, we observed TLEE in five subjects. Four of these patients were also reported to have TLEE in our prior study [8]. After re-exposure

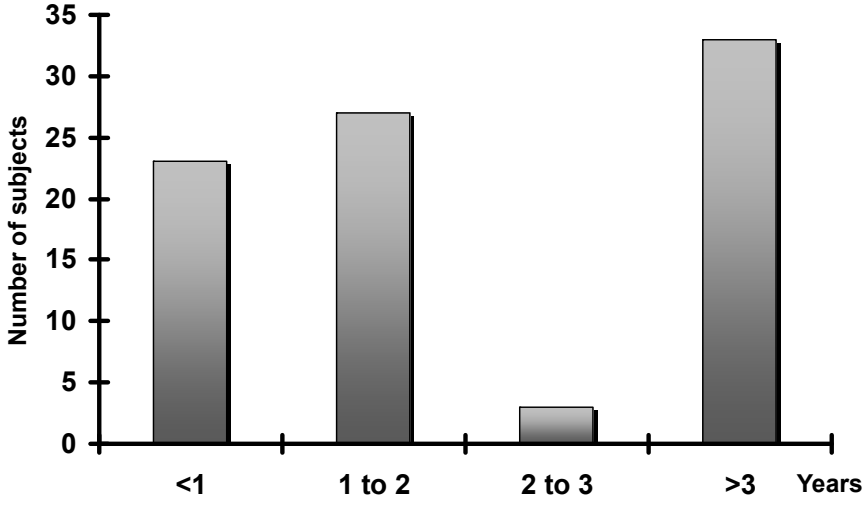

Figure 1: Number of subjects treated with twice weekly PEG-V and SRIF analog, divided over treatment periods of one, two, three, or more than 3 years.
SRIF = somatostatin, PEG-V = pegvisomant

Table 1. Clinical and biochemical parameters of 23 patients with elevated liver enzymes during combination therapy.

Sex	Age (yrs)	Time between start of PEG-V and LFT (weeks)	Weekly PEG-V dose at onset of LFT elevations	Peak LFT ALT	AST	γGT ----(xULN)----	DM	Follow up In all patients PEG-V was continued
M	53	17	60	31.6	14.2	18.4	+	MRCP showed gallstones, LFT declined rapidly and normalized < 8 weeks
M	30	86	60	8.8	4.7	5.7	-	Galway obstruction. LFT normalized after cholecystectomy < 1 week
M	62	18	40	2.8	2.0	10.4	+	LFT normalized < 12 weeks
M	38	20	60	1.8	1.3	1.1	-	LFT normalized < 18 weeks
M	48	46	160	2.4	1.1	1.2	+	LFT normalized < 18 weeks
M	61	24	60	3.0	1.8	1.5	+	LFT normalized < 12 weeks
M	44	48	60	4.8	2.9	5.1	-	LFT normalized < 18 weeks
M	30	50	200	1.5	0.4	0.8	+	LFT normalized < 6 weeks
M	46	8	80	4.3	3.3	2.2	-	LFT normalized < 12 weeks
M	47	25	60	31.9	7.1	7.6	+	PEG-V withdrawn, LFT normalized
M	47	16	80	14.5	5.9	5.4	-	LFT normalized < 12 weeks
M	61	21	60	4.0	2.2	2.0	-	LFT normalized < 6 weeks
M	44	60	60	2.9	1.3	2.9	+	LFT normalized < 10 weeks
M	54	16	40	4.3	2.4	1.8	-	LFT normalized < 8 weeks
F	27	14	60	5.3	3.8	1.7	-	LFT normalized < 12 weeks
F	68	8	40	5.5	3.6	2.2	+	LFT normalized < 18 weeks
F	66	12	40	2.0	1.2	2.0	+	LFT normalized < 18 weeks
F	57	24	60	5.1	2.6	3.0	-	LFT normalized < 12 weeks
F	72	40	40	2.1	1.6	0.4	-	LFT normalized < 12 weeks
F	45	92	40	2.3	1.2	1.0	-	LFT normalized < 3 weeks
F	45	16	40	14.6	8.5	1.4	-	LFT normalized < 26 weeks
F	74	68	40	1.8	1.4	2.5	-	LFT normalized < 8 weeks
F	47	33	80	1.7	1.3	0.7	+	LFT normalized <8 week

F, Female; M, male; LFT, liver function tests; xULN, times upper limit of normal; DM, diabetes mellitus; MRCP=Magnetic resonance cholangiopancreatography.

with PEG-V during dose finding for an optimal QoL, TLEE reoccurred in all subjects. In Fig. 2, TLEE of two of these patients are presented, while the two other subjects had similar results.

Another known side effect of PEG-V treatment, at the injection site, which did not occur during our previous follow-up is lipohypertrophy [11]. Reversible lipohypertrophy was observed in three patients at the abdominal injection sites, after at least 3 months

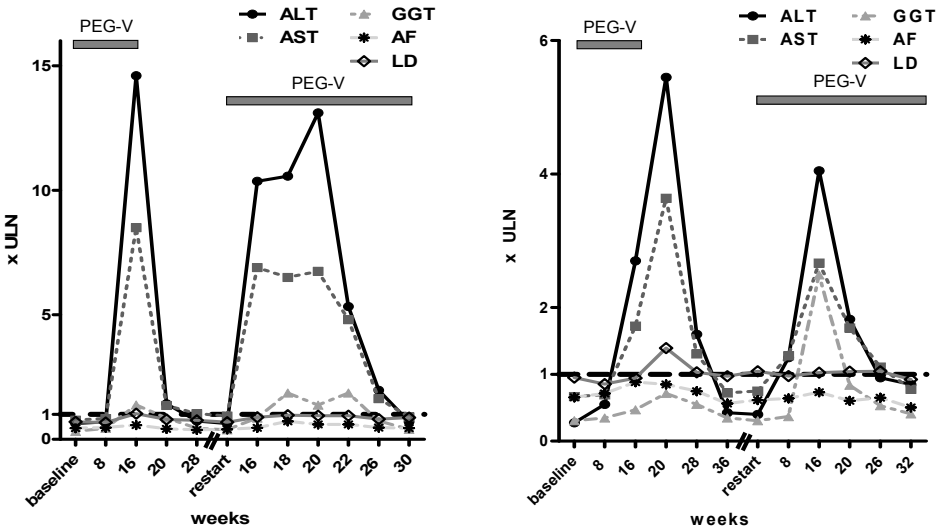

Figure 2: Elevated liver enzymes during combination therapy in 2 patients. Both patients were treated with combination treatment for 2 consecutive periods divided by a wash-out period of more than 4 months. PEG-V = pegvisomant, ALT = alanine aminotransferase, AST = aspartate aminotransaminase, AF = alkaline phosphatase, GGT = γ –glutamyltranspeptidase, LD = lactate dehydrogenase and xULN = times upper limit of normal.

of treatment. By changing the site of injection more frequently, the lipohypertrophy disappeared after 8 months. The PEG-V dose injected by these patients ranged from 60 mg once weekly to 60 mg twice weekly. None of these patients injected insulin or discontinued treatment.

In 12 out of the 86 patients, no tumor size decrease could be assessed due to empty sella prior to the start of combined treatment. In 14 (19%) patients out of the remaining 74 (86-12) patients with an assessable tumor size, the size of the tumor decrease by more than 20%, which is considered to be clinically significant. Two patients with tumor shrinkage underwent TSS in the past and one also received radiotherapy. The other 12 patients were on primary medical therapy. In none of the 86 patients was a tumor size increase observed.

DISCUSSION

We report that long-term combined treatment of long-acting SRIF analog and (twice) weekly PEG-V appears to be safe up to more than 57 months. TLEE do occur during combined therapy, but they appear to be completely reversible without decreasing the dose of PEG-V. When PEG-V is withdrawn and reintroduced after a complete wash-out of more than 2 months, TLEE reoccur in all our patients. The frequency of TLEE in

our series, with twice weekly PEG-V, is 27% (23/86) versus a report on daily PEG-V 9% (21/229) [6]. The frequency is lower when only TLEE of 3x ULN are taken into account (in our series in 15.0% (13/86) of the subjects versus 5.2% (12/229) in daily PEG-V [6]. The elevated transaminases of 3x ULN all occur within the first year of combined treatment. Therefore, assessment of the liver enzymes, as indicated by the package insert of PEG-V, is mandatory. Our advice is, close monitoring of the liver enzymes especially in the first year, because within this time period the TLEE seem to occur.

Previously, we reported that diabetic acromegalics have a 5.1 (odds ratio) higher risk to developing TLEE [2]. In the present study, we observed that diabetic acromegalics have a 2.3 times higher risk for developing TLEE. Significance was just lost when TLEE of 3 xULN were used for the analysis, probably due to the small number of subjects. Apparently, diabetic acromegalic patients should be monitored even more closely, but in our series TLEE in diabetic acromegalic subjects appear to be also transient and completely reversible without discontinuation or dose adjustment of PEG-V. The moment of occurrence in diabetic subjects tends to be earlier; however, this was not significant. The mechanism behind these PEG-V-induced elevated transaminases is still unknown; however, since diabetic acromegalics are more prone to develop the TLEE, it might be hypothesized that insulin resistance and lipid accumulation in the liver may predispose for these transient elevated transaminases. During the TLEE, no deterioration of the glycemic control occurred. It seems to be the other way around. The glycemic control is improving, and when there is no further improvement of the glycemic control the TLEE rarely occur. Most of these diabetics were on oral drug therapy and no correlation between any medication and the occurrence of TLEE could be found.

In this present series, there is no relationship between cumulative dose of PEG-V and the TLEE. Patients treated longer than 2 years had no TLEE even though many of them have had much higher cumulative dose than patients with TLEE. Patients with higher weekly dose of PEG-V did not have higher change for developing TLEE than patients with a lower dose.

In our previous QoL study, five subjects developed TLEE [8]. Four of these patients participated in our present dose-finding series. All developed TLEE again after the re-exposure to PEG-V and TLEE disappeared during continuation. In our hands, the combined therapy appears to be safe with regard to TLEE. TLEE are reversible in all our patients and seem not to re-occur during continuous PEG-V treatment. Therefore, we do not support the drug-warning by the Canadian health authorities that states that combination therapy might not be safe due to elevated transaminases.

Our recommendation for the work-up of a patient with TLEE is to closely monitor patients in which TLEE of more than 3 times ULN is observed. We advise to exclude cholelithiasis in all of these patients. However, in patients with more than 10 times ULN,

we advise to perform not only an ultrasound of the liver and bile ducts but also a liver biopsy and discontinue PEG-V treatment in the case of drug-induced hepatitis.

Another side effect that was observed was a painless lipohypertrophy in three patients. In all patients, it resolved within 8 months after changing the injection sites more frequently. None of these patients were dissatisfied with their treatment and insisted continuation with PEG-V. This is in contrast with another report, in which PEG-V was discontinued [11].

An important observation is that in 19% of our patients, we observed a clinically significant tumor shrinkage of >20%. The patients with the highest percentage of tumor shrinkage are the ones that receive primary medical treatment. This is most likely the effect of the continuous treatment with long-acting SRIF analogs. Primary medical-treated acromegaly patients might show more tumor shrinkage than patients treated with adjuvant SRIF analogs [7]. Therefore, we can conclude that long-acting SRIF analogs are still able to induce tumor shrinkage, even in the presence of PEG-V. The most important aspect of our present analyses was safety; however, efficacy is also available. In the uncontrolled group of 63 acromegaly patients, 60 (95%) had an IGF1 within the age-adjusted normal limits. All three patients with elevated IGF1 started recently with combination therapy (range 1.2–4.0) months.

An argument for combined therapy might be the improved QoL that we observed when PEG-V was added to long-acting SRIF analog treatment [8]. In addition, the improved insulin sensitivity compared with SRIF analogs alone [12,2], the advantage of tumor size control over PEG-V monotherapy [2,6,13], and lower necessary dose of PEG-V, which can lead to a cost reduction in at least some patients [1], favor the combined use of SRIF analogs and PEG-V. Therefore, we believe that combination therapy deserves a prominent place in the medical treatment of acromegaly.

In conclusion, although further research is necessary, our series indicate that combination therapy with long-acting SRIF analogs and twice weekly PEG-V is safe. Mild and transient elevated liver enzyme levels were observed in 15% of the patients. During continuation of PEG-V therapy, these liver enzyme abnormalities do not re-occur. However, re-exposure to PEG-V treatment after a wash-out period for whatever reason will again induce another episode with elevated liver enzymes in those subjects who are sensitive for this side effect. Finally, diabetic patients seem to be more prone to develop these episodes of transient elevated liver enzyme tests.

REFERENCES

1. Feenstra J, de Herder WW, ten Have SM, van den Beld AW, Feelders RA, Janssen JA, van der Lely AJ (2005) Combined therapy with somatostatin analogues and weekly pegvisomant in active acromegaly. Lancet 365 (9471):1644-1646
2. Neggers SJ, van Aken MO, Janssen JA, Feelders RA, de Herder WW, van der Lely AJ (2007) Long-term efficacy and safety of combined treatment of somatostatin analogs and pegvisomant in acromegaly. The Journal of clinical endocrinology and metabolism 92 (12):4598-4601
3. Trainer PJ, Drake WM, Katznelson L, Freda PU, Herman-Bonert V, van der Lely AJ, Dimaraki EV, Stewart PM, Friend KE, Vance ML, Besser GM, Scarlett JA, Thorner MO, Parkinson C, Klibanski A, Powell JS, Barkan AL, Sheppard MC, Malsonado M, Rose DR, Clemmons DR, Johannsson G, Bengtsson BA, Stavrou S, Kleinberg DL, Cook DM, Phillips LS, Bidlingmaier M, Strasburger CJ, Hackett S, Zib K, Bennett WF, Davis RJ (2000) Treatment of acromegaly with the growth hormone-receptor antagonist pegvisomant. The New England journal of medicine 342 (16):1171-1177
4. van der Lely AJ, Hutson RK, Trainer PJ, Besser GM, Barkan AL, Katznelson L, Klibanski A, Herman-Bonert V, Melmed S, Vance ML, Freda PU, Stewart PM, Friend KE, Clemmons DR, Johannsson G, Stavrou S, Cook DM, Phillips LS, Strasburger CJ, Hackett S, Zib KA, Davis RJ, Scarlett JA, Thorner MO (2001) Long-term treatment of acromegaly with pegvisomant, a growth hormone receptor antagonist. Lancet 358 (9295):1754-1759
5. Sheppard MC (2003) Primary medical therapy for acromegaly. Clinical endocrinology 58 (4):387-399
6. Schreiber I, Buchfelder M, Droste M, Forssmann K, Mann K, Saller B, Strasburger CJ, German Pegvisomant I (2007) Treatment of acromegaly with the GH receptor antagonist pegvisomant in clinical practice: safety and efficacy evaluation from the German Pegvisomant Observational Study. European journal of endocrinology / European Federation of Endocrine Societies 156 (1): 75-82
7. Bevan JS (2005) Clinical review: The antitumoral effects of somatostatin analog therapy in acromegaly. The Journal of clinical endocrinology and metabolism 90 (3):1856-1863
8. Neggers SJ, van Aken MO, de Herder WW, Feelders RA, Janssen JA, Badia X, Webb SM, van der Lely AJ (2008) Quality of life in acromegalic patients during long-term somatostatin analog treatment with and without pegvisomant. The Journal of clinical endocrinology and metabolism 93 (10): 3853-3859
9. Webb SM, Prieto L, Badia X, Albareda M, Catala M, Gaztambide S, Lucas T, Paramo C, Pico A, Lucas A, Halperin I, Obiols G, Astorga R (2002) Acromegaly Quality of Life Questionnaire (ACROQOL) a new health-related quality of life questionnaire for patients with acromegaly: development and psychometric properties. Clinical endocrinology 57 (2):251-258
10. Feenstra J, van Aken MO, de Herder WW, Feelders RA, van der Lely AJ (2006) Drug-induced hepatitis in an acromegalic patient during combined treatment with pegvisomant and octreotide long-acting repeatable attributed to the use of pegvisomant. European journal of endocrinology / European Federation of Endocrine Societies 154 (6):805-806
11. Bonert VS, Kennedy L, Petersenn S, Barkan A, Carmichael J, Melmed S (2008) Lipodystrophy in patients with acromegaly receiving pegvisomant. The Journal of clinical endocrinology and metabolism 93 (9):3515-3518
12. Jorgensen JO, Feldt-Rasmussen U, Frystyk J, Chen JW, Kristensen LO, Hagen C, Orskov H (2005) Co-treatment of acromegaly with a somatostatin analog and a growth hormone receptor antagonist. The Journal of clinical endocrinology and metabolism 90 (10):5627-5631

13. van der Lely AJ, Muller A, Janssen JA, Davis RJ, Zib KA, Scarlett JA, Lamberts SW (2001) Control of tumor size and disease activity during cotreatment with octreotide and the growth hormone receptor antagonist pegvisomant in an acromegalic patient. The Journal of clinical endocrinology and metabolism 86 (2):478-481

Chapter 4

Conversion of daily Pegvisomant to weekly Pegvisomant combined with long-acting Somatostatin Analogs, in controlled acromegaly patients

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ABSTRACT

Background: The efficacy of combined treatment in active acromegaly with both long-acting somatostatin analogs (SRIF) and pegvisomant (PEG-V).
Objective: To describe the PEG-V dose reductions after the conversion from daily PEG-V to combination treatment. To clarify the individual beneficial and adverse effects, in two acromegaly patients, who only normalized their insulin like growth factor (IGF1) levels with high-dose pegvisomant therapy.
Design: We present two cases of a 31 and 44 yrs old male with gigantism and acromegaly that were controlled subsequently by surgery, radiotherapy, SRIF analogs and daily PEG-V treatment. They were converted to combined treatment of monthly SRIF analog and (twice) weekly PEG-V. High dose SRIF analog treatment was added while the PEG-V dose was decreased during carful monitoring of the IGF1.
Results: After switching from PEG-V monotherapy to SRIF analogs plus pegvisomant combination therapy IGF1 remained normal. However the necessary PEG-V dose, to normalize IGF1 differed significantly between these two patients. One patient needed twice weekly 100 mg, the second needed 60 mg once weekly on top of their monthly lanreotide Autogel injections of 120 mg. The weekly dose reduction was 80 and 150 mg. After the introducing of lanreotide, fasting glucose and glycosylated haemoglobin concentrations increased. Diabetic medication had to be introduced or increased. No changes in liver tests or in pituitary adenoma size were observed.
Conclusion: In these two patients, PEG-V in combination with long-acting SRIF analogs was as effective as PEG-V monotherapy in normalizing IGF1 levels, although significant dose-reductions in PEG-V could be achieved. However, there seems to be a wide variation in the reduction of PEG-V dose, which can be obtained after conversion to combined treatment.

INTRODUCTION

Pegvisomant, a growth hormone (GH) receptor antagonist is a highly effective medical therapy for acromegaly. Studies have reported on high efficacy of pegvisomant (PEG-V), alone or in combination with somatostatin analogs (SRIF) of >90% to control insulin like growth factor (IGF1) [1-4]. The necessary PEG-V dose to control IGF1 seems to be lower during combination therapy than with PEG-V alone [5,2]. The combination treatment is probably therefore more effective at equal weekly dose than PEG-V alone [5,3,2]. There is a necessity to control IGF1 and GH has been well established since this will predict mortality [6-7]. So combined treatment seems to be an attractive option.

We present two cases of acromegaly patients who were controlled during daily, mono-therapy of PEG-V and were converted to combination treatment.

SUBJECTS AND METHODS

Patients

Case A; A 31-yr-old man was diagnosed with diabetes and gigantism since 1995, with a random GH of 220 mU/l and an IGF1 4.5 times the upper limit of normal (x ULN). A macro adenoma of 3 cm with suprasellar extension, impingement of the chiasm and invasion in the left cavernous sinus which was assessed by the Magnetic Resonance Imaging (MRI). In 1996, the initial treatment was transsphenoidal surgery. Pathologist reported a pituitary adenoma which a positive immunohistochemical staining for GH. Postoperative the GH levels and IGF1 levels remained uncontrolled (table 1). In 1997, 4 times daily subcutaneous injection of Octreotide 300 µg was started and later converted to Octreotide LAR 30 mg monthly. In 1997, additional radiotherapy of 45 Gy in 25 fractions was given. In 1998, despite all these efforts IGF1 and GH remained elevated while other anterior pituitary hormones became deficient, which needed replacement therapy. Therefore, this patient was switched to pegvisomant therapy. Octreotide LAR was stopped in 1998 and daily PEG-V was increased up to 40 mg daily until IGF1 was within the age adjusted normal limits [4]. In October 2006 Lanreotide autosolution (LAN) was added and PEG-V was decreased in dose and frequency according to protocol. On pituitary MRI prior to the introduction of the LAN a remnant (8 mm) of the adenoma was present in and around left cavernous sinus.

Case B; A 44-yr-old man was diagnosed with acromegaly and osteoporosis since 1994. At the time of diagnose random GH serum levels were around 120 mU/l while IGF1 serum levels were around 3.5 x ULN. On MRI, he had a pituitary tumor of 2-cm with suprasellar extension, but without impingement of the chiasm and possible invasion of the right cavernous sinus. In 1994 he underwent transsphenoidal surgery. A tumor

Table 1.

Patient A date	**1998**	**2000**	**2002**	**2003**	**jan-07**	**apr-07**	**jul-07**	**oct-07**
acromegaly medication	Octreotide LAR 30	PEG-V 40 OD	PEG-V 40 OD	PEG-V 40 OD	LAN 120 PEG-V 70 mg TW	LAN 120 PEG-V 80 mg TW	LAN 120 PEG-V 100 mg TW	LAN 120 PEG-V 100 MG TW
GH µg/l	42	N/A	N/A	N/A	N/A	N/A	N/A	N/A
IGF1 ULN (nmol/l)	1.9 (73,9)	0.98 (39.3)	0.93 (37.3)	0.93 (37.0)	1.3 (55.7)	1.0(40.9)	0.8(31.4)	0.5 (20.8)
HbA1c	6.9	6.4	6.1	6.2	8.4	8.7	9.0	7.7
Fasting glucose	7.3	4.9	5.9	6.5	8.0	9.4	5.7	3.8
DM medication	INN TD 5	INN TD 5	INN TD 5	INN TD 5	INN TD 5	INN TrD 5	INN TrD 5	INN TD 5 Glargin 48 IE OD
Patient B date	2003	2004	2005	2006	jun-07	**sep-07**	**dec-07**	**mar-07**
acromegaly medication	Octreotide LAR 40	PEG-V 30 OD	PEG-V 30 OD	PEG-V 30 OD	LAN 120 PEG-V 50 mg TW	LAN 120 PEG-V 80 mg OW	LAN 120 PEG-V 60 mg OW	LAN 120 PEG-V 60 mg OW
GH µg/l	2.3	N/A	N/A	N/A	N/A	N/A	N/A	N/A
IGF1 ULN (nmol/l)	1.93 (67.5)	0.82 (28.6)	0.96 (33.6)	0.96 (32.9)	0.3 (12.1)	0.5 (16.6)	0.9 (31.7)	0.8 (29.8)
HbA1c	N/A	6.6	6.3	6.4	6.9	7.8	8.9	7.4
Fasting glucose	5.7	4.0	4.6	5.0	6.9	10.7	8.9	7.8
DM medication	none	none	none	none	none	MT 500 TD	MT 850 TrD	MT 850 TrD

LAN = Lanreotide Autogel (mg), PEG-V = Pegvisomant (mg), OD = Once Daily, OW = Once Weekly, TW = Twice Weekly, TD Twice Daily, TrD Trice Daily, INN = glibenclamide (mg), MT = metformin (mg) and N/A

specimen revealed positive immunohistochemical staining for GH. After surgery, he was treated with radiotherapy (42 Gray in 32 fractions). After surgery and RT GH serum levels dropped to around 17 µg/l. Three months post surgery IGF1 serum levels were decreased to 3.0 x ULN. In 1995, Octreotide was started (100 µg t.i.d.) and converted to monthly Octreotide LAR 40 mg in 1998. Although he developed panhypopituitarism and GH levels normalized, IGF1 levels remained elevated, around 1.9 x ULN (table 1). In 2003, Octreotide LAR therapy was switched into PEG-V monotherapy. Daily PEG-V dosages of 30 mg PEG-V were necessary to decrease IGF1 levels to within the age adjusted normal limits. In Marche 2007, LAN was added and PEG-V was decreased in dose and

frequency. On pituitary MRI prior to the introduction of the LAN, a small (5 mm) remnant of the adenoma was present close to the right cavernous sinus.

Methods

After monthly LAN 120 mg was started the intervals between visits were 8 weeks to assess efficacy and safety. The initial dose of PEG-V was decreased with 50% and later a further stepwise decrease until IGF1 levels escaped (see results section). If IGF1 was above the upper limit of normal the dose was increased again until IGF1 levels were within the age adjusted normal range. If IGF1 fell below 0.5 ULN PEG-V dose was decreased. After normalization of IGF1 during the combination treatment phase, subjects visited our outpatient clinic every 12-16 weeks. If a pegvisomant injection dose reached 100 mg or more, the administration of pegvisomant was divided into 2 (equal) dosages that were injected twice weekly.

From 2004 to 2009, IGF1 and GH concentrations were measured by an immunometric assays (Diagnostic Products Corporation, Los Angeles USA) and before by an immunoradiometric assays (Diagnostic Systems Laboratories, Texas USA). The IGF1 age adjusted reference ranges were used [8]. But during PEG-V treatment the GH serum levels were not assessed.

Safety assessment included: Fasting glucose, glycosylated haemoglobin, EKG, serum concentrations of alkaline phosphatase , γ –glutamyltranspeptidase (γ-GT), alanine aminotransferase (ALT), aspartate aminotransaminase (AST), lactate dehydrogenase, total bilirubin and change in pituitary tumor volume. Tumor volume was assessed at baseline by MRI which was repeated every 12 months. Change in pituitary adenoma size was assessed by the same neuro-radiologist.

RESULTS

LAN 120 mg was given every 4 weeks. After the second administration of LAN 120 mg PEG-V dose was decreased by 50% of the normal total weekly dose of the subjects. PEG-V was increase until IGF1 normalized after 3 and/or 6 months (table1). Patient A was on 40 mg PEG-V, daily. Patient's B original dose was 30 mg a day of PEG-V. The dose reduction of PEG-V in patient A started with a dose decrease to twice weekly 70 mg of PEG-V. Patient B decreased the PEG-V to twice weekly 50 mg. After 3 months patient A increased the dose to twice weekly 80 mg and patient B decreased the dose to 80 mg once weekly. After 6 months the final dose for patient A was twice weekly 100 mg and for patient B once weekly 60 mg of PEG-V. The weekly PEG-V dose reduction for patient A was 80 mg and patient B, 150 mg weekly, both with a normalized IGF1 (figure 1).

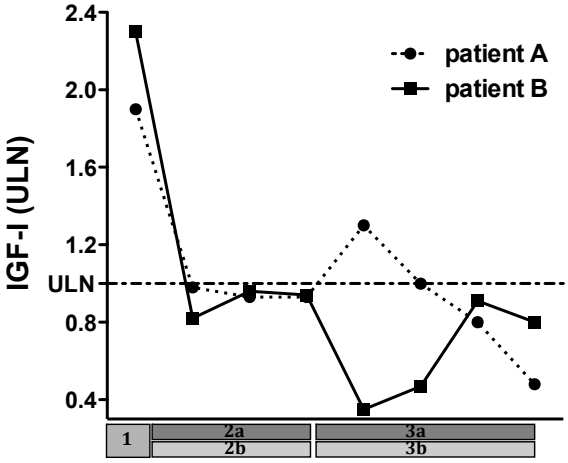

Figure 1: Course of IGF1 during different treatment modalities.
1: Octreotide LAR, 2a Pegvisomant 40 mg daily, 2b Pegvisomant 30 mg daily, 3a & 3b Somatuline autogel and Pegvisomant

SAFETY

Fasting glucose and glycosylated haemoglobin levels increased after introduction of LAN (Table 1). In patient 1, additional diabetic medication was necessary. Glargine up to 48 IE was added on top of glibenclamide (5 mg b.i.d). Patient 2 had to start diabetic medication (metformin 850 mg t.i.d.; table 1). In these patients no transient elevated liver enzyme tests or tumor size increase was observed.

DISCUSSION

We report two adequate controlled acromegaly patients during PEG-V monotherapy. Both could also be controlled after the conversion to combined treatment of PEG-V with long-acting SRIF analogs. During combined treatment, a dose reduction of PEG-V could be obtained between 80-150 mg weekly to keep IGF1 levels within the age-adjusted reference range.

During long-term combined therapy the weekly mean dose of PEG-V was 77 mg [2,9] while with PEG-V monotherapy a weekly mean dose of 130 mg was necessary to reach an equal efficacy rate of more than 90% [4]. Data from the long-term surveillance AcroStudy^{tm} indicate that the mean patient on PEG-V therapy (of which around 1 out of 4 also use SRIF analogs) needs more than 21 mg of PEG-V daily with an efficacy rate of around 60% [10]. In our 2 patients that needed high-dose PEG-V during monotherapy, we observed dose reductions of 80 & 150 mg per week, which reflect reductions in an-

nual costs of around 35.000 - 65.000 € respectively. When we take into consideration that high-dose SRIF analogs treatment costs between 19.000 – 25.000 €, depending on the country, our data indicate that in patients who need high-dose PEG-V during monotherapy, a significant reduction in costs can be achieved without loosing efficacy in normalizing IGF1 levels. The ratio behind the dose reduction in PEG-V during combined therapy of LAN and PEG-V is an increase of about 20% in PEG-V serum levels [11,4] and the decrease in GH serum levels due to SRIF analog treatment. Also, a direct and indirect inhibition of IGF1 generation of the liver by SRIF analogs [11,4,12,2,13-14] has been reported. Thus, during combined treatment PEG-V, which is a competitive antagonist of the GH receptor (GHR), has less GH to compete with, which reduces the necessary dose of PEG-V. Moreover, experiments in rodents, observed a decrease in the number of GHR on the liver due to a decrease of portal insulin concentration, which is a direct effect of SRIF analogs [12,15-16]. Therefore a further reduction in PEG-V dose can be possible.

The difference in dose reduction we observed between both patients might be explained by the expression level of subtype of the somatostatin receptor (SSTR) on the pituitary adenoma. Patient 1 might have a lower expression of sst2 than patient B, as the efficacy of SRIF analogs in suppressing pathological GH secretion depends on the expression level of the sst subtypes on the pituitary adenoma [17]. The expression level of sst2 on human pancreatic beta cells is high, but there remains controversy on the expression of sst3 and sst5 [18]. In vivo, octreotide, mainly an sst2 agonist, decreases insulin concentration in contrast to Pasireotide (SOM 230) [17]. Pasireotide, with high affinity to the sst1 and sst5 as well , has as Octreotide hyperglycemic effect. There for it unclear what the effect of SOM 230 is on the GHR expression on the liver. The alternative treatment with SOM 230 in patient 1 might lead to a dose reduction since GH levels will be lower but because of the possible higher expression of GHR a higher dose could be needed. Thus it is unclear what the net effect will be beneficial for the PEG-V dose reduction. Finally most studies which have been conducted with SOM230 where with diabetic patient that had an optimal control. Therefore it is questionable if this patient would really benefit from SOM 230.

SRIF analogs inhibits both exocrine and endocrine hormone secretion and to a lesser extent neuroendocrine tumor cell proliferation [19]. Activation of sst receptors decreases intra-cellular c-AMP generation through the inhibition of adenylyl cyclase. There seems to be a role of the GHRH –cAMP signaling pathway in somatotroph tumorgenesis. Ectopic GHRH production leads to somatotroph hyperplasia, however rarely to adenoma formation [20]. GHRH induces c-AMP via a G-protein coupled receptor, the GHRH receptor, which induces GH production. Guanine nucleotide-binding protein (GNAS) mutation can lead to constitutively elevated c-AMP, protein kinase A activity, GH synthesis and secretion [21]. About 40% of sporadic somatotroph adenomas harbor this mutation. Although the percentage of patients using PEG-V that develop a clinical

significant increase in tumor size is reassuring low [22-23], the inhibitory effects of SRIF analogs on these mechanisms that are related to tumor growth also would suggest a more prominent role for the combination of both SRIF analogs and PEG-V, especially in patients with aggressive tumors and active disease that need high dose PEG-V during PEG-V monotherapy.

The disadvantage of SRIF analogs treatment compared to PEG-V treatment with respect to carbohydrate metabolism is clearly demonstrated here. In healthy volunteers, administration of PEG-V did not influence the fasting insulin and glucose or response to oral glucose loading, in contrast with octreotide [24]. Short term combined treatment resulted in a lower fasting glucose than with SRIF analogs alone, without a change in insulin levels [11]. During long-term studies a decrease in glycosylated haemoglobin could be observed despite the reduction of insulin or oral diabetic medication [3,2]. It seems that in the hierarchy of the beneficial effects on carbohydrate metabolism, PEG-V as monotherapy is superior to combined treatment. However, the combination is better than SRIF analogs monotherapy.

In the past, both patients were treated with conventional radiotherapy. This was the pre-PEG-V era. To date, radiotherapy has become redundant from efficacy point of view. After a mean duration of 10 years remission rates of 50-60% are reported, but also 50-80% of subjects develop hypopituitarism as well [25]. Radiotherapy has some more disadvantages. A decreased quality of life has been reported which seems to be progressive over time [26] and radiotherapy might also increase mortality (SMR 2.68) mainly due to cerebrovascular mortality [27]. Our patients already showed some long-term side effects of radiotherapy as both have hypopituitarism. Cerebrovascular accidents have not occurred to date.

These two cases are different from our previous reports, because these two patients were previously treated controlled with PEG-V daily monotherapy. Our previous studies included patients on SRIF analogs that still had an elevated IGF1 and therefore we did not have a direct comparison for dose reduction of PEG-V.

In this case-study we start immediately with high-dose SRIF analogs treatment and we reduce weekly dose of pegvisomant by 50%. After 3 and 6 months, the PEG-V can be adjusted according to the serum IGF1 levels, If elevated, a higher weekly dose is necessary, while in case of normal IGF1 levels, one could further reduce the PEG-V dosages to find the lowest effective dose.

In conclusion, these two case-reports show that a dose reduction in PEG-V is possible after the addition of a monthly high dose SRIF analogs. This might significantly reduce the annual costs. However, deterioration in the carbohydrate metabolism should be taken into account.

REFERENCES

1. Feenstra J, de Herder WW, ten Have SM, van den Beld AW, Feelders RA, Janssen JA, van der Lely AJ (2005) Combined therapy with somatostatin analogues and weekly pegvisomant in active acromegaly. Lancet 365 (9471):1644-1646
2. Neggers SJ, de Herder WW, Janssen JA, Feelders RA, van der Lely AJ (2009) Combined treatment for acromegaly with long-acting somatostatin analogs and pegvisomant: long-term safety for up to 4.5 years (median 2.2 years) of follow-up in 86 patients. European journal of endocrinology / European Federation of Endocrine Societies 160 (4):529-533
3. Neggers SJ, van Aken MO, Janssen JA, Feelders RA, de Herder WW, van der Lely AJ (2007) Long-term efficacy and safety of combined treatment of somatostatin analogs and pegvisomant in acromegaly. The Journal of clinical endocrinology and metabolism 92 (12):4598-4601
4. van der Lely AJ, Hutson RK, Trainer PJ, Besser GM, Barkan AL, Katznelson L, Klibanski A, Herman-Bonert V, Melmed S, Vance ML, Freda PU, Stewart PM, Friend KE, Clemmons DR, Johannsson G, Stavrou S, Cook DM, Phillips LS, Strasburger CJ, Hackett S, Zib KA, Davis RJ, Scarlett JA, Thorner MO (2001) Long-term treatment of acromegaly with pegvisomant, a growth hormone receptor antagonist. Lancet 358 (9295):1754-1759
5. Hodish I, Barkan A, Medscape (2008) Long-term effects of pegvisomant in patients with acromegaly. Nature clinical practice 4 (6):324-332
6. Dekkers OM, Biermasz NR, Pereira AM, Romijn JA, Vandenbroucke JP (2008) Mortality in acromegaly: a metaanalysis. The Journal of clinical endocrinology and metabolism 93 (1):61-67
7. Holdaway IM, Bolland MJ, Gamble GD (2008) A meta-analysis of the effect of lowering serum levels of GH and IGF-I on mortality in acromegaly. European journal of endocrinology / European Federation of Endocrine Societies 159 (2):89-95
8. Elmlinger MW, Kuhnel W, Weber MM, Ranke MB (2004) Reference ranges for two automated chemiluminescent assays for serum insulin-like growth factor I (IGF-I) and IGF-binding protein 3 (IGFBP-3). Clin Chem Lab Med 42 (6):654-664
9. Neggers SJ, van der Lely AJ (2009) Somatostatin analog and pegvisomant combination therapy for acromegaly. Nat Rev Endocrinol 5 (10):546-552
10. Trainer PJ, Ezzat S, D'Souza GA, Layton G, Strasburger CJ (2009) A randomized, controlled, multicentre trial comparing pegvisomant alone with combination therapy of pegvisomant and long-acting octreotide in patients with acromegaly. Clinical endocrinology 71 (4):549-557
11. Jorgensen JO, Feldt-Rasmussen U, Frystyk J, Chen JW, Kristensen LO, Hagen C, Orskov H (2005) Co-treatment of acromegaly with a somatostatin analog and a growth hormone receptor antagonist. The Journal of clinical endocrinology and metabolism 90 (10):5627-5631
12. Leung KC, Doyle N, Ballesteros M, Waters MJ, Ho KK (2000) Insulin regulation of human hepatic growth hormone receptors: divergent effects on biosynthesis and surface translocation. The Journal of clinical endocrinology and metabolism 85 (12):4712-4720
13. Neggers SJ, van Aken MO, de Herder WW, Feelders RA, Janssen JA, Badia X, Webb SM, van der Lely AJ (2008) Quality of life in acromegalic patients during long-term somatostatin analog treatment with and without pegvisomant. The Journal of clinical endocrinology and metabolism 93 (10): 3853-3859
14. Murray RD, Kim K, Ren SG, Chelly M, Umehara Y, Melmed S (2004) Central and peripheral actions of somatostatin on the growth hormone-IGF-I axis. The Journal of clinical investigation 114 (3): 349-356

15. Shishko PI, Dreval AV, Abugova IA, Zajarny IU, Goncharov VC (1994) Insulin-like growth factors and binding proteins in patients with recent-onset type 1 (insulin-dependent) diabetes mellitus: influence of diabetes control and intraportal insulin infusion. Diabetes research and clinical practice 25 (1):1-12
16. Wurzburger MI, Prelevic GM, Sonksen PH, Balint-Peric LA, Wheeler M (1993) The effect of recombinant human growth hormone on regulation of growth hormone secretion and blood glucose in insulin-dependent diabetes. The Journal of clinical endocrinology and metabolism 77 (1): 267-272
17. van der Hoek J, de Herder WW, Feelders RA, van der Lely AJ, Uitterlinden P, Boerlin V, Bruns C, Poon KW, Lewis I, Weckbecker G, Krahnke T, Hofland LJ, Lamberts SW (2004) A single-dose comparison of the acute effects between the new somatostatin analog SOM230 and octreotide in acromegalic patients. The Journal of clinical endocrinology and metabolism 89 (2):638-645
18. Strowski MZ, Blake AD (2008) Function and expression of somatostatin receptors of the endocrine pancreas. Molecular and cellular endocrinology 286 (1-2):169-179
19. Melmed S (2009) Acromegaly pathogenesis and treatment. The Journal of clinical investigation 119 (11):3189-3202. doi:39375 [pii] 10.1172/JCI39375
20. Thorner MO, Perryman RL, Cronin MJ, Rogol AD, Draznin M, Johanson A, Vale W, Horvath E, Kovacs K (1982) Somatotroph hyperplasia. Successful treatment of acromegaly by removal of a pancreatic islet tumor secreting a growth hormone-releasing factor. The Journal of clinical investigation 70 (5):965-977
21. Vallar L, Spada A, Giannattasio G (1987) Altered Gs and adenylate cyclase activity in human GH-secreting pituitary adenomas. Nature 330 (6148):566-568
22. Buhk JH, Jung S, Psychogios MN, Goricke S, Hartz S, Schulz-Heise S, Klingebiel R, Forsting M, Bruckmann H, Dorfler A, Jordan M, Buchfelder M, Knauth M (2010) Tumor volume of growth hormone-secreting pituitary adenomas during treatment with pegvisomant: a prospective multicenter study. The Journal of clinical endocrinology and metabolism 95 (2):552-558. doi: jc.2009-1239 [pii] 10.1210/jc.2009-1239
23. Trainer PJ (2009) ACROSTUDY: the first 5 years. European journal of endocrinology / European Federation of Endocrine Societies 161 Suppl 1:S19-24. doi:EJE-09-0322 [pii]10.1530/EJE-09-0322
24. Parkinson C, Drake WM, Roberts ME, Meeran K, Besser GM, Trainer PJ (2002) A comparison of the effects of pegvisomant and octreotide on glucose, insulin, gastrin, cholecystokinin, and pancreatic polypeptide responses to oral glucose and a standard mixed meal. The Journal of clinical endocrinology and metabolism 87 (4):1797-1804
25. Castinetti F, Morange I, Dufour H, Regis J, Brue T (2009) Radiotherapy and radiosurgery in acromegaly. Pituitary 12 (1):3-10. doi:10.1007/s11102-007-0078-y
26. van der Klaauw AA, Biermasz NR, Hoftijzer HC, Pereira AM, Romijn JA (2008) Previous radiotherapy negatively influences quality of life during 4 years of follow-up in patients cured from acromegaly. Clinical endocrinology 69 (1):123-128
27. Ayuk J, Clayton RN, Holder G, Sheppard MC, Stewart PM, Bates AS (2004) Growth hormone and pituitary radiotherapy, but not serum insulin-like growth factor-I concentrations, predict excess mortality in patients with acromegaly. The Journal of clinical endocrinology and metabolism 89 (4):1613-1617

Chapter 5

Quality of Life in Acromegalic Patients during Long-Term Somatostatin Analog Treatment with and without Pegvisomant

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ABSTRACT

Objective: The objective of the study was to assess whether weekly administration of 40 mg pegvisomant (PEG-V) improves quality of life (QoL) and metabolic parameters in acromegalic patients with normal age-adjusted IGF1 concentrations during long-acting somatostatin analog (SRIF) treatment.

Design: This was a prospective, investigator-initiated, double blind, placebo-controlled, crossover study. Twenty acromegalic subjects received either PEG-V or placebo for two consecutive treatment periods of 16 wks, separated by a washout period of 4 wks. Efficacy was assessed as change between baseline and end of each treatment period. QoL was assessed by the Acromegaly Quality of Life Questionnaire (AcroQoL) and the Patient-Assessed Acromegaly Symptom Questionnaire (PASQ).

Results: The AcroQoL (P = 0.008) and AcroQoL physical (P = 0.002) improved significantly after PEG-V was added. The addition of PEG-V also significantly improved the PASQ (P = 0.038) and the single PASQ questions, perspiration (P = 0.024), soft tissue swelling (P = 0.036), and overall health status (P = 0.035). No significant change in Z-score of IGF1 (P = 0.34) was observed during addition of PEG-V. Transient liver enzyme elevations were observed in five subjects (25%).

Conclusion: Improvement in quality of life was observed without significant change in IGF1 after the addition of 40 mg pegvisomant weekly to monthly SRIF analogs therapy in acromegalic patients who had normalized IGF1 on SRIF analogs monotherapy. These data question the current recommendations in how to assess disease activity in acromegaly. Moreover, the findings question the validity of the current approach of medical treatment in which pegvisomant is used only when SRIF analogs therapy has failed to normalize IGF1.

INTRODUCTION

Recent improvement in the medical treatment of acromegaly has resulted in better biochemical disease control in virtually every acromegaly patient [1-4]. By normalizing both IGF1 and GH, the elevated long-term mortality will decrease [5-8]. However, normalization does not completely relieve patients from their symptoms [9]. From the patient's perspective an important parameter of disease control is the quality of life (QoL). Indeed, these residual symptoms result in an impaired QoL [10-13]. To quantify the symptoms and QoL in patients with acromegaly, the Patient-Assessed Acromegaly Symptom Questionnaire (PASQ) [3-4] and the Acromegaly Quality of Life Questionnaire (AcroQoL) have been developed [14].

We recently reported that the combination of somatostatin analog (SRIF) and (twice) weekly pegvisomant (PEG-V) is an effective treatment for patients in whom the IGF1 remains elevated during SRIF analog monotherapy [1-2]. The rationale for this combined treatment is based on the concept that SRIF analogs will decrease GH secretion in acromegalic subjects. When there is less endogenous GH to compete for the GH receptor (GHR), less of the GHR antagonist PEG-V is needed. Moreover, in vitro data suggest that the inhibition of insulin secretion by SRIF analogs in the portal vein results in a reduction of GHR on the cell surface of hepatocytes [15]. So when there are fewer hepatic GHRs and less endogenous GH, less PEG-V is necessary. Finally, SRIF analogs can also directly inhibit IGF1 production by hepatocytes [16]. Therefore, SRIF analogs makes the liver less GH sensitive, as the rest of the body might still be slightly acromegalic. If these extrahepatic GH actions could be antagonized by the addition of pegvisomant, one might observe an improvement of QoL.

We therefore performed a randomized, double-blind, placebo-controlled, crossover study in which we assessed QoL, using AcroQoL and PASQ, in acromegalic patients in biochemical remission on long-term SRIF analog monotherapy before and after the addition of a weekly dose of 40 mg PEG-V for a period of 16 wks.

PATIENTS AND METHODS

Patients

Twenty acromegalic patients, median age 56 (range 39–74) yr, with an IGF1 within the age adjusted normal range during long-term long-acting SRIF analog therapy, were enrolled in this study [17]. All subjects were on a stable long-acting monthly SRIF analog treatment for at least 36 months. The single GH levels assessed before the initiation of PEG-V therapy were less than 2.5 µg/liter in all but one subject. Patients' characteristics

Table 1. Baseline characteristics

	Number[#]	(%)[#]
Patients	20	(100)
Sex (female)	9	(45)
Age (years)		
Mean (SD, range)	55	(10.0, 39-74)
Diabetes Mellitus	3	(15)
Growth hormone (µg/l) at baseline		
Mean (SD)	1.12	(0.7)
Median (range)	1.0	(0.2-3.1)
Previous treatment		
TSS	15	(75)
Both TSS and radiotherapy	6	(30)
Primary medical therapy	5	(25)
Pituitary insufficiency		
Panhypopituitarism	5	(25)
1-2 axis	11	(55)
No hypopituitarism	4	(20)
Long-acting somatostatin analogs		
Lanreotide	8	(40)
Octreotide	12	(60)

[#]Unless otherwise specified TSS= Transsphenoidal Surgery RT= radiotherapy

are presented in Table 1. All patients gave their written informed consent, and the study was approved by the local ethics committee.

Design

The study was a prospective, investigator-initiated, double-blind, placebo-controlled, single-center, crossover study. After enrollment [visit (V)], patients were randomized to receive a single weekly sc injection of 40 mg PEG-V or placebo. QoL assessment and biochemical evaluation were performed at baseline and after 8 (V2) and 16 wks (V3) of combined treatment. After a 4-wks washout period (V4), patients were switched to either placebo or PEG-V for another 16 wks (V5 at 28 wks and V6 at 36 wks).

At all visits efficacy and safety data were assessed including two QoL-questionnaires, IGF1, glycosylated hemoglobin, fasting glucose, insulin, lipids and homeostasis model assessment insulin resistance. Additional safety including serum alkaline phosphatase, -glutamyltranspeptidase, alanine aminotransferase, aspartate aminotransaminase, lactate dehydrogenase, and total bilirubin were measured, and each subject had an electrocardiogram. IGF1, insulin, and GH concentrations were measured by immuno-

metric assays (Immulite 2000; Diagnostic Products Corp., Los Angeles, CA). For IGF1 the intra- and interassay coefficients of variation are 4.9–6.3 and 3.5–7.5%, respectively [18]. IGF1 age-adjusted reference ranges were used to calculate the Z-score of the IGF1 [17].

At baseline and last visit of the study, a magnetic resonance imaging of the pituitary was performed to detect any change in pituitary volume during the study. An independent physician, who did not participate in the study and was unblinded for the medication, evaluated the laboratory findings after each visit to ensure safety and the double-blind set-up of the study. If elevated liver enzyme tests were higher than 4 times the upper limit or normal (ULN) during the treatment periods, the patients were withdrawn from the study.

Questionnaires

AcroQoL

AcroQoL comprises 22 questions. Each question has five possible answers scored 1–5, with a total maximum score of 110 and quoted as a percentage. The score of 110 reflects the best possible QoL. The 22 questions are divided into two main categories: physical and psychological function. The psychological dimension is subdivided into appearance and personal relationships [19,14]. The AcroQoL has a good internal consistency (Cronbach's > 0.7) [20].

PASQ

The PASQ is a disease-specific questionnaire, which consists of six questions scoring 0–8 and the seventh question addressing the overall health status, based on the other six questions, scoring 0–10 [3-4]. The first six questions evaluate symptoms as headache, excessive sweating, joint pain, fatigue, soft tissue swelling, and numbness or tingling of the extremities. The maximum score of these six questions is 48 and indicates severe signs and symptoms, with lower scores reflecting improved QoL.

Statistics

For the analysis of QoL questionnaires, the change between baseline and end of each treatment period with pegvisomant (PEG-V) and with placebo (placebo) was calculated. By assessing the change in QoL-questionnaire scores, between baseline and follow-up, possible confounder were taken into account when the paired of these patients were analyzed. The IGF1 results are expressed as Z-score. Therefore, they are reported in SD units. Sample size calculation was made, based on expected PASQ scores, anticipating that SD of each group (assuming they are equal) is 2.5 on the scale of 0–8. When choosing = 0.05, two-tailed, and power = 80%, a number of 20 patients per group will enable detection of differences of 1.82.

The paired data were analyzed with the Wilcoxon's signed rank test. The correlation between the nonparametric data was assessed by the Spearman's rank correlation. Statistical analyses were performed by GraphPad Prism (version 5.00 for Windows; GraphPad Software, San Diego, CA). Statistical significance was accepted at $P < 0.05$ (two tailed). Data are nonparametric and therefore expressed as median ± SD unless otherwise specified.

RESULTS

All 20 patients completed the study. At baseline, all IGF1 levels were within the age-adjusted normal range and GH was 2.5 µg/liter or less in all except for one subject (GH of 3.1 µg/liter). The QoL scores, parameters of metabolic control, and body weight are presented in Table 2.

Table 2. Quantitative changes in QoL in two groups induced by the addition of 40 mg PEG-V once weekly in two different groups of acromegalic patients (PEG-V-placebo, Table 2A, and placebo-PEG-V, Table 2B) with normal IGF1 during long-term treatment with SRIF analogs.

2A	Baseline	PEG-V	After wash-out	Placebo
PEG-V Placebo group				
Z-score of IGF1	1.50 (1.04-1.73)	1.46 (-0.09-1.58)	1.45 (0.89-1.84)	1.64 (1.03-1.99)
Body weight (Kg)	83.1 (69.7-107.7)	82.1 (70.3-106.6)	81.6 (70.4-107.3)	83.0 (69.4-106.2)
AcroQol **global** (%)	64.2 (44.3-79.5)	69.9 (39.8-87.5)	66.5 (31.8-88.6)	68.2 (30.7-86.0)
AcroQol **physical** (%)	51.7 (37.5-92.0)	62.5 (40.6-93.8)	53.1 (31.3-96.9)	59.4 (28.1-87.5)
AcroQol **psychological** (%)	65.2 (48.3-87.5)	70.5 (39.3-85.8)	70.5 (32.1-89.3)	72.3 (32.2-85.7)
AcroQol **pers, relation** (%)	82.1 (53.6-96.4)	82.1 (46.4-100.0)	80.4 (42.9-100.0)	82.1 (35.7-100.0)
AcroQol **appearance** (%)	50.0 (21.4-85.7)	62.5 (28.6-82.1)	64.3 (21.4-82.1)	62.5 (28.6-82.1)
PASQ	14.0 (2.0-21.0)	10.0 (0.0-17.0)	7.5 (0.0-20.0)	10.0 (3.0-22.0)
PASQ **headache**	1.0 (0.0-3.0)	1.0 (0.0-4.0)	0.0 (0.0-3.0)	1.5 (0.0-4.0)
PASQ **excessive sweating**	1.0 (0.0-5.0)	0.0 (0.0-3.0)	0.0 (0.0-1.0)	1.0 (0.0-4.0)
PASQ **joint pain**	4.0 (0.0-6.0)	3.0 (0.0-8.0)	2.5 (0.0-8.0)	3.0 (0.0-7.0)

Table 2. (continued)

	Baseline	PEG-V	After wash-out	Placebo
PASQ **fatigue**	3.5 (1.0-6.0)	4.0 (0.0-6.0)	2.0 (0.0-6.0)	2.5 (1.0-6.0)
PASQ **soft tissue swelling**	2.0 (1.0-3.0)	0.5 (0.0-2.0)	0.5 (0.0-3.0)	0.5 (0.0-3.0)
PASQ **numbness or tingling**	1.5 (0.0-5.0)	1.0 (0.0-1.0)	1.0 (0.0-1.0)	0.5 (0.0-3.0)
PASQ **overall health status**	3.0 (2.0-7.0)	1.5 (0.0-6.0)	1.5 (0.0-6.0)	3.0 (1.0-6.0)
2B	**Baseline (median (range))**	**Placebo (median (range))**	**After wash-out**	**PEG-V (median (range))**
Placebo PEG-V group				
Z-score of IGF1	1.66 (0.82-2.03)	1.52 (0.80-1.92)	1.57 (0.89-1.86)	1.67 (1.26-1.93)
Body weight (Kg)	96.8 (69.7-115.8)	95.5 (70.3-117.3)	95.4 (70.4-115.8)	93.7 (69.4-115.1)
AcroQol **global** (%)	52.9 (22.7-93.2)	57.4 (22.7-96.6)	54.0 (23.9-95.5)	59.4 (28.4-96.7)
AcroQol **physical** (%)	43.9 (18.8-87.5)	40.7 (18.8-90.6)	43.9 (15.6-90.6)	45.3 (34.4-94.4)
AcroQol **psychological** (%)	60.7 (21.5-98.2)	69.6 (21.4-100.0)	64.3 (25.0-98.2)	68.8 (25.0-98.0)
AcroQol **pers, relation** (%)	71.4 (25.0-100.0)	66.1 (25.0-100.0)	64.3 (25.0-100.0)	71.1 (25.0-100.0)
AcroQol **appearance** (%)	55.4 (17.9-96.4)	64.3 (17.9-100.0)	55.3 (25.0-96.4)	64.3 (25.0-96.6)
PASQ	23.5 (9.0-37.0)	26.5 (3.0-39.0)	25.0 (4.0-40.0)	22.0 (5.0-39.0)
PASQ **headache**	5.0 (0.0-6.0)	3.5 (0.0-7.0)	5.0 (0.0-7.0)	3.0 (0.0-7.0)
PASQ **excessive sweating**	4.5 (0.0-6.0)	4.5 (0.0-7.0)	3.5 (0.0-7.0)	4.0 (0.0-7.0)
PASQ **joint pain**	4.5 (2.0-7.0)	5.0 (0.0-8.0)	5.0 (2.0-8.0)	4.5 (0.0-8.0)
PASQ **fatigue**	4.5 (2.0-8.0)	5.5 (1.0-7.0)	5.0 (0.0-8.0)	5.0 (0.0-7.0)
PASQ **soft tissue swelling**	1.5 (0.0-6.0)	3.0 (0.0-5.0)	2.5 (0.0-7.0)	3.0 (0.0-5.0)
PASQ **numbness or tingling**	3.5 (0.0-8.0)	4.0 (0.0-7.0)	3.5 (0.0-7.0)	3.5 (0.0-7.0)
PASQ **overall health status**	4.5 (1.0-7.0)	4.5 (1.0-8.0)	4.5 (1.0-8.0)	4.5 (0.0-7.0)

All value are expressed as (median (range)); IGF1 = insulin-like growth factor-I; PEG-V = pegvisomant; PASQ = Patient-assessed; AcroQol = Acromegaly Quality of Life Questionnaire

AcroQoL

The AcroQoL improved significantly after the addition of PEG-V (PEG-V 6.4 ± 4.25%, placebo –1.1 ± 7.12%, P = 0.008, Fig. 1 and Table 2). PEG-V increased QoL in the AcroQoL physical (PEG-V 8.0 ± 7.88%, placebo 0.0 ± 6.25%, P = 0.002, Fig. 1 and Table 2). However, the AcroQoL psychological (PEG-V 3.6 ± 6.09%, placebo –0.9 ± 9.36%, P = 0.185), Acro-QoL appearance (PEG-V 4.0 ± 7.97%, placebo –2.0 ± 12.09%, P = 0.409), and personal relations (PEG-V 0.0 ± 6.14%, placebo –4.0 ± 9.66%, P = 0.109) tended to increase with the addition of PEG-V but failed to reach significance.

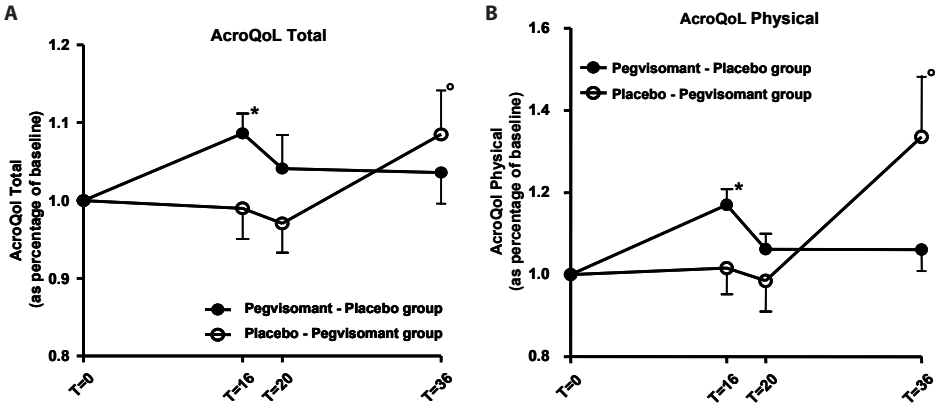

Figure 1: A, Changes in AcroQoL induced by the addition of 40 mg PEG-V once weekly in two different groups of acromegalic patients (PEG-V-placebo and placebo-PEG-V) with normal IGF1 during long-term treatment with SRIF analogs. *, $P = 0.013$ for T_0 *vs.* T_{16}; °, $P = 0.001$ for T_{20} *vs.* T_{36}. B, Changes in AcroQol physical after the addition of placebo or PEG-V for the two different groups PEG-V-placebo and placebo-PEG-V. *, $P = 0.006$ for T_0 *vs.* T_{16}; °, $P = 0.019$ for T_{20} *vs.* T_{36}. The mean and SEM values are given for each phase of the study. The baseline AcroQoL score is expressed as 1 and the other intervals (T 16, T 20, and T 36 wks) as ratio of baseline QoL.

PASQ

The PASQ changed significantly (P = 0.038) after cotreatment with PEG-V (PEG-V –2.0 ± 6.60, placebo 1.5 ± 5.02, Fig. 2). In the six questions of the PASQ addressing the different symptoms, a significant decrease in signs and symptoms was observed in the questions soft tissue swelling (PEG-V –0.5 ± 1.37, placebo 0.0 ± 1.28, P = 0.024), excessive sweating (PEG-V 0.0 ± 1.79, placebo 0.5 ± 0.98, P = 0.036), and overall health status (PEG-V –1.0 ± 1.99, placebo 0.5 ± 1.36, P = 0.035, Fig. 2) when PEG-V was added. The joint pain did not improve significantly (PEG-V –1.0 ± 1.47, placebo 0.0 ± 1.49, P = 0.083). In the other parameters, headache (P = 0.899), fatigue (P = 0.662), or numbness or tingling of the extremities (P = 0.175), no significant improvement was observed.

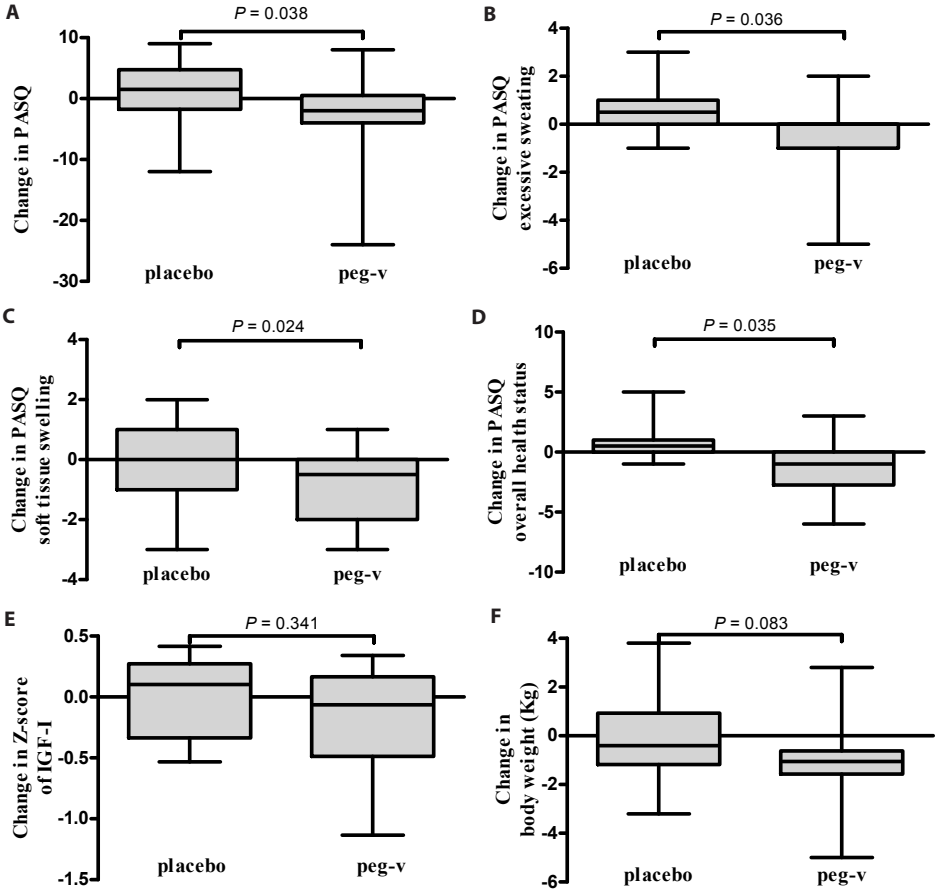

Figure 2: Change in PASQ and metabolic parameters induced by the addition of 40 mg PEG-V once weekly in two different groups of acromegalic patients (PEG-V-placebo and placebo-PEG-V) with normal IGF1 during long-term treatment with SRIF analogs. A, Total PASQ score. B, PASQ subscore excessive sweating. C, PASQ subscore soft tissue swelling. D, PASQ overall health status. E, Change in Z-score of IGF1 induced by the addition of 40 mg PEG-V once weekly in two different groups of acromegalic patients (PEG-V-placebo and placebo-PEG-V) with normal IGF1 during long-term treatment with SRIF analogs. F, Change in body weight induced by the addition of 40 mg PEG-V once weekly in two different groups of acromegalic patients (PEG-V-placebo and placebo-PEG-V) with normal IGF1 during long-term treatment with somatostatin analogs. Box whisker plots are expressed in minimum, median, and maximum.

Metabolic parameters

The combined treatment of PEG-V and SRIF analog did not result in a significant change the Z-score of IGF1 (PEG-V –0.064 ± 0.380, placebo 0.102 ± 0. 317, P = 0.341) or in absolute IGF1 concentration (P = 0.444, Fig. 2). The insulin-dependent metabolic parameters as homeostasis model assessment insulin resistance (P = 0.808, Fig. 3C), glycosylated hemoglobin (P = 0.241), and fasting glucose (P = 0.955) did not change significantly either. During PEG-V treatment, total cholesterol (TC) and low-density lipoprotein tended

to decrease; however, these changes were not significant; TC PEG-V was –0.35 ± 1.04 (median ± SD) and TC placebo was 0.10 ± 1.12 (P = 0.091). The low-density lipoprotein PEG-V was –0.19 ± 0.72 and placebo 0.20 ± 0.75 (P = 0.055). In the other lipids (P > 0.05) and the free fatty acids (P = 0.231), no change was observed. We observed no decrease in body weight in the whole group of subjects treated with PEG-V and SRIF analog (PEG-V –0.95 ± 1.70, placebo –0.40 ± 1.60, P = 0.081, Fig. 2), although some individuals showed a remarkable decrease in their body weight during their treatment period with both SRIF analog and PEG-V.

Correlations

The correlations between the parameters of QoL and biochemical and phenotypical parameters are presented in Fig. 3. Neither the change in the score of IGF1 nor the baseline GH levels correlated with changes in QoL. Changes in body weight correlated well with the observed improvements in QoL by the AcroQoL physical (r = –0.449, P = 0.047, Fig. 3) and the PASQ joint pain (r = 0.489, P = 0.029), excessive sweating, and soft tissue swelling, which are all QoL entities that reflect increased GH actions.

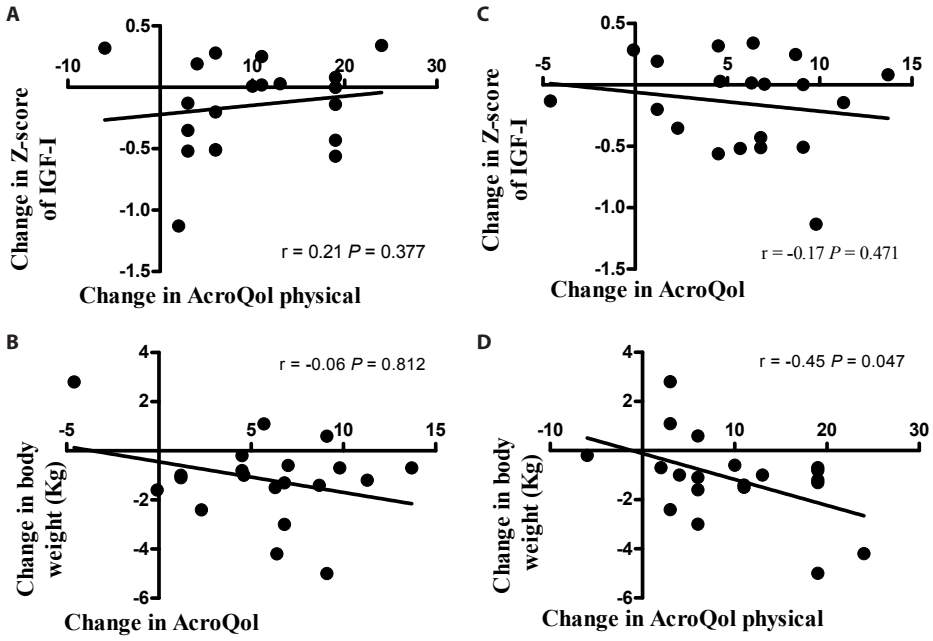

Figure 3: Correlation between the change in AcroQoL and the Z-score of IGF1 (A) and change in body weight (B). Correlation between the change in AcroQoL, category physical (AcroQoL physical) and the Z-score of IGF1 (C) and change in body weight (D).

Safety

Transient elevated liver enzyme tests (TLETs) were observed in five patients (25%) but did not necessitate discontinuation of PEG-V treatment. In one patient TLETs up to 9 times the ULN were observed during her last visit of the PEG-V treatment period. Because it was at the end of the treatment period, she was not withdrawn from the study. TLETs up to a maximum of 4 ULN were observed in another four subjects. The duration of TLETs was 8.0 wks median with a SD of ± 5.4. Of these five patients with TLETs, two patients also suffered from diabetes mellitus (40%). As expected, no change in pituitary volume was observed in any of the subjects.

DISCUSSION

Our study has two important messages. The first is that a significant improvement in QoL can be observed without a significant decrease in IGF1 after the addition of 40 mg PEG-V weekly in acromegalic patients with IGF1 concentrations within the age-adjusted range during SRIF analog treatment. This observation questions the current strategy in which PEG-V is used only as monotherapy or in combination with SRIF analog after IGF1 failed to normalize during monotherapy with long-acting SRIF analogs. It is noteworthy that the magnitude of the improvement in AcroQoL-score of 6.4% in this study is equal to the observations of Paisley et al. [21] [6.8% (–11.4 to 26.1)]. In their study, this change in QoL was observed when elevated IGF1 was reduced to the age adjusted normal range.

Although in some individuals IGF1 levels clearly decreased during PEG-V cotreatment, for the whole group, IGF1 did not decrease significantly. This observation might be explained by an observation by Segev et al. [22], who reported that a GH receptor antagonist in rodents was able to block GH actions, in their case in the kidney at lower concentrations than were necessary to decrease serum IGF1 and somatic growth. However, our study was powered to detect a difference in PASQ score and not designed to detect a difference in IGF1. Therefore, it is possible that studies in larger populations will observe a significant decrease.

The current consensus on the goals of treatment of acromegaly has focused on normalization of IGF1 and GH and thereby reducing long-term morbidity and mortality [23-24]. However, the normalization of levels of total serum IGF1 and GH do not necessarily reflect optimal QoL in acromegalic patients [10-12,9,13]. The second important message of our study is that total serum IGF1 levels, assessed by the commercially available IGF1 assays, do not correlate well enough with the QoL of the patient to use them for defining proper biochemical control.

In our study the improvement of QoL correlated with other GH-dependent parameters such as loss of body weight, perspiration, soft tissue swelling, and the AcroQoL

physical, strongly suggesting that in these patients, integrated GH action is too high, despite normal IGF1 levels. GH action is known to increase extracellular volume [25]. GH activates the renin-angiotensin-aldosterone system, which leads to fluid retention when GH concentrations are high [26-27]. These observations could explain the changes we observed in GH dependent parameters. We observed a significant improvement in the physical dimension of the AcroQoL and not in the dimension appearance, which is mostly affected in the chronic phase of acromegaly. This might be explained by the short duration (16 wks) of coadministration of PEG-V, resulting in an acute change that is perceived physically but not as a change in appearance.

The mode of action of SRIF analogs might also explain why the addition of PEG-V improves QoL. The effects of SRIF analog therapy on GH actions are not only mediated by the reduction of pathological GH secretion by the pituitary adenoma, but SRIF analogs also reduces insulin secretion in the portal vein. This mechanism will most likely reduce the available GH receptors on the liver [15]. Finally, SRIF analogs are able to directly reduce IGF1 production by hepatocytes [16]. These mechanisms are the basis of the concept that the combined use of SRIF analogs and pegvisomant should be able to reduce the dosage and frequency of pegvisomant administration, and indeed they do [1]. These mechanisms also suggest that whereas the liver is made relatively GH resistant, the rest of the body is still slightly acromegalic during SRIF analog treatment. One might then expect that the treatment of this extrahepatic acromegaly with low-dose weekly PEG-V administration would therefore improve the GH-dependent signs and symptoms and QoL, and indeed this was observed. The dose of 40 mg of PEG-V per week was pragmatically chosen as being about half the starting dose of PEG-V monotherapy, and the optimal dose has not been determined by this study. Nor do we know whether a further increase in QoL can be achieved by another dose of PEG-V that would decrease serum IGF1 levels.

We believe that our data might be important for treatment of acromegaly when medical treatment is concerned. The available consensus statements aim at just normalizing IGF1 to within the age-adjusted normal range [23-24]. They more or less ignore signs and symptoms of patients the moment IGF1 has become normal. However, from a patient's perspective, just normalization of serum IGF1 levels might not be enough. Our data suggest that most patients seem to know exactly what optimal treatment is, and they favor a combined approach by which both SRIF analogs and PEG-V play their specific roles. This suggests that we should abandon the step-up approach and that we should investigate the role of the combined approach in larger series of patients. Apart from the statistical outcome of the study, what really impressed us was the fact that 80% of subjects knew in retrospect which of the two treatment periods was the one in which they had received PEG-V, which is striking in a double-blind study design. They not only recognized the presence of PEG-V but also insisted in getting it back the moment the study was finished.

TLETs were observed in five patients (25%) of which two subjects also suffered from diabetes. Furthermore, as expected in this short study of 4 months of PEG-V exposure, no change in pituitary adenoma size was observed. No change in overall insulin sensitivity or change in lipids in the whole study population was observed. However, this could be due to a type 2 error or to our short treatment period of 16 wks because others have observed improvement [28]. One patient had to reduce her oral diabetic medication due to frequent hypoglycemic episodes during PEG-V treatment period. Five weeks after withdrawal of PEG-V, the dose of diabetic medication had to be increased again.

It is internationally accepted that in most studies, a 2-wks period between test and retest is enough to avoid for the memory effect [29-30]. In our study we assessed QoL during a 4-month period so we think that memory could not have any impact on the scores.

In conclusion, QoL in acromegalic patients who normalized serum IGF1 concentrations during long-term treatment with long-acting SRIF analogs can be significantly improved by the addition of a weekly dose of 40 mg pegvisomant. This improvement is not accompanied by a significant decrease in serum IGF1 levels, which questions the importance of total serum IGF1 as a reliable parameter for QoL from a patient's perspective. Our data also question the step-up approach in which patients are treated only with pegvisomant when somatostatin analog monotherapy was not able to normalize IGF1 levels. These findings warrant further investigation on the efficacy and safety of adding pegvisomant to SRIF analog therapy in most acromegalic patients.

REFERENCES

1. Feenstra J, de Herder WW, ten Have SM, van den Beld AW, Feelders RA, Janssen JA, van der Lely AJ (2005) Combined therapy with somatostatin analogues and weekly pegvisomant in active acromegaly. Lancet 365 (9471):1644-1646
2. Neggers SJ, van Aken MO, Janssen JA, Feelders RA, de Herder WW, van der Lely AJ (2007) Long-term efficacy and safety of combined treatment of somatostatin analogs and pegvisomant in acromegaly. The Journal of clinical endocrinology and metabolism 92 (12):4598-4601
3. Trainer PJ, Drake WM, Katznelson L, Freda PU, Herman-Bonert V, van der Lely AJ, Dimaraki EV, Stewart PM, Friend KE, Vance ML, Besser GM, Scarlett JA, Thorner MO, Parkinson C, Klibanski A, Powell JS, Barkan AL, Sheppard MC, Malsonado M, Rose DR, Clemmons DR, Johannsson G, Bengtsson BA, Stavrou S, Kleinberg DL, Cook DM, Phillips LS, Bidlingmaier M, Strasburger CJ, Hackett S, Zib K, Bennett WF, Davis RJ (2000) Treatment of acromegaly with the growth hormone-receptor antagonist pegvisomant. The New England journal of medicine 342 (16):1171-1177
4. van der Lely AJ, Hutson RK, Trainer PJ, Besser GM, Barkan AL, Katznelson L, Klibanski A, Herman-Bonert V, Melmed S, Vance ML, Freda PU, Stewart PM, Friend KE, Clemmons DR, Johannsson G, Stavrou S, Cook DM, Phillips LS, Strasburger CJ, Hackett S, Zib KA, Davis RJ, Scarlett JA, Thorner MO (2001) Long-term treatment of acromegaly with pegvisomant, a growth hormone receptor antagonist. Lancet 358 (9295):1754-1759
5. Beauregard C, Truong U, Hardy J, Serri O (2003) Long-term outcome and mortality after transsphenoidal adenomectomy for acromegaly. Clinical endocrinology 58 (1):86-91
6. Holdaway IM, Rajasoorya CR, Gamble GD, Stewart AW (2003) Long-term treatment outcome in acromegaly. Growth Horm IGF Res 13 (4):185-192. doi:S1096637403000303 [pii]
7. Holdaway IM, Rajasoorya RC, Gamble GD (2004) Factors influencing mortality in acromegaly. The Journal of clinical endocrinology and metabolism 89 (2):667-674
8. Swearingen B, Barker FG, 2nd, Katznelson L, Biller BM, Grinspoon S, Klibanski A, Moayeri N, Black PM, Zervas NT (1998) Long-term mortality after transsphenoidal surgery and adjunctive therapy for acromegaly. The Journal of clinical endocrinology and metabolism 83 (10):3419-3426
9. Hua SC, Yan YH, Chang TC (2006) Associations of remission status and lanreotide treatment with quality of life in patients with treated acromegaly. European journal of endocrinology / European Federation of Endocrine Societies 155 (6):831-837
10. Biermasz NR, Dekker FW, Pereira AM, van Thiel SW, Schutte PJ, van Dulken H, Romijn JA, Roelfsema F (2004) Determinants of survival in treated acromegaly in a single center: predictive value of serial insulin-like growth factor I measurements. The Journal of clinical endocrinology and metabolism 89 (6):2789-2796
11. Biermasz NR, Pereira AM, Smit JW, Romijn JA, Roelfsema F (2005) Morbidity after long-term remission for acromegaly: persisting joint-related complaints cause reduced quality of life. The Journal of clinical endocrinology and metabolism 90 (5):2731-2739
12. Bonapart IE, van Domburg R, ten Have SM, de Herder WW, Erdman RA, Janssen JA, van der Lely AJ (2005) The 'bio-assay' quality of life might be a better marker of disease activity in acromegalic patients than serum total IGF-I concentrations. European journal of endocrinology / European Federation of Endocrine Societies 152 (2):217-224
13. Rowles SV, Prieto L, Badia X, Shalet SM, Webb SM, Trainer PJ (2005) Quality of life (QOL) in patients with acromegaly is severely impaired: use of a novel measure of QOL: acromegaly quality of life questionnaire. The Journal of clinical endocrinology and metabolism 90 (6):3337-3341

14. Webb SM, Prieto L, Badia X, Albareda M, Catala M, Gaztambide S, Lucas T, Paramo C, Pico A, Lucas A, Halperin I, Obiols G, Astorga R (2002) Acromegaly Quality of Life Questionnaire (ACROQOL) a new health-related quality of life questionnaire for patients with acromegaly: development and psychometric properties. Clinical endocrinology 57 (2):251-258
15. Leung KC, Doyle N, Ballesteros M, Waters MJ, Ho KK (2000) Insulin regulation of human hepatic growth hormone receptors: divergent effects on biosynthesis and surface translocation. The Journal of clinical endocrinology and metabolism 85 (12):4712-4720
16. Murray RD, Kim K, Ren SG, Chelly M, Umehara Y, Melmed S (2004) Central and peripheral actions of somatostatin on the growth hormone-IGF-I axis. The Journal of clinical investigation 114 (3): 349-356
17. Elmlinger MW, Kuhnel W, Weber MM, Ranke MB (2004) Reference ranges for two automated chemiluminescent assays for serum insulin-like growth factor I (IGF-I) and IGF-binding protein 3 (IGFBP-3). Clin Chem Lab Med 42 (6):654-664
18. Granada ML, Ulied A, Casanueva FF, Pico A, Lucas T, Torres E, Sanmarti A (2008) Serum IGF-I measured by four different immunoassays in patients with adult GH deficiency or acromegaly and in a control population. Clinical endocrinology 68 (6):942-950
19. Badia X, Webb SM, Prieto L, Lara N (2004) Acromegaly Quality of Life Questionnaire (AcroQoL). Health Qual Life Outcomes 2:13. doi:10.1186/1477-7525-2-13 1477-7525-2-13 [pii]
20. Webb SM (2006) Quality of life in acromegaly. Neuroendocrinology 83 (3-4):224-229
21. Paisley AN, Rowles SV, Roberts ME, Webb SM, Badia X, Prieto L, Shalet SM, Trainer PJ (2007) Treatment of acromegaly improves quality of life, measured by AcroQol. Clinical endocrinology 67 (3): 358-362
22. Segev Y, Landau D, Rasch R, Flyvbjerg A, Phillip M (1999) Growth hormone receptor antagonism prevents early renal changes in nonobese diabetic mice. J Am Soc Nephrol 10 (11):2374-2381
23. Biochemical assessment and long-term monitoring in patients with acromegaly: statement from a joint consensus conference of the Growth Hormone Research Society and the Pituitary Society (2004). The Journal of clinical endocrinology and metabolism 89 (7):3099-3102
24. Giustina A, Barkan A, Casanueva FF, Cavagnini F, Frohman L, Ho K, Veldhuis J, Wass J, Von Werder K, Melmed S (2000) Criteria for cure of acromegaly: a consensus statement. The Journal of clinical endocrinology and metabolism 85 (2):526-529
25. Bengtsson BA, Brummer RJ, Eden S, Bosaeus I, Lindstedt G (1989) Body composition in acromegaly: the effect of treatment. Clinical endocrinology 31 (4):481-490
26. Hansen TK, Moller J, Thomsen K, Frandsen E, Dall R, Jorgensen JO, Christiansen JS (2001) Effects of growth hormone on renal tubular handling of sodium in healthy humans. American journal of physiology 281 (6):E1326-1332
27. Moller J, Nielsen S, Hansen TK (1999) Growth hormone and fluid retention. Hormone research 51 Suppl 3:116-120
28. Jorgensen JO, Feldt-Rasmussen U, Frystyk J, Chen JW, Kristensen LO, Hagen C, Orskov H (2005) Cotreatment of acromegaly with a somatostatin analog and a growth hormone receptor antagonist. The Journal of clinical endocrinology and metabolism 90 (10):5627-5631
29. Keenan AM, McKenna SP, Doward LC, Conaghan PG, Emery P, Tennant A (2008) Development and validation of a needs-based quality of life instrument for osteoarthritis. Arthritis and rheumatism 59 (6):841-848
30. Linde L, Sorensen J, Ostergaard M, Horslev-Petersen K, Hetland ML (2008) Health-related quality of life: validity, reliability, and responsiveness of SF-36, 15D, EQ-5D [corrected] RAQoL, and HAQ in patients with rheumatoid arthritis. The Journal of rheumatology 35 (8):1528-1537

Chapter 6

Growth Hormone Receptor mRNA expression and the effect of Pegvisomant on Growth Hormone secretion by Somatotroph Pituitary adenomas

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ABSTRACT

Background: Of the currently available treatment regimes for acromegaly, pegvisomant (PEG-V) has the highest efficacy. During PEG-V treatment, growth hormone (GH) serum levels increase. The exact mechanism behind this is remains unclear. It could be explained by an ultra-short feedback loop via GH receptors (GHR) in the anterior pituitary gland, but than GHR should be present in the adenoma

Objective: To assess the level of GHR mRNA expression in somatotroph adenomas and to evaluate whether GHR blockade by PEG-V can lead to an increase in GH levels.

Design: In 32 somatotroph adenomas and 4 samples of human liver tissue, after RNA extraction, mRNA expression of the full length GHR and d3GHR variant were assessed by real time PCR. After 24 and 72 h incubation with PEG-V, in different concentration (10^{-6}-10^{-9}M), GH secretion was assessed in three primary GH-secreting cultures. With a two-site immunofluorometric assay having no cross-reaction with PEG-V, GH concentrations were determined.

Results: In somatotroph adenomas, detectable levels of full length GHR and the d3GHR variant were found in 27 of 32 (84%) and in 23 of 32 (72%) cases respectively. GHR mRNA levels, full length and d3GHR, in the somatotroph adenomas amounted 0.8 and 4.8% of the expression level in human liver. The addition of PEG-V did not increase GH secretion by the cultured GH-secreting pituitary adenoma cells.

Conclusion: In human somatotroph pituitary adenomas the expression of GHR mRNA is low and PEG-V does not increase GH secretion in vitro. There was no relationship between expression of GHR in the adenoma and the effect of PEG-V. Therefore, the increase in GH level during PEG-V treatment in acromegaly patients does not seems to be mediated by interference of PEG-V with an ultra-short feedback loop via GHR in the somatotroph adenoma, but possible via reduced feedback due to the lowered circulating IGF1 levels.

INTRODUCTION

Acromegaly is a rare disease that is caused, in at least 95% of cases by a benign, growth-hormone (GH) secreting pituitary tumor [1]. More than 75% of these pituitary tumors are macro-adenomas, which often extent dorsally of the suprasellar region or laterally to the cavernous sinus [1]. The elevated levels of GH [2-4] and insulin-like growth factor 1 (IGF1) [5-7] seem to be the most important factors that determine reduced life expectancy, in this disease. By normalizing these parameters the life expectancy can return to normal [8]. The most effective available treatment modalities to achieve these goals are surgery and medical therapy. Medical therapy with somatostatin analogs (SRIF) normalizes IGF1 and growth hormone levels in about 50% of patients [9]. Pharmacotherapy with SRIF analogs have greater efficacy than surgical treatment in patients with macro-adenomas. However, compared with pegvisomant (PEG-V) therapy, SRIF analog therapy and surgery have lower efficacies. Treatment with PEG-V, either as a monotherapy or in combination with a SRIF, is effective in more than 90% of cases. During combined treatment a reduced cumulative dose of PEG-V seems to be necessary to achieve a normal IGF1 [10-14].

In patients circulating GH levels increase during PEG-V treatment, but when PEG-V is combined with a SRIF analogs, elevations in the serum concentration of GH are lower than they are observed during PEG-V monotherapy [10,15]. The increase in GH during PEG-V could possibility be explained by a blockade of the peripheral GH receptor (GHR) in the liver that leads to a reduction in IGF1 thereby reducing the negative feedback of IGF1 on the hypothalamus and pituitary gland [10,16]. By disrupting this regulatory mechanism of the GH/IGF1 feedback, a potential tumor growth might occur [17,10].

Different areas in the brain, as well as the pituitary, express GHR, as assessed by binding studies [18] and GHR m-RNA analysis [19]. PEG-V seems to be incapable of penetrating the blood brain barrier [20], making an effect of PEG-V via the central nervous system unlikely. However, the pituitary gland is outside the blood brain barrier, which means that PEG-V should be able to reach the pituitary. GH itself could influence the pituitary via an ultra short feedback loop as shown for thyroid stimulating hormone (TSH) [21]. Via a similar mechanism PEG-V blockade of the pituitary GHR could increase GH production further and influence tumor growth. In this study we therefore assessed mRNA expression of the GHR in somatotroph adenomas and the effect of PEG-V on GH secretion by somatotroph adenomas in vitro.

MATERIAL AND METHODS

Patients

Pituitary adenoma tissue samples were directly used for cell culture. A representative piece of tissue was snap frozen on dry ice and used for RNA isolation. The samples derived from thirty-two patients with clinically proven somatotroph adenomas and were obtained from surgical specimens after transsphenoidal surgery between 2009 and 2010 at the Department of Neurosurgery, Erasmus University Medical Centre Rotterdam. At immunohistochemical examination all these adenomas were positive for GH. As a reference for GHR expression, four hepatic tissue specimens were obtained for the Department of Pathology Erasmus University Medical Centre Rotterdam. All patients gave their informed consent to use the adenoma tissue for research purposes.

Cell dispersion and cell culture

Single-cell suspensions of the pituitary adenoma tissues were prepared by enzymatic dissociation with dispase as described in detail previously [22]. For short-term incubation of monolayer cultures, the dissociated cells were plated in 48-well plates (Corning, Cambridge, MA) at a density of 10^5 cells per well ^{in} 1 ml culture medium. After 3–4 d, the medium was refreshed and 24 to 72-h incubations without or with PEG-V (10^{-6},10^{-7},10^{-8},10^{-9}M) were initiated in quadruplicate. At the end of the incubation, the medium was removed and stored at –20°C until analysis of the GH concentrations.

The cells were cultured at 37°C in a CO_2 incubator with humidified air. The culture medium consisted of MEM supplemented with nonessential amino acids, sodium pyruvate (1 mmol/liter), 10% fetal calf serum, penicillin (1 x 10^5 U/liter), fungizone (0.5 mg/liter), L-glutamine (2 mmol/liter), and sodium bicarbonate (2.2 g/liter, pH 7.6). Media and supplements were obtained from Gibco Bio-cult Europe (Invitrogen, Breda, The Netherlands).

Quantitative PCR

Total RNA was isolated using a commercially available kit (high pure RNA isolation kit; Roche, Almere, The Netherlands). cDNA synthesis and quantitative PCR using the Taqman Gold Nuclease assay and the ABI PRISM 7900 sequence detection system (Perkin Elmer Applied Biosystems, Groningen, The Netherlands) were performed as described in detail previously [23]. The primers and probes sequences were obtained from Sigma-Aldrich (St. Louis, USA). The primer sequences were as follows for GHR full length; forward: 5'-ATCTTTGGAATATTTGGGCTAACAGT-3', reverse: 5'-GGATCGATTCCTTTAATCTTTGGA-3', probe 5'-fam-aggattaaaatgctgattctgcccccag-TAMRA-3' and for d3GHR variant; forward: 5'-TCTTTGGAATATTTGGGCTAACAGT-3', reverse: 5'-AGCTATCATGAATGGCTAAGATTGTG-3', probe: 5'-FAM-TAAACAGCAAAGGAAGGAAAATTAGAGGAGGTGA-TAMRA-3'. Dilution curves were

constructed for calculating the PCR efficiency for every primer set. Efficiency values were similar for GHR full length and the d3GHR variant. The estimated copy numbers were calculated using the comparative threshold method with efficiency correction [24].The detection of hypoxanthine-phosphoribosyl-transferase (hprt) mRNA served as a control and was used for normalization of the d3GHR and full length GHR mRNA levels. Primers and probes to detect hprt have been described previously [25]. To exclude contamination of the PCR mixtures, the reactions were also performed in the absence of cDNA template, in parallel with cDNA samples.

Growth Hormone assay

GH concentrations were assessed by a PEG-V-insensitive two-site competitive monoclonal immunofluorometric assay as described before [26]. To assess PEG-V and GH in supernatants of the pituitary adenoma cell cultures, the assay was slightly modified. Cell culture medium was used as the matrix for the calibrators. All samples were analyzed in a single run. Inter- and intra-assay coefficients of variation were 8.7% and 6.5%.

Statistics

All data on m-RNA levels are expressed as mean ±SE or as percentage of the mean m-RNA level of the liver. The groups were compared by Mann-Whitney test or when data was paired by Wilcoxon matched pairs test. The GH levels were analyzed by ANOVA for repeated measurements. Analysis was performed by GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California USA. Statistical significance was accepted at $P<0.05$.

RESULTS

In the somatotroph adenomas, GHR full length mRNA and d3GHR were detectable 27 of 32 (84%) and in 23 of 32 (7%) cases respectively. Full length and d3GHR mRNA expression levels were 0.036 ±0.006 and 0.018 ±0.005 (ratio/hprt), respectively (fig.1). The full length and d3GHR mRNA expression levels of the liver were 4.274 ±2.434 and 0.373 ±0.193 (ratio/hprt) respectively (fig.1). Compared to total GHR expression, expression of full length vs. d3GHR in the somatotroph adenomas was 67% vs. 33%. For the liver this ratio was 92% vs. 8%. mRNA expression of GHR full length in somatotroph adenomas, as the percentage of the liver expression amounted 0.8%. The mRNA expression of the d3GHR variant amounted 4.8% of the expression level detected in human liver tissue. The overall mRNA expression of the GHR in the somatotroph adenomas was only 1.2% compared to the liver expression level.

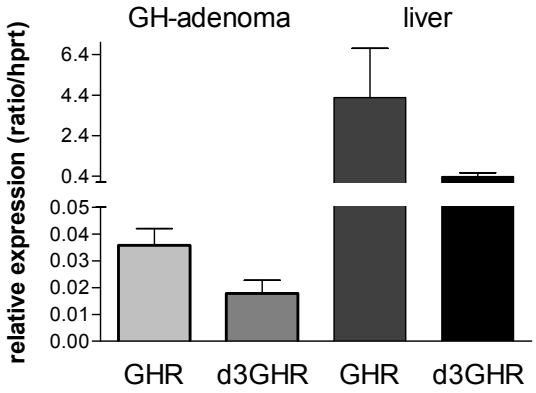

Figure 1: Comparison between mRNA expression levels of the full length GHR and the d3GHR variant, as determined by quantitative PCR in GH-secreting pituitary adenomas and liver tissue. All values are expressed as mean ± SE (Standard Error). Expression levels are normalized to the expression levels of hprt.

Three somatotroph adenoma cultures were incubated for 24 h and 72 h with PEG-V in various concentrations (control,10^{-6},10^{-7},10^{-8},10^{-9}M). After 24 h and 72 h incubation with PEG-V, GH secretion did not change significantly at any of the PEG-V concentrations tested (fig. 2). The lack of responsiveness appeared not to be related to the absence of GHR expression, except for one of the adenomas. Expression levels (ratio/hprt) of the adenomas of patients 1, 2 and 3 were 0.0120, undetectable and 0.039 for GHR full length and 0.0020, undetectable and 0.018 for d3GHR, respectively.

DISCUSSION

In the present study low mRNA expression of full length GHR and the d3GHR was found in 32 somatotroph pituitary adenomas. After the addition of PEG-V, no increase in GH concentration of supernatant was observed. Therefore, it seems very unlikely that the increase in GH during PEG-V treatment in humans is due to a blockade of an ultra-short feedback loop via GHR in the somatotroph pituitary adenomas by PEG-V.

The % of adenomas expressing GHR mRNA in our cohort of patients was comparable with that described by Beuschlein et al. [19]. However, they did not did not assess the relative mRNA expression to that of the human liver. Moreover, Kola et al. observed that GH-R mRNA expression levels in GH-secreting pituitary adenomas were lower compared those in normal pituitary [27].

A dose dependent increase in circulating GH levels during PEG-V treatment in acromegaly was already observed in the early clinical studies [10]. The increase in GH levels is probably not caused by an altered circulating halftime of GH and/or clearance during PEG-V treatment, but by a true increase in GH production [26]. Another possibil-

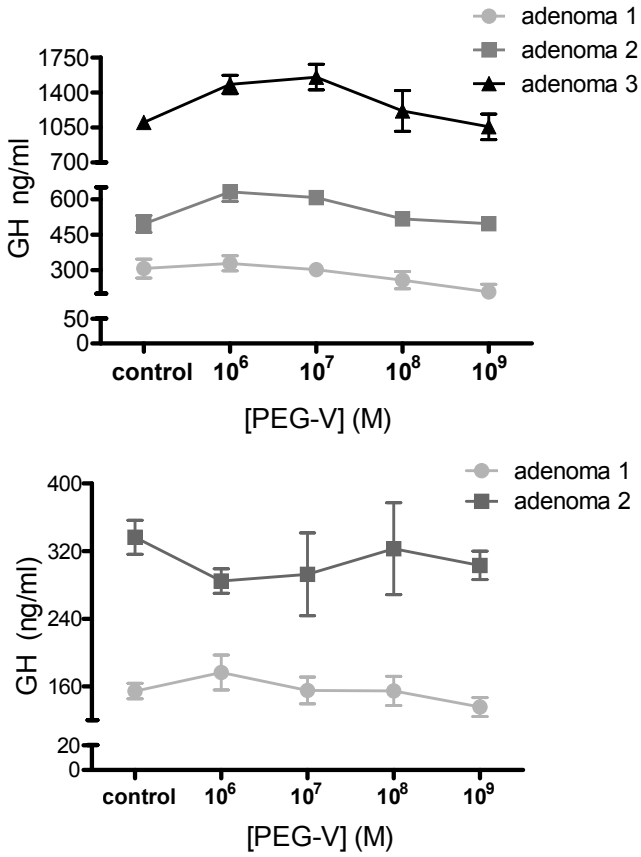

Figure 2: The effect of PEG-V at decreasing dose on GH secretion by cultured somatotroph adenomas. 2A: 24 hr incubation (adenoma 1 and 2) and 2B: 72 hr incubation (adenomas 1,2 and 3). All values are expressed as mean ± SE (Standard Error).

ity is that PEG-V blocks an ultra-short feedback loop as was observed with TSH on the pituitary [21]. Until now, this hypothesis has not been evaluated. However, since the mRNA levels of the GHR are very low compared with liver GHR expression level [19] and because PEG-V did not increase GH secretion in vitro by somatotroph adenomas, this suggests that modification of the putative ultra-short feedback loop is very unlikely, in these adenomas. In other parts of the brain GHR are present as well, such as in the hypothalamus, [18] which could also partially explain the increase in GH levels during PEG-V treatment in acromegaly. However, Veldhuis and co-workers recently assessed PEG-V in the Cerebrospinal fluid (CSF). The uptake of GH was 1500 fold higher than that of PEG-V[20]. Therefore, it seems very unlikely that a direct effect via the central nervous system could explain the increase in GH levels during PEG-V treatment.

As indicated above, the lack of increase in GH secretion in vitro seems most likely explained by the low number of GHR on the somatotroph adenomas. Although not

investigated, a dysfunctional GHR [28] on the somatotroph adenoma cannot be fully excluded. It would be of great scientific interest to assess if the apparent absence of an ultra-short loop feedback is also observed in primary normal human pituitary cell cultures.

To antagonize the GH effect in an acromegalic patient, an excess of PEG-V is required. The highest dose of PEG-V that we used in our in vitro experiments was more than 10 times the amount of GH produced by the adenoma cells. Even at this highest PEG-V dose, no effect of PEG-V on GH secretion by the somatotroph adenomas was observed. Therefore, the most rational explanation for the increase in GH level during PEG-V treatment in acromegalic patients is the decrease in IGF1 resulting in less feedback suppression at the adenoma and/or normal somatotrophs. IGF1 was previously shown to inhibit GH secretion by primary cultures of GH secreting pituitary adenomas, as well as in normal rat anterior pituitary cell cultures [29].

In conclusion, our study demonstrates that GHR mRNA levels in somatotroph adenomas are low and that the addition of PEG-V is not able to increase the GH production by the adenomas in vitro. Therefore, the increase in GH levels during PEG-V treatment in acromegalic patients does not seem be mediated by interference of PEG-V with an ultra-short feedback loop via GHR in the somatotroph adenoma, but possible via a reduced feedback due to the lowered circulating IGF1 levels.

REFERENCES

1. Melmed S (2006) Medical progress: Acromegaly. The New England journal of medicine 355 (24): 2558-2573
2. Orme SM, McNally RJ, Cartwright RA, Belchetz PE (1998) Mortality and cancer incidence in acromegaly: a retrospective cohort study. United Kingdom Acromegaly Study Group. The Journal of clinical endocrinology and metabolism 83 (8):2730-2734
3. Bates AS, Van't Hoff W, Jones JM, Clayton RN (1993) An audit of outcome of treatment in acromegaly. The Quarterly journal of medicine 86 (5):293-299
4. Ayuk J, Clayton RN, Holder G, Sheppard MC, Stewart PM, Bates AS (2004) Growth hormone and pituitary radiotherapy, but not serum insulin-like growth factor-I concentrations, predict excess mortality in patients with acromegaly. The Journal of clinical endocrinology and metabolism 89 (4):1613-1617
5. Swearingen B, Barker FG, 2nd, Katznelson L, Biller BM, Grinspoon S, Klibanski A, Moayeri N, Black PM, Zervas NT (1998) Long-term mortality after transsphenoidal surgery and adjunctive therapy for acromegaly. The Journal of clinical endocrinology and metabolism 83 (10):3419-3426
6. Holdaway IM, Rajasoorya RC, Gamble GD (2004) Factors influencing mortality in acromegaly. The Journal of clinical endocrinology and metabolism 89 (2):667-674
7. Biermasz NR, van Thiel SW, Pereira AM, Hoftijzer HC, van Hemert AM, Smit JW, Romijn JA, Roelfsema F (2004) Decreased quality of life in patients with acromegaly despite long-term cure of growth hormone excess. The Journal of clinical endocrinology and metabolism 89 (11):5369-5376
8. Holdaway IM, Bolland MJ, Gamble GD (2008) A meta-analysis of the effect of lowering serum levels of GH and IGF-I on mortality in acromegaly. European journal of endocrinology / European Federation of Endocrine Societies 159 (2):89-95
9. Holdaway IM (2004) Treatment of acromegaly. Hormone research 62 Suppl 3:79-92
10. van der Lely AJ, Hutson RK, Trainer PJ, Besser GM, Barkan AL, Katznelson L, Klibanski A, Herman-Bonert V, Melmed S, Vance ML, Freda PU, Stewart PM, Friend KE, Clemmons DR, Johannsson G, Stavrou S, Cook DM, Phillips LS, Strasburger CJ, Hackett S, Zib KA, Davis RJ, Scarlett JA, Thorner MO (2001) Long-term treatment of acromegaly with pegvisomant, a growth hormone receptor antagonist. Lancet 358 (9295):1754-1759
11. Neggers SJ, van Aken MO, Janssen JA, Feelders RA, de Herder WW, van der Lely AJ (2007) Long-term efficacy and safety of combined treatment of somatostatin analogs and pegvisomant in acromegaly. The Journal of clinical endocrinology and metabolism 92 (12):4598-4601
12. Neggers SJ, de Herder WW, Janssen JA, Feelders RA, van der Lely AJ (2009) Combined treatment for acromegaly with long-acting somatostatin analogs and pegvisomant: long-term safety for up to 4.5 years (median 2.2 years) of follow-up in 86 patients. European journal of endocrinology / European Federation of Endocrine Societies 160 (4):529-533
13. Higham CE, Chung TT, Lawrance J, Drake WM, Trainer PJ (2009) Long-term experience of pegvisomant therapy as a treatment for acromegaly. Clinical endocrinology 71 (1):86-91
14. Biering H, Saller B, Bauditz J, Pirlich M, Rudolph B, Johne A, Buchfelder M, Mann K, Droste M, Schreiber I, Lochs H, Strasburger CJ, German pegvisomant i (2006) Elevated transaminases during medical treatment of acromegaly: a review of the German pegvisomant surveillance experience and a report of a patient with histologically proven chronic mild active hepatitis. European journal of endocrinology / European Federation of Endocrine Societies 154 (2):213-220

15. Jorgensen JO, Feldt-Rasmussen U, Frystyk J, Chen JW, Kristensen LO, Hagen C, Orskov H (2005) Co-treatment of acromegaly with a somatostatin analog and a growth hormone receptor antagonist. The Journal of clinical endocrinology and metabolism 90 (10):5627-5631
16. Trainer PJ, Drake WM, Katznelson L, Freda PU, Herman-Bonert V, van der Lely AJ, Dimaraki EV, Stewart PM, Friend KE, Vance ML, Besser GM, Scarlett JA, Thorner MO, Parkinson C, Klibanski A, Powell JS, Barkan AL, Sheppard MC, Malsonado M, Rose DR, Clemmons DR, Johannsson G, Bengtsson BA, Stavrou S, Kleinberg DL, Cook DM, Phillips LS, Bidlingmaier M, Strasburger CJ, Hackett S, Zib K, Bennett WF, Davis RJ (2000) Treatment of acromegaly with the growth hormone-receptor antagonist pegvisomant. The New England journal of medicine 342 (16):1171-1177
17. van der Lely AJ, Muller A, Janssen JA, Davis RJ, Zib KA, Scarlett JA, Lamberts SW (2001) Control of tumor size and disease activity during cotreatment with octreotide and the growth hormone receptor antagonist pegvisomant in an acromegalic patient. The Journal of clinical endocrinology and metabolism 86 (2):478-481
18. Nyberg F (2000) Growth hormone in the brain: characteristics of specific brain targets for the hormone and their functional significance. Front Neuroendocrinol 21 (4):330-348. doi:10.1006/frne.2000.0200 S0091-3022(00)90200-0 [pii]
19. Beuschlein F, Hancke K, Petrick M, Gobel H, Honegger J, Reincke M (2005) Growth hormone receptor mRNA expression in non-functioning and somatotroph pituitary adenomas: implications for growth hormone substitution therapy? Exp Clin Endocrinol Diabetes 113 (4):214-218. doi: 10.1055/s-2005-837668
20. Veldhuis JD, Bidlingmaier M, Bailey J, Erickson D, Sandroni P (2010) A pegylated growth hormone receptor antagonist, pegvisomant, does not enter the brain in humans. The Journal of clinical endocrinology and metabolism 95 (8):3844-3847. doi:jc.2010-0538 [pii] 10.1210/jc.2010-0538
21. Brokken LJ, Scheenhart JW, Wiersinga WM, Prummel MF (2001) Suppression of serum TSH by Graves' Ig: evidence for a functional pituitary TSH receptor. The Journal of clinical endocrinology and metabolism 86 (10):4814-4817
22. Oosterom R, Blaauw G, Singh R, Verleun T, Lamberts SW (1984) Isolation of large numbers of dispersed human pituitary adenoma cells obtained by aspiration. J Endocrinol Invest 7 (4):307-311
23. Hofland LJ, van der Hoek J, Feelders R, van Aken MO, van Koetsveld PM, Waaijers M, Sprij-Mooij D, Bruns C, Weckbecker G, de Herder WW, Beckers A, Lamberts SW (2005) The multi-ligand somatostatin analogue SOM230 inhibits ACTH secretion by cultured human corticotroph adenomas via somatostatin receptor type 5. European journal of endocrinology / European Federation of Endocrine Societies 152 (4):645-654. doi:152/4/645 [pii] 10.1530/eje.1.01876
24. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25 (4):402-408. doi:10.1006/meth.2001.1262 S1046-2023(01)91262-9 [pii]
25. Hofland LJ, van der Hoek J, van Koetsveld PM, de Herder WW, Waaijers M, Sprij-Mooij D, Bruns C, Weckbecker G, Feelders R, van der Lely AJ, Beckers A, Lamberts SW (2004) The novel somatostatin analog SOM230 is a potent inhibitor of hormone release by growth hormone- and prolactin-secreting pituitary adenomas in vitro. The Journal of clinical endocrinology and metabolism 89 (4):1577-1585
26. Veldhuis JD, Bidlingmaier M, Anderson SM, Evans WS, Wu Z, Strasburger CJ (2002) Impact of experimental blockade of peripheral growth hormone (GH) receptors on the kinetics of endogenous and exogenous GH removal in healthy women and men. The Journal of clinical endocrinology and metabolism 87 (12):5737-5745

27. Kola B, Korbonits M, Diaz-Cano S, Kaltsas G, Morris DG, Jordan S, Metherell L, Powell M, Czirjak S, Arnaldi G, Bustin S, Boscaro M, Mantero F, Grossman AB (2003) Reduced expression of the growth hormone and type 1 insulin-like growth factor receptors in human somatotroph tumours and an analysis of possible mutations of the growth hormone receptor. Clinical endocrinology 59 (3): 328-338. doi:1851 [pii]
28. Asa SL, Digiovanni R, Jiang J, Ward ML, Loesch K, Yamada S, Sano T, Yoshimoto K, Frank SJ, Ezzat S (2007) A growth hormone receptor mutation impairs growth hormone autofeedback signaling in pituitary tumors. Cancer Res 67 (15):7505-7511. doi:67/15/7505 [pii] 10.1158/0008-5472.CAN-07-0219
29. Lamberts SW, van Koetsveld P, Hofland L (1989) A close correlation between the inhibitory effects of insulin-like growth factor-I and SMS 201-995 on growth hormone release by acromegalic pituitary tumours in vitro and in vivo. Clinical endocrinology 31 (4):401-410

Chapter 7

Hypothesis: Extra-hepatic acromegaly: a new paradigm?

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ABSTRACT

Medical treatment of acromegaly with long-acting somatostatin analogs (SRIF) and the GH receptor antagonist, pegvisomant (PEG-V), has made it possible to achieve normal serum IGF1 concentrations in a majority of patients with acromegaly. These two compounds, however, impact the GH–IGF1 axis differently, which challenges the traditional biochemical assessment of the therapeutic response. We postulate that long-acting SRIF analogs in certain patients normalizes serum IGF1 levels in the presence of elevated GH actions in extra-hepatic tissues. This may result in persistent disease activity for which we propose the term extra-hepatic acromegaly. PEG-V, on the other hand, blocks systemic GH actions, which are not necessarily reliably reflected by serum IGF1 levels, and this treatment causes a further elevation of serum GH levels. Medical treatment is therefore difficult to monitor with the traditional biomarkers. Moreover, the different modes of actions of SRIF analogs and PEG-V make it attractive to use the two drugs in combination. We believe that it is time to challenge the existing concepts of treatment and monitoring of patients with acromegaly.

INTRODUCTION

Acromegaly is a rare disease, most often caused by a GH-producing tumor of the anterior pituitary [1]. Available treatment modalities to date aim at normal- izing serum insulin-like growth factor 1 (IGF1) levels via reduction of either GH overproduction or GH actions [2-5]. The obvious advantage is that the efficacy of different treatments can be easily compared by means of serum IGF1 measurements, as this is more practical than frequent GH measurements. This also applies to comparisons between the effects of long-acting somatostatin therapies (SRIF) and the GH receptor (GHR) antagonist, pegvisomant (PEG-V). This approach, however, is based on the assumption that serum IGF1 levels adequately and uniformly reflect disease activity. This assumption, however, is not necessarily valid. In this paper, we address the relationship between the GH–IGF1 axis with a specific emphasis on the significant differences in the modes of action of SRIF analogs and PEG-V. In doing so, we will introduce the novel hypothetic paradigm of hepatic and extra-hepatic acromegaly and its potential clinical implications.

THE EFFECTS OF GH ARE TISSUE SPECIFIC AND CONCENTRATION DEPENDENT

The physiological effects of GH versus IGF1 remain controversial. Historically, it has been difficult to isolate the individual effects of GH and IGF1 at the tissue level during physiological conditions. But the fact that GH possesses a diabetogenic or 'anti-insulin' activity [6], while IGF1 (as the name implies) is similar to insulin in its actions, clearly demonstrates that physiological differences exist between the actions of the two peptide hormones. Below, we cite results of animal studies that address specific effects of GH and physiological effects of GH versus IGF1.

ANIMAL STUDIES OF THE ACTIONS OF GH VERSUS IGF1

Since GH is a diabetogenic molecule, it would not be predicted to be used as a pharmaceutical to treat type 2 diabetes. Yet its lipolytic and anti-lipogenic actions could have potential positive outcomes in type 2 diabetic indi- viduals. Two reports have documented beneficial effects of GH on glucose metabolism in type 2 diabetic patients [7-8].

A mouse model attempting to determine the effect of GH on diet-induced type 2 diabetes parameters has been presented [9]. In this model, male C57BL/6J mice were placed on a high-fat diet to induce obesity and type 2 diabetes. During the studies, mice were treated with various doses of GH. Body weight and composition, fasting blood

glucose, insulin and IGF1 levels, glucose tolerance, liver triacylglycerol, tissue weights, and blood chemistries were determined. Several important findings were reported [9]. First, a GH dose-dependent decrease in fat and an increase in lean mass were found. These effects on body composition were seen at the highest two doses of GH administered, even though only the highest dose of GH resulted in elevated circulating IGF1 levels. These results indicate that certain effects of GH are independent of circulating IGF1 levels. Second, the increase in lean mass was observed before the decrease in white adipose tissue (WAT); thus, physiological effects of GH were not observed at the same time points. Third, GH-induced WAT loss was specific to subcutaneous and mesenteric fat. This result agrees with previously published work in which subcutaneous WAT depots were found to be increased in mice that lacked GH action [10-12]. Thus, these data further support the notion that 'not all WAT depots are treated equally' in terms of GH action and should be evaluated independently in studies with GH or other treatments.

The finding that GH can affect body composition independent of elevations in total serum IGF1 levels is important. However, we must point out that these GH-dependent changes in body composition may be due to the autocrine/paracrine actions of IGF1 and not the direct action of GH. The importance of the autocrine or paracrine production of IGF1 has been documented in the liver-specific IGF1 gene-deficient mouse [13].

Other mouse studies attempting to discriminate the effect of GH versus IGF1 were carried out nearly 20 years ago. These studies showed that animals that have high GH and IGF1 levels display glomerulosclerosis; however, glomerulosclerosis is not observed in mice with increased levels of IGF1 alone [14-15].

A continuation of these studies was carried out employing transgenic mice that express analogs of GH. For example, when a bovine (b) GH analog containing the following changes (L121P and E126G) is expressed in the transgenic mice, the resulting animals are of normal size with normal levels of IGF1; yet they display kidney glomerulosclerosis as severe as mice that express wild-type bGH [16]. These data suggest that GH can affect the kidney independent of increases in IGF1.

Additionally, diabetic kidney disease can be induced in mice using streptozotocin. This kidney pathology was not seen in mice that express a GHR antagonist [17-18] or in mice injected with PEG-V (19). Important in this latter study was the fact that kidney pathology was prevented by PEG-V, even in the absence of a decrease in serum IGF1 [19]. Again, this implies that GH has a direct effect on the kidney independent of serum IGF1 levels.

The above data derived from mouse models of GH action suggest that GH can have temporal and tissue- specific effects independent of elevations of serum IGF1.

THE RELATIONSHIP BETWEEN PORTAL INSULIN AND GH SENSITIVITY OF THE LIVER

Human and other mammals are capable of prolonged fasting because they can recruit and utilize lipid stores when they exhaust readily available carbohydrates [20-22]. Prolonged fasting is associated with a gradual decline in hepatic IGF1 production, which makes teleological sense due to the insulin-like effects of IGF1. A study by Leung et al. suggests that GH-induced hepatic IGF1 production is regulated by portal insulin levels [23]. They reported that insulin promotes the translocation of the hepatic GHR to the surface.

When portal insulin levels are high, the liver becomes GH sensitive, regardless of the cause of the elevation in insulin production [23]. In addition, portal insulin also inhibits hepatic IGFBP1 production, which may increase the bioavailability of circulating IGF1 [24-25].

In conclusion, high portal insulin levels increase liver GH sensitivity (via up-regulation of surface GHRs) and, therefore, ultimately increase liver IGF1 production with concomitant increases in serum IGF1 levels. In contrast, low portal insulin levels reduce the sensitivity of liver for GH and, therefore, reduce serum IGF1 levels.

WHY DO ACROMEGALY PATIENTS HAVE ELEVATED IGF1 LEVELS

Acromegaly patients have elevated IGF1 levels as a consequence of GH hypersecretion [1]. In addition, the elevated GH levels stimulate lipolysis and induce resistance to the effects of insulin on glucose metabolism in liver and muscle. The net result is a hypermetabolic state characterized by elevated levels of glucose, free fatty acids, and insulin [26-27].

The GH-induced hyperinsulinemia, in turn, is likely to further stimulate hepatic IGF1 production and to lower IGFBP1 levels [28-29]. The importance of this effect is supported by the observation that prolonged fasting- induced hypoinsulinemia can completely normalize serum IGF1 levels in acromegaly patients [30].

In conclusion, acromegaly patients have elevated serum IGF1 levels because of the pathological hyperse- cretion of GH by the pituitary tumor, which is aggravated by the accompanying hyperinsulinemia.

HOW SOMATOSTATIN ANALOGS WORK

Somatostatin analogs (SRIF) bind to somatostatin receptors of which subtypes 2 (sst2) and 5 (sst5) are the most important ones for mediating the actions of the available long-acting SRIF analogs [31-33]. Because of the expression of sst2 and sst5 on the somatotroph cells, pathological GH secretion can be inhibited by SA, which translates into reduced hepatic IGF1 production [31-32,34,33]. When this reduction is sufficient to normalize IGF1 levels, the treatment is traditionally considered adequate [31,35-37].

However, SRIF analog also binds to sst2 and sst5 receptors on the pancreatic islet, which will reduce glucagon and insulin secretion (38, 39). This occasionally results in a worsening of the glycemic control in acromegaly patients during long-term long-acting SRIF analog [38]. Moreover, a suppression of insulin secretion by SRIF analog also selectively results in hepatic GH resistance, which itself decreases hepatic IGF1 production [23]. Therefore, the ensuing reduction in circulating IGF1 does not necessarily reflect GH activity in peripheral tissues.

A GH-independent suppressive effect of SRIF analog on serum IGF1 levels has been documented in two studies in humans (41, 42). Both studies involved adminis- tration of octreotide for 7 days during continued GH treatment in adult GH deficiency (GHD) patients. This resulted in a significant 16–18% reduction in serum IGF1 levels with a concomitant reduction in insulin levels and elevated levels of IGFBP1 [39-40].

In the context of acromegaly, it is therefore plausible that normalization of serum IGF1 levels during long-acting SRIF analog not necessarily implies control of disease activity in peripheral tissues, i.e. a condition for which we propose the term 'extra-hepatic acromegaly' (Figure 1a). This is further supported by a recent report by Rubeck et al. [41]. They compared traditional and novel biomarkers and health status in patients with acromegaly treated with either surgery alone or SRIF analog. They reported that despite similar and normalized IGF1 levels, SRIF analog treatment compared with surgery alone was associated with less suppressed GH levels and less symptom relief. They concluded that this discordance may be due to specific suppression of hepatic IGF1 production by SRIF analogs [41].

In the absence of a convenient bioassay for disease activity in acromegaly, it is not easy to validate whether extra-hepatic acromegaly is a clinical entity rather than a semantic issue, but it is noteworthy that impaired quality of life (QoL) has been reported in long-acting SRIF analog patients despite normal IGF1 levels [42].

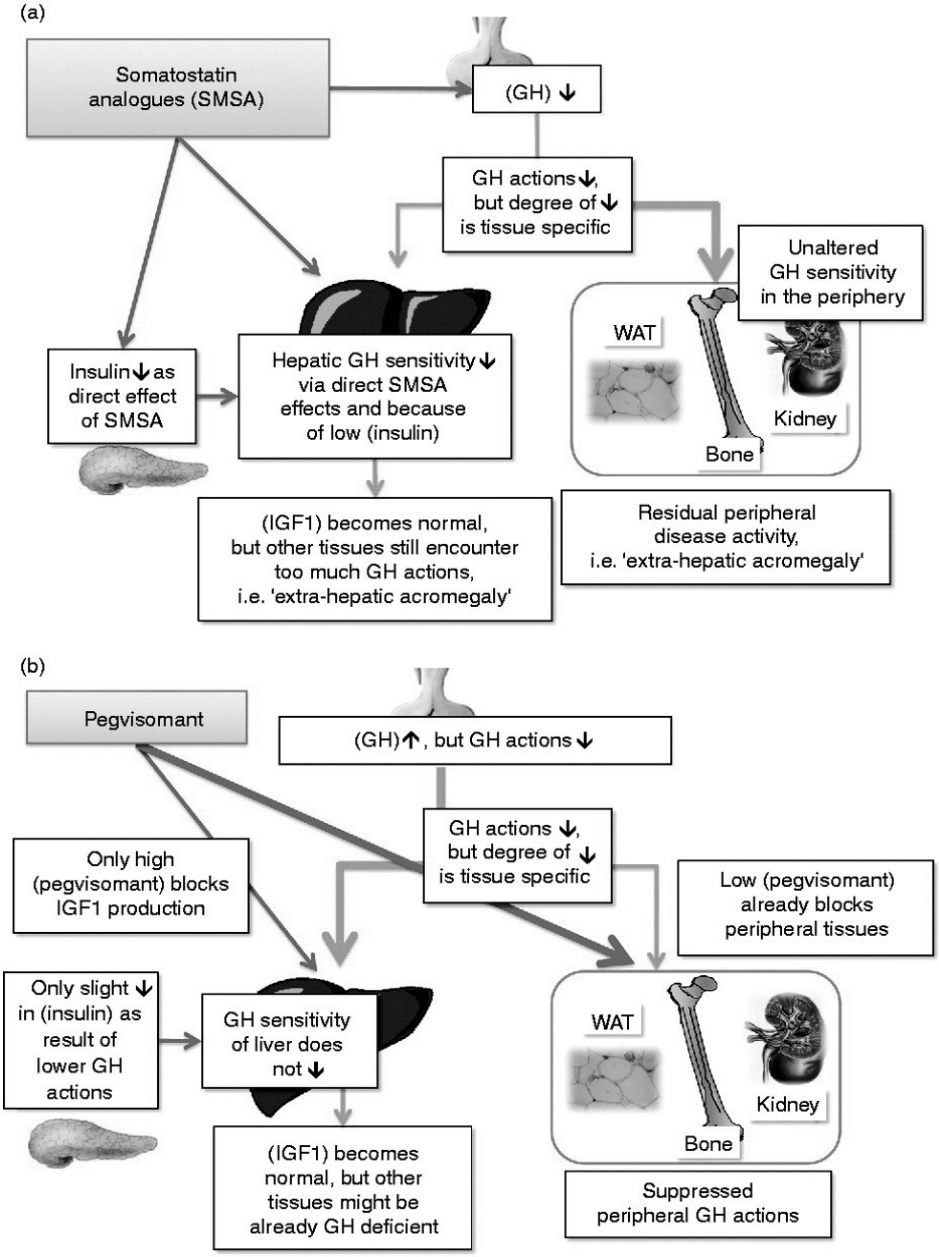

Figure 1: (a) Effects of somatostatin analogs (SRIF) in SRIF analog-sensitive acromegalic subjects. Red arrows indicate inhibitory effects; green arrows indicate stimulatory effects, while thickness of arrow indicates level of inhibition. (b) Effects of pegvisomant in acromegalic subjects. Red arrows indicate inhibitory effects; green arrows indicate stimulatory effects, while thickness of arrow indicates level of inhibition.

HOW GHR ANTAGONISTS WORK

As described above, the GHR antagonist, PEG-V, competitively blocks the GHRs in all peripheral tissues [43-45]. Thus, the higher the endogenous GH level, the more PEG-V is needed to effectively block GH actions [5]. PEG-V, however, does not block all tissues equally effective for the actions of GH. Adipose tissue, the kidneys, and skeletal muscle seem to require less PEG-V to reduce GH actions compared to the liver where more PEG-V is required in order to reduce IGF1 production [19]. In further support of this, it was recently reported that short-term PEG-V administration in healthy subjects can suppress lipolysis without affecting either circulating or local IGF1 [46].

It is therefore possible that PEG-V treatment in acromegaly is subject to tissue-specific differences in a dose-dependent manner. In particular, it is possible that peripheral suppression of GH activity is obtained prior to normalization of hepatic IGF1 production. Such a condition during PEG-V therapy could be denoted 'hepatic acromegaly', which in essence is reciprocal to the putative conditions during long-acting SRIF analog (Fig. 1b). However, unlike extra-hepatic acromegaly, data in humans are lacking to suggest that hepatic acromegaly indeed does occur during PEG-V monotherapy.

LESSONS FROM DIABETES TYPE I AND II

The effects of restoring portal insulin levels on serum IGF1 have been studied in type I diabetes [47]. Only with portal insulin administration, did IGF1 levels increase to within the normal range, which resulted in a decrease in GH levels. However, diabetic patients on conventional insulin therapy had low IGF1 and elevated GH levels [47].

Wurzburger et al. [48] also studied GH-stimulated IGF1 levels in type 1 diabetes. The patients were divided into C-peptide-negative patients without residual b-cell activity and C-peptide-positive patients with preserved b-cell activity. A GH-induced increase in serum IGF1 levels was only observed in patients with remnant b-cell activity [48].

There are similarities between type I diabetes and long-acting SRIF analog-treated acromegalic subjects: both exhibit elevated systemic GH activity together with relative hepatic GH resistance due to low portal insulin levels. The difference is that type I diabetic subjects have low IGF1 levels, while long-acting SRIF analog-treated acromegalic subjects have normal or elevated IGF1 levels. There are also similarities between type 2 diabetes and PEG-V-treated acromegalic subjects: both exhibit low systemic GH activity in the presence of relatively high hepatic GH sensitivity due to normal or elevated portal insulin levels.

WHAT ABOUT COMBINING SRIF AND PEG-V

Several papers have presented data on combination therapy with long-acting SRIF analog and PEG-V. To date, the focus in these reports has been on patients with an insufficient response to long-acting SRIF analog [49-50], but we believe that combination treatment may offer benefit to other patients. The strongest evidence for this is presented by Neggers et al. [51]. They hypothesized that weekly administration of 40 mg PEG-V could improve QoL and metabolic parameters in acromegalic patients with normal age-adjusted IGF1 concentrations during long-acting SRIF analog treatment. In a double-blind, placebo-controlled, crossover study, 20 acromegalic subjects received either PEG-V or placebo for two consecutive treatment periods of 16 weeks, separated by a wash-out period of 4 weeks. Efficacy was assessed as a significant change in disease-specific QoL between baseline and at the end of each treatment period. QoL was assessed by the acromegaly QoL questionnaire (AcroQoL) and the patient-assessed acromegaly symptom questionnaire (PASQ). Interestingly, the AcroQoL and AcroQol improved significantly after PEG-V was added. The addition of PEG-V also significantly improved the PASQ and the single PASQ questions dealing with perspiration, soft tissue swelling, and overall health status. By contrast, no significant changes in IGF1 levels were observed during the addition of PEG-V. As the age- dependent normal range for IGF1 is still relatively wide, it might be possible, however, that some patients may have a statistically normal IGF1 level that is in fact too high for them. Addition of weekly PEG-V might induce a short-lived decline of IGF1 for 2 days, which does not register if the blood is drawn 7 days after PEG-V administration, but the clinical effects might be manifested in QoL questionnaires. Thus, low-dose PEG-V treatment improved the signs and symptoms of 'extra-hepatic acromegaly' without impacting hepatic IGF1 production consistent with our hypothesis of extra- hepatic acromegaly. It is noteworthy that the largest improvement in QoL was observed in patients who also responded to PEG-V with alleviation of fluid retention [51]. It remains to be studied whether the same favorable effects could be obtained by an increase in the dose of long-acting SRIF analog.

CONCLUSIONS AND FUTURE DIRECTIONS

SRIF analog have stood the test of time as a safe and effective treatment for acromegaly; however, adequate control of the disease is not always achieved. With the recent introduction of PEG-V, it is now possible to obtain biochemical control of the disease in most patients. Thus, now is an appropriate moment for critical evaluation of the proper assessment of the therapeutic outcome with these two different treatment modalities. In particular, we postulate that circulating IGF1 is not necessarily the most reliable

biomarker of disease activity. SRIF analog have at least three tissue-specific effects: i) decreased GH secretion from the pituitary tumor, ii) decreased insulin secretion from the pancreas, and iii) decreased hepatic IGF1 production that may lead to a normalization of serum IGF1 levels despite insufficient control of disease activity in peripheral tissues. The combination of these effects may lead to a state of normalized serum IGF1 levels and residual peripheral disease activity, i.e. extra-hepatic acromegaly. Whether this is of clinical significance and whether it may be overcome by simply increasing the dose of long-acting SRIF analog merits are to be addressed in a controlled clinical trial. It would be obvious to compare the outcome of LA-SRIF analog in patients who are randomized to dosing according to either serum IGF1 levels or GH levels.

The use of PEG-V also is challenging since this treatment is accompanied by a further elevation in GH levels. Moreover, there is evidence to suggest that PEG-V in some cases may induce significant blockade of peripheral GH actions prior to blockade of the hepatic GHRs. In this context, it is also noteworthy that the dose requirements of PEG-V are subject to a wide inter-individual variation. Novel biomarkers in addition to IGF1 for this individual variation are needed. It also remains to be determined whether assessment of serum PEG-V levels would be useful. We believe that patients using combination therapy of SRIF analog and PEG-V should be monitored with more specific ways. This might include procollagen II levels or another parameter that can integrate GH actions on the 'extra-hepatic' tissues such as bone.

The fact that long-acting SRIF analog and PEG-V exert complementary suppressive effects on the GH–IGF1 axis makes combination therapy with the two modalities an interesting option. Indeed, there is evidence to suggest that combination therapy is superior to monotherapy with long-acting SRIF analog in terms of glucose homeostasis [50] and disease-specific QoL [51]. The latter observation also suggests that assessment of QoL could be considered as routine practice during medial therapy. Our hope is that the introduction of the hypothetic paradigm of extra-hepatic acromegaly will challenge basic scientists, clinicians, and pharmaceutical industries to design and perform studies that show that we are wrong, because if we are not, medical treatment of acromegalic patients might need a significant update. Last but not least, we believe there is a need for novel biomarkers (either genomic, metabolomic, proteomic, or others), which ideally integrate hepatic as well as peripheral disease activity.

REFERENCES

1. Melmed S (2009) Acromegaly pathogenesis and treatment. The Journal of clinical investigation 119 (11):3189-3202. doi:39375 [pii] 10.1172/JCI39375
2. Castinetti F, Nagai M, Morange I, Dufour H, Caron P, Chanson P, Cortet-Rudelli C, Kuhn JM, Conte-Devolx B, Regis J, Brue T (2009) Long-term results of stereotactic radiosurgery in secretory pituitary adenomas. The Journal of clinical endocrinology and metabolism 94 (9):3400-3407. doi: jc.2008-2772 [pii] 10.1210/jc.2008-2772
3. Melmed S, Colao A, Barkan A, Molitch M, Grossman AB, Kleinberg D, Clemmons D, Chanson P, Laws E, Schlechte J, Vance ML, Ho K, Giustina A (2009) Guidelines for acromegaly management: an update. The Journal of clinical endocrinology and metabolism 94 (5):1509-1517
4. Trainer PJ, Drake WM, Katznelson L, Freda PU, Herman-Bonert V, van der Lely AJ, Dimaraki EV, Stewart PM, Friend KE, Vance ML, Besser GM, Scarlett JA, Thorner MO, Parkinson C, Klibanski A, Powell JS, Barkan AL, Sheppard MC, Malsonado M, Rose DR, Clemmons DR, Johannsson G, Bengtsson BA, Stavrou S, Kleinberg DL, Cook DM, Phillips LS, Bidlingmaier M, Strasburger CJ, Hackett S, Zib K, Bennett WF, Davis RJ (2000) Treatment of acromegaly with the growth hormone-receptor antagonist pegvisomant. The New England journal of medicine 342 (16):1171-1177
5. van der Lely AJ, Hutson RK, Trainer PJ, Besser GM, Barkan AL, Katznelson L, Klibanski A, Herman-Bonert V, Melmed S, Vance ML, Freda PU, Stewart PM, Friend KE, Clemmons DR, Johannsson G, Stavrou S, Cook DM, Phillips LS, Strasburger CJ, Hackett S, Zib KA, Davis RJ, Scarlett JA, Thorner MO (2001) Long-term treatment of acromegaly with pegvisomant, a growth hormone receptor antagonist. Lancet 358 (9295):1754-1759
6. Rabinowitz D, Merimee TJ, Burgess JA, Riggs L (1966) Growth hormone and insulin release after arginine: indifference to hyperglycemia and epinephrine. The Journal of clinical endocrinology and metabolism 26 (10):1170-1172
7. Ahn CW, Kim CS, Nam JH, Kim HJ, Nam JS, Park JS, Kang ES, Cha BS, Lim SK, Kim KR, Lee HC, Huh KB (2006) Effects of growth hormone on insulin resistance and atherosclerotic risk factors in obese type 2 diabetic patients with poor glycaemic control. Clinical endocrinology 64 (4):444-449. doi: CEN2490 [pii] 10.1111/j.1365-2265.2006.02490.x
8. Nam SY, Kim KR, Cha BS, Song YD, Lim SK, Lee HC, Huh KB (2001) Low-dose growth hormone treatment combined with diet restriction decreases insulin resistance by reducing visceral fat and increasing muscle mass in obese type 2 diabetic patients. Int J Obes Relat Metab Disord 25 (8): 1101-1107. doi:10.1038/sj.ijo.0801636
9. List EO, Palmer AJ, Berryman DE, Bower B, Kelder B, Kopchick JJ (2009) Growth hormone improves body composition, fasting blood glucose, glucose tolerance and liver triacylglycerol in a mouse model of diet-induced obesity and type 2 diabetes. Diabetologia 52 (8):1647-1655. doi:10.1007/s00125-009-1402-z
10. Berryman DE, List EO, Coschigano KT, Behar K, Kim JK, Kopchick JJ (2004) Comparing adiposity profiles in three mouse models with altered GH signaling. Growth Horm IGF Res 14 (4):309-318. doi:10.1016/j.ghir.2004.02.005 S1096637404000292 [pii]
11. Berryman DE, List EO, Kohn DT, Coschigano KT, Seeley RJ, Kopchick JJ (2006) Effect of growth hormone on susceptibility to diet-induced obesity. Endocrinology 147 (6):2801-2808. doi: en.2006-0086 [pii] 10.1210/en.2006-0086
12. Berryman DE, List EO, Palmer AJ, Chung MY, Wright-Piekarski J, Lubbers E, O'Connor P, Okada S, Kopchick JJ (2010) Two-year body composition analyses of long-lived GHR null mice. J Gerontol A Biol Sci Med Sci 65 (1):31-40. doi:glp175 [pii] 10.1093/gerona/glp175

13. Yakar S, Liu JL, Stannard B, Butler A, Accili D, Sauer B, LeRoith D (1999) Normal growth and development in the absence of hepatic insulin-like growth factor I. Proceedings of the National Academy of Sciences of the United States of America 96 (13):7324-7329
14. Yang CW, Striker GE, Chen WY, Kopchick JJ, Striker LJ (1997) Differential expression of glomerular extracellular matrix and growth factor mRNA in rapid and slowly progressive glomerulosclerosis: studies in mice transgenic for native or mutated growth hormone. Lab Invest 76 (4):467-476
15. Yang CW, Striker LJ, Kopchick JJ, Chen WY, Pesce CM, Peten EP, Striker GE (1993) Glomerulosclerosis in mice transgenic for native or mutated bovine growth hormone gene. Kidney Int Suppl 39: S90-94
16. Yang CW, Striker LJ, Pesce C, Chen WY, Peten EP, Elliot S, Doi T, Kopchick JJ, Striker GE (1993) Glomerulosclerosis and body growth are mediated by different portions of bovine growth hormone. Studies in transgenic mice. Lab Invest 68 (1):62-70
17. Chen NY, Chen WY, Kopchick JJ (1996) A growth hormone antagonist protects mice against streptozotocin induced glomerulosclerosis even in the presence of elevated levels of glucose and glycated hemoglobin. Endocrinology 137 (11):5163-5165
18. Esposito C, Liu ZH, Striker GE, Phillips C, Chen NY, Chen WY, Kopchick JJ, Striker LJ (1996) Inhibition of diabetic nephropathy by a GH antagonist: a molecular analysis. Kidney Int 50 (2):506-514
19. Flyvbjerg A, Bennett WF, Rasch R, Kopchick JJ, Scarlett JA (1999) Inhibitory effect of a growth hormone receptor antagonist (G120K-PEG) on renal enlargement, glomerular hypertrophy, and urinary albumin excretion in experimental diabetes in mice. Diabetes 48 (2):377-382
20. Boyle PJ, Shah SD, Cryer PE (1989) Insulin, glucagon, and catecholamines in prevention of hypoglycemia during fasting. Am J Physiol 256 (5 Pt 1):E651-661
21. Moller J, Nielsen S, Hansen TK (1999) Growth hormone and fluid retention. Hormone research 51 Suppl 3:116-120
22. Salgin B, Marcovecchio ML, Humphreys SM, Hill N, Chassin LJ, Lunn DJ, Hovorka R, Dunger DB (2009) Effects of prolonged fasting and sustained lipolysis on insulin secretion and insulin sensitivity in normal subjects. American journal of physiology 296 (3):E454-461. doi:90613.2008 [pii] 10.1152/ajpendo.90613.2008
23. Leung KC, Doyle N, Ballesteros M, Waters MJ, Ho KK (2000) Insulin regulation of human hepatic growth hormone receptors: divergent effects on biosynthesis and surface translocation. The Journal of clinical endocrinology and metabolism 85 (12):4712-4720
24. Frystyk J, Delhanty PJ, Skjaerbaek C, Baxter RC (1999) Changes in the circulating IGF system during short-term fasting and refeeding in rats. Am J Physiol 277 (2 Pt 1):E245-252
25. Ogilvy-Stuart AL, Hands SJ, Adcock CJ, Holly JM, Matthews DR, Mohamed-Ali V, Yudkin JS, Wilkinson AR, Dunger DB (1998) Insulin, insulin-like growth factor I (IGF-I), IGF-binding protein-1, growth hormone, and feeding in the newborn. The Journal of clinical endocrinology and metabolism 83 (10):3550-3557
26. Jorgensen JO, Krag M, Jessen N, Norrelund H, Vestergaard ET, Moller N, Christiansen JS (2004) Growth hormone and glucose homeostasis. Hormone research 62 Suppl 3:51-55. doi: HRE2004062S03051 [pii] 10.1159/000080499
27. Moller N, Jorgensen JO (2009) Effects of growth hormone on glucose, lipid, and protein metabolism in human subjects. Endocrine reviews 30 (2):152-177. doi:er.2008-0027 [pii]10.1210/er.2008-0027
28. Jorgensen JO, Moller N, Moller J, Weeke J, Blum WF (1994) Insulin-like growth factors (IGF)-I and -II and IGF binding protein-1, -2, and -3 in patients with acromegaly before and after adenomectomy. Metabolism 43 (5):579-583. doi:0026-0495(94)90199-6 [pii]

29. Parkinson C, Flyvbjerg A, Trainer PJ (2004) High levels of 150-kDa insulin-like growth factor binding protein three ternary complex in patients with acromegaly and the effect of pegvisomant-induced serum IGF-I normalization. Growth Horm IGF Res 14 (1):59-65. doi:S1096637403001333 [pii]
30. Ho PJ, Friberg RD, Barkan AL (1992) Regulation of pulsatile growth hormone secretion by fasting in normal subjects and patients with acromegaly. The Journal of clinical endocrinology and metabolism 75 (3):812-819
31. Castinetti F, Saveanu A, Morange I, Brue T (2009) Lanreotide for the treatment of acromegaly. Adv Ther 26 (6):600-612. doi:10.1007/s12325-009-0035-4
32. Freda PU, Katznelson L, van der Lely AJ, Reyes CM, Zhao S, Rabinowitz D (2005) Long-acting somatostatin analog therapy of acromegaly: a meta-analysis. The Journal of clinical endocrinology and metabolism 90 (8):4465-4473. doi:jc.2005-0260 [pii] 10.1210/jc.2005-0260
33. Melmed S, Sternberg R, Cook D, Klibanski A, Chanson P, Bonert V, Vance ML, Rhew D, Kleinberg D, Barkan A (2005) A critical analysis of pituitary tumor shrinkage during primary medical therapy in acromegaly. The Journal of clinical endocrinology and metabolism 90 (7):4405-4410. doi:jc.2004-2466 [pii] 10.1210/jc.2004-2466
34. Lamberts SW, van der Lely AJ, de Herder WW, Hofland LJ (1996) Octreotide. The New England journal of medicine 334 (4):246-254
35. Giustina A, Bonadonna S, Bugari G, Colao A, Cozzi R, Cannavo S, de Marinis L, Degli Uberti E, Bogazzi F, Mazziotti G, Minuto F, Montini M, Ghigo E (2009) High-dose intramuscular octreotide in patients with acromegaly inadequately controlled on conventional somatostatin analogue therapy: a randomised controlled trial. European journal of endocrinology / European Federation of Endocrine Societies 161 (2):331-338. doi:EJE-09-0372 [pii] 10.1530/EJE-09-0372
36. Lombardi G, Minuto F, Tamburrano G, Ambrosio MR, Arnaldi G, Arosio M, Chiarini V, Cozzi R, Grottoli S, Mantero F, Bogazzi F, Terzolo M, Tita P, Boscani PF, Colao A (2009) Efficacy of the new long-acting formulation of lanreotide (lanreotide Autogel) in somatostatin analogue-naive patients with acromegaly. J Endocrinol Invest 32 (3):202-209. doi:6337 [pii]
37. Toledano Y, Rot L, Greenman Y, Orlovsky S, Pauker Y, Olchovsky D, Eliash A, Bardicef O, Makhoul O, Tsvetov G, Gershinsky M, Cohen-Ouaqnine O, Ness-Abramof R, Adnan Z, Ilany J, Guttmann H, Sapir M, Benbassat C, Shimon I (2009) Efficacy of long-term lanreotide treatment in patients with acromegaly. Pituitary 12 (4):285-293. doi:10.1007/s11102-009-0172-4
38. Koop BL, Harris AG, Ezzat S (1994) Effect of octreotide on glucose tolerance in acromegaly. European journal of endocrinology / European Federation of Endocrine Societies 130 (6):581-586
39. Laursen T, Moller J, Fisker S, Jorgensen JO, Christiansen JS (1999) Effects of a 7-day continuous infusion of octreotide on circulating levels of growth factors and binding proteins in growth hormone (GH)-treated GH-deficient patients. Growth Horm IGF Res 9 (6):451-457. doi:10.1054/ghir.1999.0131 S1096-6374(99)90131-4 [pii]
40. Pokrajac A, Frystyk J, Flyvbjerg A, Trainer PJ (2009) Pituitary-independent effect of octreotide on IGF1 generation. European journal of endocrinology / European Federation of Endocrine Societies 160 (4):543-548. doi:EJE-08-0822 [pii] 10.1530/EJE-08-0822
41. Rubeck KZ, Madsen M, Andreasen CM, Fisker S, Frystyk J, Jorgensen JO (2010) Conventional and novel biomarkers of treatment outcome in patients with acromegaly: discordant results after somatostatin analog treatment compared with surgery. European journal of endocrinology / European Federation of Endocrine Societies 163 (5):717-726. doi:EJE-10-0640 [pii] 10.1530/EJE-10-0640

42. Bonapart IE, van Domburg R, ten Have SM, de Herder WW, Erdman RA, Janssen JA, van der Lely AJ (2005) The 'bio-assay' quality of life might be a better marker of disease activity in acromegalic patients than serum total IGF-I concentrations. European journal of endocrinology / European Federation of Endocrine Societies 152 (2):217-224
43. Chen WY, Wight DC, Mehta BV, Wagner TE, Kopchick JJ (1991) Glycine 119 of bovine growth hormone is critical for growth-promoting activity. Mol Endocrinol 5 (12):1845-1852
44. Chen XZ, Shafer AW, Yun JS, Li YS, Wagner TE, Kopchick JJ (1992) Conversion of bovine growth hormone cysteine residues to serine affects secretion by cultured cells and growth rates in transgenic mice. Mol Endocrinol 6 (4):598-606
45. Dattani MT, Hindmarsh PC, Brook CG, Robinson IC, Kopchick JJ, Marshall NJ (1995) G120R, a human growth hormone antagonist, shows zinc-dependent agonist and antagonist activity on Nb2 cells. The Journal of biological chemistry 270 (16):9222-9226
46. Moller L, Norrelund H, Jessen N, Flyvbjerg A, Pedersen SB, Gaylinn BD, Liu J, Thorner MO, Moller N, Lunde Jorgensen JO (2009) Impact of growth hormone receptor blockade on substrate metabolism during fasting in healthy subjects. The Journal of clinical endocrinology and metabolism 94 (11):4524-4532. doi:jc.2009-0381 [pii] 10.1210/jc.2009-0381
47. Shishko PI, Dreval AV, Abugova IA, Zajarny IU, Goncharov VC (1994) Insulin-like growth factors and binding proteins in patients with recent-onset type 1 (insulin-dependent) diabetes mellitus: influence of diabetes control and intraportal insulin infusion. Diabetes research and clinical practice 25 (1):1-12
48. Wurzburger MI, Prelevic GM, Sonksen PH, Balint-Peric LA, Wheeler M (1993) The effect of recombinant human growth hormone on regulation of growth hormone secretion and blood glucose in insulin-dependent diabetes. The Journal of clinical endocrinology and metabolism 77 (1): 267-272
49. Feenstra J, de Herder WW, ten Have SM, van den Beld AW, Feelders RA, Janssen JA, van der Lely AJ (2005) Combined therapy with somatostatin analogues and weekly pegvisomant in active acromegaly. Lancet 365 (9471):1644-1646
50. Jorgensen JO, Feldt-Rasmussen U, Frystyk J, Chen JW, Kristensen LO, Hagen C, Orskov H (2005) Cotreatment of acromegaly with a somatostatin analog and a growth hormone receptor antagonist. The Journal of clinical endocrinology and metabolism 90 (10):5627-5631
51. Neggers SJ, van Aken MO, de Herder WW, Feelders RA, Janssen JA, Badia X, Webb SM, van der Lely AJ (2008) Quality of life in acromegalic patients during long-term somatostatin analog treatment with and without pegvisomant. The Journal of clinical endocrinology and metabolism 93 (10): 3853-3859

Chapter 8

General Discussion and Conclusion

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INTRODUCTION

With the introduction of PEG-V, new possibilities became available for the treatment of acromegaly. Initially, PEG-V was used as a single agent in the treatment of acromegaly, but in view of its possible involvement in tumour growth [1], the high cost of this new medication and the broad use of SRIF analogs inspired investigators to assess the combined use of SRIF analogs and PEG-V [2]. One of these investigators, AJ van der Lely started by combining high dose SRIF analogs with weekly PEG-V. The first part of this thesis, chapters 2, 3 and 4, is focused on the efficacy, safety and costs of this combination treatment in up to that time uncontrolled acromegalic patients during SRIF analog treatment.

The second part of this thesis, chapters 5 and 7, concentrates on QoL in long-term controlled acromegaly patients during long-acting SRIF analogue treatment, with or without PEG-V. Furthermore, this part of the thesis describes the tissue specific physiology of the GH-IGF1 axis, which may explain the benefit from combining PEG-V and SRIF analogs.

Finally, in chapter 6 a possible explanation for the increase in GH levels during PEG-V monotherapy is studied. If a GH receptor would be present in the pituitary adenoma, PEG-V should be able to block these and thereby increase GH levels. A Similar ultra-short feedback loop is found in other pituitary hormone system as TSH.

PART 1

EFFICACY OF COMBINED TREATMENT AND COST REDUCTION

Clinically, acromegaly is characterized by soft tissue enlargement, excessive skeletal growth and reduced life expectancy [3]. In at least 95 % of cases it is caused by excessive growth hormone (GH) secretion by a benign pituitary tumour. More than 75 % of these pituitary adenomas are macroadenomas, which often extends dorsally of the suprasellar region or laterally to the cavernous sinus [3]. Depending on the size, localization of the pituitary adenoma and the patients' characteristics, a treatment modality should be chosen.

Available treatment modalities to date aim at normalizing serum IGF1 levels via reduction of either GH overproduction or GH actions. [4-7]. The obvious advantage is that the efficacy of different treatments can be easily compared by means of serum IGF1 measurements, as this is more practical than frequent GH measurements.

Over 90 % of the patients are treated by transsphenoidal surgery. Transsphenoidal surgery is a well-established first line treatment for microadenomas in acromegaly. In

patients with a microadenoma, remission can be achieved in about 80% [8] and recurrence is reported in 3-10% of the patients [9-12]. Long-term efficacy largely depends on the experience of the neurosurgeon, while GH levels at diagnosis, tumour size and extrasellar invasion also play a major role in this. In hospitals without highly specialized neurosurgeons, remission rates of less than 40 % are not uncommon in patients with microadenomas [13]. Compared to treatment with ,somatostatin (SRIF) analogs, that have an efficacy rate of 44-77% in normalising IGF1 levels, remission rates for surgery are within the same range (40%-80%). However, successful surgery is accompanied by a rapid fall in GH and IGF1 and the costs relative to long-term drug therapy (SRIF analog, PEG-V) are low. Despite this, after surgery, remission is reached in less than 50 % of patients with macroadenomas, even in centres with experienced neurosurgical staff [3]. Generally, reported remission rates are between 20-30 % for macroadenomas [13]. When compared to efficacy rates of medical treatment with SRIF analog and/or PEG-V, surgical efficacy rates in patients with macroadenomas are unacceptably low. There might be one exception, which is the intrasellar macroadenoma. In this specific case remission rates are observed of approximately the same magnitude as with the microadenomas [13].

Combination treatment with SRIF analogs and PEG-V achieve normal IGF1 levels in more than 90% of patients [14-15]. This is clearly shown in chapter 2 and 3. These efficacy rates are comparable with PEG-V monotherapy [7]. However, from the observational registry, Acrostudy^{tm}, efficacy rates of 62% are reported [16]. In this registry the majority of patients were treated with PEG-V daily. Combinations of somatostatin analogs and dopamine agonist with PEG-V were used in 4% of patients [16]. The mean weekly dose of PEG-V was 106 mg in patients with a normal IGF1, and 113 mg in those with an elevated IGF1. The mean weekly dose in the study by van der Lely was around 130 mg, with an efficacy rate of >90% [7].

With an equal efficacy rate, but at a mean weekly PEG-V dose of 77 mg, long-term combination therapy seems to help reduce the required dose of PEG-V [14,17]. This is clearly shown in chapter 2 and 3, although the magnitude of the decrease in dose can differ greatly between patients, as observed in chapter 4.

Yearly, cost reductions of up to €65.000 can be attained. Considering that treatment with high-dose SRIF analogs costs between €19.000 – 25.000, depending on the country, our data indicate that in patients who need high-dose PEG-V during monotherapy, a significant reduction in costs can be achieved without losing efficacy in normalizing IGF1 levels.

The rationale behind the dose reduction in PEG-V during combined therapy with SRIF analogs and PEG-V is an increase of about 20% in PEG-V serum levels [18,1]. Additional growth hormone levels decrease due to the use of SRIF analog treatment [18]. Thus, PEG-V, which is a competitive blocker of the growth-hormone receptor, has less

GH to compete with for receptor binding, which increases its pharmacological effect. Additionally, in experiments rodents showed that SRIF analogs decrease the number of growth hormone receptors expressed in the liver owing to a decrease in portal insulin concentration [19-21]. Finally, SRIF analogs direct inhibition of IGF1 synthesis in the liver [22]. These mechanisms suggest that the combined use of somatostatin analogs and PEG-V enables clinicians to administer PEG-V at a reduced dosage and frequency.

SAFETY OF COMBINED TREATMENT

The most commonly observed adverse effect of pegvisomant therapy or SRIF analog and PEG-V combination therapy is liver dysfunction (Chapter 2-3), which includes hepatocellular and cholestatic liver disturbances. Cholestatic abnormalities are indicated by elevated levels of alkaline phosphatase and alkaline γ-glutamyltranspeptidase and radiological signs of biliary obstruction. These deleterious effects on liver function are most often related to treatment with somatostatin analogs [23]. Many patients with such abnormalities have asymptomatic bile stones, but active disease that requires surgery or endoscopic retrograde cholangiopancreatography intervention is rare [23]. During combined treatment 3 patients had gallstones. The cholestatic disturbances were treated with cholecystectomy in two of these patients and all patients continued combination therapy. In the other patient the cholestatic disturbances were transient [14-15,17].

Hepatocellular disturbances are indicated by elevated levels of transaminases, which are probably related to the use of pegvisomant [24]. The mechanism behind the elevated levels of transaminases remains unclear. The prevalence of such elevations seems to be higher during combination treatment (11.0-15.0%) [25,14,17] than during pegvisomant monotherapy (5.2%) [24]. However, since our patients visited the outpatient clinic every 6-8 weeks, and patients from the German Pegvisomant observational Study did attend a regular follow up, underreporting could explain the difference in prevalence. The elevations in transaminase levels are usually mild and transient and occur within the first year of treatment [14,26,15,17]. Of note, most patients who had elevated levels of transaminases during combination treatment continued taking pegvisomant, whereas many of those who had such symptoms during pegvisomant monotherapy did not [17]. One patient on combination treatment, who received weekly PEG-V discontinued treatment [2,27]. He was re-challenged with pegvisomant alone, levels of transaminases increased again, so PEG-V treatment was stopped [27]. In a series of four patients who previously had elevated levels of transaminases, this type of liver dysfunction occurred again when pegvisomant was reintroduced after a drug-free period of more than 4 months [26,17]. A subgroup of patients with acromegaly, those with diabetes mellitus, seem to have an increased risk of developing elevated levels of transaminases [14-15,17].

The average rise in transaminase levels in these patients is approximately 2.3-fold that in those without diabetes mellitus (DM). [14]. In some studies, however, no association was found between the presence of DM and elevated levels of transaminases [28,24,16]. The impact of DM seems to fade in the large cohort of patients [14]. A common polymorphism, UGT1A1*28, associated with Gilbert's syndrome seems to be associated with the occurrence of elevated levels of transaminases during monotherapy PEG-V, as was male gender. [29]. The incidence of homozygous and heterozygous genotypes of UGT1A1*28 in acromegalic patients was 54% [29]. Currently, no other factors are known to increase levels of transaminases in these patients, as transaminase levels do not seem to be related to the cumulative dose of pegvisomant (or to concomitant medication). Further studies into the cause of the elevated transaminases should be aimed at the UGT1A1*28 polymorphism which may be correlated with efficacy parameters. The observation of increased risk of developing these liver enzyme disturbances in diabetes, and the need for a higher dose of PEG-V to normalize IGF1, could well be a clue. However, the cumulative dose of PEG-V does not increase the risk of elevated transaminases.

Tumour size

Pegvisomant is unable to prevent tumour growth, but in only a few cases has clinically important tumour growth been reported [30,25]. In the German Pegvisomant Observation Study tumour increase was carefully and systematically reviewed in over 300 patients. After this systematic review, only 3 patients of the 8 patients reported initially had a real, but minor, increase in tumour size after PEG-V was started [30]. In the other 3 of the 8 subjects, the originally reported increase in tumour-size already started before the initiation of PEG-V treatment. In 2 subjects a known rebound of tumour-size was present after cessation of SRIF analogs [30]. During combination treatment no tumour size increase was observed in about 99 patients [17]. Only in the study by Jorgensen and co-workers was an increase in tumour size observed in one of the 11 patients studied [18]. Some of the patients in this study, however, received a high dose of long-acting octreotide (30 mg every 2 weeks) prior to study entry. There was no data on the tumour size increase prior to study entry and the study also consisted of a period with PEG-V monotherapy as well. Therefore, it is impossible to determine if the increase in tumour size is caused by a rebound effect after discontinuation of SRIF analogs or by continued growth of the adenoma which started prior to study entry. Moreover during combination treatment, in about 19% of the subjects a decrease in tumour size was observed [14,17].

Lipodystrophy

Lipodystrophy has been described by several reports in both combined and monotherapy with PEG-V [31-32,14]. The prevalence is low, and when lipodystrophy does occur,

frequent change of injection site has been shown to reverse local lipohypertrophy after 8 months [14]. The explanation for this phenomenon is probably the local severe GH deficiency due to very high tissue levels at the injection site in the presence of insulin. This misbalance leads to accumulation of adipose tissue around the injection sites [28,17]. In some patients this leads to discontinuation, in others frequent change of injection site reversed the local reaction.

Glucose metabolism

The advantage of PEG-V treatment over SRIF analog therapy with respect to carbohydrate metabolism has been established in a study of healthy volunteers [33]. In this study, administration of PEG-V did not influence levels of fasting glucose and insulin or response to an oral glucose tolerance test; in contrast, octreotide augmented glucose levels and impaired the insulin response. Combination treatment resulted in lower fasting glucose levels than were achieved with octreotide treatment alone, but no significant difference was observed in insulin levels [18]. In long-term studies, combination treatment decreased HbA1c levels in patients with diabetes mellitus and acromegaly, despite reduced dose requirements for insulin or oral anti-diabetic medication [14-15,17]. This finding was not confirmed, however, in a study by De Marinis and co-workers [34]. It seems that in the hierarchy of the beneficial effects on carbohydrate metabolism, PEG-V as monotherapy is superior to combined treatment. However, the combination is better than SRIF analog monotherapy (chapter4).

In conclusion; combined treatment with SRIF analogs and PEG-V has similar efficacy rates as PEG-V monotherapy. However, the cumulative mean weekly dose seems to be significantly lower during combined treatment. This results in a cost reduction that differs between individual patients. The safety profile of the combination therapy is more or less comparable with PEG-V monotherapy. Although transiently elevated levels of transaminases seem to occur at a higher rate. On the other hand, during combined treatment no tumour size increase was observed and in a substantial group of patients tumour size decreased. Finally, the combined treatment provides the best of both worlds (SRIF and PEG-V) since glucose homeostasis seems to be improved when compared with SRIF analog monotherapy.

PART 2

Recent improvements in the medical treatment of acromegaly has resulted in better biochemical disease control in virtually every acromegaly patient, as described above. The current consensus on the goals of treatment of acromegaly has focused on normalization of IGF1 and GH and thereby a reduction in long-term morbidity and mortality

[35-36]. However, normalization of levels of total serum IGF1 and GH do not necessarily reflect optimal quality of life (QoL) nor relief of symptoms, in acromegalic patients [37-41]. From the patient's perspective an important parameter of disease control is QoL. To quantify the symptoms and QoL in patients with acromegaly, the Patient-Assessed Acromegaly Symptom Questionnaire (PASQ) [6-7] and the Acromegaly Quality of Life Questionnaire (AcroQoL) have been developed [42] (Table 1).

Table 1. Quality of life questionnaires

AcroQoL comprises 22 questions. Each question has five possible answers scored 1–5, with a total maximum score of 110 and quoted as a percentage. The score of 110 reflects the best possible QoL. The 22 questions are divided into two main categories: physical and psychological function. The psychological dimension is subdivided into appearance and personal relationships [42-44]. The AcroQoL has a good internal consistency (Cronbach's > 0.7) [43].

The PASQ is a disease-specific questionnaire, which consists of six questions scoring 0–8 and the seventh question addressing the overall health status, based on the other six questions, scoring 0–10 [6-7]. The first six questions evaluate symptoms as headache, excessive sweating, joint pain, fatigue, soft tissue swelling, and numbness or tingling of the extremities. The maximum score of these six questions is 48 and indicates severe signs and symptoms, with lower scores reflecting improved QoL.

During treatment SRIF analogs reduce portal insulin concentration and the number of available growth hormone receptors in the liver [21], and can directly inhibit IGF1 production by hepatocytes [22]. These mechanisms suggest that although the liver becomes relatively resistant to growth hormone during somatostatin analog treatment, acromegalic symptoms still persist in other parts of the body. One might expect that treatment of this 'extrahepatic acromegaly' with low-dose, weekly pegvisomant would improve the growth-hormone-dependent signs and symptoms and the patient's quality of life. [21].

In a prospective, double-blind, placebo-controlled, crossover trial QoL was assessed by AcroQoL and PASQ to assess the effects of the addition of a weekly low dose of pegvisomant in patients with acromegaly whose levels of IGF1 were within the age-adjusted normal limits during long-term SRIF analog therapy [26]. After 16 weeks of treatment with 40 mg pegvisomant weekly, the patients' quality of life improved, as indicated by increases in the AcroQoL total score and the AcroQoL score's physical dimension. These improvements were accompanied by a reduction in the total PASQ score and in improvement of perspiration, soft-tissue swelling and overall health status. Moreover, these symptoms; perspiration and soft-tissue swelling can also be provoked during overdosing GH treatment in patients with GH deficiency.

The improvements in patients' quality of life, and signs and symptoms of acromegaly were not accompanied by a significant decrease in IGF1 level. Only change in body weight correlated with the improvement in the AcroQoL score's physical dimension, but the treatment-related decrease in body weight was not significant. The pegvisomant-related improvement in patients' quality of life might be also explained by the mode

of action of somatostatin analogs. As mentioned above, somatostatin analogs reduce portal insulin concentration and the number of available growth hormone receptors in the liver, and can directly inhibit IGF1 production by hepatocytes. These mechanisms suggest that whereas the liver becomes relatively resistant to growth hormone during somatostatin analog treatment, acromegalic symptoms still persist in other parts of the body. One might expect that treatment of this 'extrahepatic acromegaly' with low-dose, weekly pegvisomant could improve the growth-hormone-dependent signs and symptoms and the patient's quality of life. The observed improvement in quality of life with combination therapy calls into question the widely used step-up approach, according to which patients are only treated with pegvisomant if somatostatin analog monotherapy is not able to normalize IGF1 levels.

Although in some individuals IGF1 levels clearly decreased during PEG-V co-treatment, for the whole group, IGF1 did not decrease significantly. This observation might be explained by an observation by Segev et al. [45], who reported that a GH receptor antagonist in rodents was able to block (in this case, renal) GH actions at lower concentrations than were necessary to decrease serum IGF1 and somatic growth. However, our study was powered to detect a difference in PASQ score and not designed to detect a difference in IGF1. Therefore, it is possible that studies in larger populations will observe a significant decrease.

The improvement in QoL can not be explained by a recall phenomenon of the questions. Our study assessed QoL over a 4-month period and it is internationally accepted that in most studies, a 2-wk period between test and retest is enough to circumvent the memory effect [46-47]. More intervention studies are needed to assess the value of QoL measurements in clinical practice. Whereas physicians would like to apply QoL measurements in patients' treatment, this is not readily realized. QoL determination is time consuming, and single questionnaires in a single patient cannot be interpreted. Thus, physicians tend to ask a few questions and call this QoL, resulting in large differences between different doctors. Thus, more studies evaluating the value of the addition of PEG-V in treatment are needed, but also a feasible tool to assess the QoL in daily medical practice. This is a prerequisite for the implementation of the studies described in this thesis every day medical practice.

In conclusion; QoL in acromegalic patients who normalized serum IGF1 concentrations during long-term treatment with long-acting SRIF analogs can be significantly improved by the addition of a low weekly dose of PEG-V. This improvement does not seem to be accompanied by a decrease in serum IGF1 levels. This brings into question the importance of total serum IGF1 as a reliable parameter for QoL. The improvement in QoL after adding PEG-V, raises questions about the step-up approach in which patients are treated only with pegvisomant when SRIF analog monotherapy was not able to normalize IGF1 levels.

New paradigm?

Available treatment modalities to date aim at normalizing serum IGF1 levels via reduction of GH action or GH overproduction [4-7]. A tangible advantage is that the efficacy of different treatments can be easily compared by means of serum IGF1 measurements, as this is more practical than time consuming, and costly, 24h GH measurements. This also applies to comparisons between the effects of long-acting SRIF analog therapies and PEG-V. This approach, however, is based on the assumption that serum IGF1 levels adequately and uniformly reflect disease activity. This assumption, however, is not necessarily valid.

Although GH regulates IGF1 and vice versa, they are not interchangeable. It is difficult to isolate individual effects of GH and IGF1 at the tissue level during physiological conditions. But the fact that GH possesses a diabetogenic or 'anti-insulin' counter-regulatory activity [48], while IGF1 (as the name implies) is similar to insulin in its actions, clearly demonstrates that physiological differences exist between the actions of the two peptide hormones [49]. In mouse models, GH has temporal and tissue specific effects independent of elevations of serum levels. In humans these tissue specific and without a change in IGF1 serum levels are hard to study. In adipose, kidney and skeletal muscle local activity of GH can be blocked with PEG-V without any change in serum IGF1 [50]. Therefore treatment with PEG-V monotherapy in acromegaly will lead to a blockade of peripheral GH action without decreasing IGF1 levels, which we could call "hepatic acromegaly". In order to normalize IGF1, with PEG-V, peripheral tissues could become GH deficient. SRIF analogs have an opposite effect since they reduce GH and thereby IGF1. However, SRIFs also decrease insulin and glucagon. This can result in a worsening of glycemic control during long-term SRIF analog use [51]. The lower portal insulin level selectively results in hepatic GH resistance [21]. This resistance causes a reduction in circulating IGF1 [21]. IGF1 suppression by SRIF can be GH independent in human [52-53]. Therefore normal IGF1 levels in acromegaly during SRIF analog treatment does not imply control of the disease in peripheral tissues. Since SRIFs make the liver GH-resistant, but do not control the acromegaly in the peripheral tissues, patients still have a decreased QoL and their symptoms remain [26]. By combining SRIF analogs and PEG-V this extra-hepatic acromegaly can be treated even though IGF1 levels are normal during SRIF treatment [26,49]. But the necessary dose of PEG-V might have a wide inter-individual variation. Since GH and IGF1 levels are far from optimal endpoints in assessing this effect, novel biomarkers are necessary. These novel markers should not only reflect GH action in specific tissues, but should reflect GH action in all or most peripheral tissues and the liver. This will be a challenging quest since to date these markers are not available.

Where should we look for this "grail"? Liver, muscle, kidney and fat are the tissues to go for. The kind of marker should be stable over long periods. GH and cytokines seem to interact on different levels. However these cytokines are highly variable and typically hard

to assess. There is a relationship between the GH-IGF1 system and cytokines. Both receptors, GH and IGF1 use for signalling, suppressor of cytokine signalling (SOCS) proteins [54]. These SOCS proteins increase after stimulation of the cell by cytokines. Imbalance of SOCS2 activity results in excessive somatic growth stimulated by the GH/IGF1 system. SOCS2 regulates GH and IGF1 signalling by preventing activation of signal transducers and activators of transcription (STATs). For GH this is STAT5 and for IGF1 STAT3 [54]. SOCS2 knockout mice do not have an increased GH or IGF1, although phenotypically they are lean giants [55]. In SOCS2 knockout mice, in which GH is overactive, insulin seems to enhance GH action further without IGF1 change [56]. Lastly, SOCS2 seems to play a role in the development of diabetes mellitus type 2 [57]. Thus, the SOCS KO mouse model could be a good candidate to use for future study of our concept of hepatic acromegaly and peripheral acromegaly. However, these mice have an altered immune response and are more sensitive to the actions of GH. Therefore, it is questionable if SRIFs or PEG-V will have any effects in SOCS2 KO mice. Concluding, a thorough examination of the tissues mentioned above appears the best step to take. Possibly the SOCS2 knock out can help in the search for a relevant biomarker, but the ideal mouse model is not available yet.

In conclusion; SRIF and PEG-V exert complementary suppressive effects on the GH-IGF1 axis and this makes combination treatment an interesting option. This is especially the case, since the addition of PEG-V to SRIF controlled acromegaly patients results in an increased QoL. However novel biomarkers are necessary to assess disease activity peripherally and in the liver.

Chapter 6

Circulating serum GH levels increase during PEG-V treatment, but when PEG-V is combined with an SRIF analog, elevations in the serum concentration of GH are lower than they are observed during PEG-V monotherapy [7,18]. The increase in GH during PEG-V treatment could possibly be explained by its blockade of hepatic GHR leading to suppression of IGF1 levels and a reduction in its negative feedback effects at the hypothalamus and pituitary gland [7,6]. Disruption of this regulatory mechanism could lead to tumour growth [1,7].

Different areas in the brain, as well as the pituitary, express GHR, as assessed by binding studies [58] and GHR mRNA analysis [59]. PEG-V seems to be incapable of penetrating the blood brain barrier [60], making an effect of PEG-V via the central nervous system unlikely. However, the pituitary gland is outside the blood brain barrier, which means it is accessible to PEG-V. GH itself could influence the pituitary via an ultra-short feedback loop as shown for thyroid stimulating hormone (TSH) [61]. Via a similar mechanism, PEG-V blockade of the pituitary GHR could increase GH production further and influence tumour growth.

Messenger RNA expression of somatotroph adenomas were assessed as well as GH levels in the supernatant of primary pituitary cell cultures after the addition of PEG-V. Low mRNA expression of full length GHR and the d3GHR was found in 32 somatotroph pituitary adenomas. The addition of PEG-V in different concentrations (10^{-6}-10^{-9}M) during 24 and 72 h incubation did not increase GH concentrations in the supernatant. The observed GH increase during PEG-V treatment *in vivo* could be due to GHR blockade in other parts of the brain, such as the hypothalamus [58]. However, Veldhuis and coworkers recently assessed PEG-V in the cerebrospinal fluid (CSF). The uptake of GH was 1500 fold higher than that of PEG-V [60]. Therefore, it seems very unlikely that a direct effect via the central nervous system could explain the increase in GH levels. The GHR in our somatotroph pituitary adenomas could be dysfunctional [62]. This could explain the lack of GH increase after the addition of PEG-V. The doses of PEG-V to assess the GH response *in vitro* was of the same magnitude as those used in acromegalic patients to control IGF1, more than 10 times the amount of GH produced by the adenoma cells. Finally, IGF1 decreases GH levels secreted by the pituitary [63], which has been observed by many others [64-65].

In conclusion, our study of somatotroph adenoma GHR mRNA indicates very low levels of expression, and that the addition of PEG-V was not able to increase GH production by primary adenoma cells in vitro. Therefore, the increase in GH level during PEG-V treatment in acromegalic patients seems not be mediated by interference of an ultra-short feedback loop via GHR in the somatotroph adenoma, but rather via a reduced feedback due to the lowered circulating IGF1 levels.

REFERENCES

1. van der Lely AJ, Muller A, Janssen JA, Davis RJ, Zib KA, Scarlett JA, Lamberts SW (2001) Control of tumor size and disease activity during cotreatment with octreotide and the growth hormone receptor antagonist pegvisomant in an acromegalic patient. The Journal of clinical endocrinology and metabolism 86 (2):478-481
2. Feenstra J, de Herder WW, ten Have SM, van den Beld AW, Feelders RA, Janssen JA, van der Lely AJ (2005) Combined therapy with somatostatin analogues and weekly pegvisomant in active acromegaly. Lancet 365 (9471):1644-1646
3. Melmed S (2006) Medical progress: Acromegaly. The New England journal of medicine 355 (24): 2558-2573
4. Castinetti F, Nagai M, Morange I, Dufour H, Caron P, Chanson P, Cortet-Rudelli C, Kuhn JM, Conte-Devolx B, Regis J, Brue T (2009) Long-term results of stereotactic radiosurgery in secretory pituitary adenomas. The Journal of clinical endocrinology and metabolism 94 (9):3400-3407. doi: jc.2008-2772 [pii] 10.1210/jc.2008-2772
5. Melmed S, Colao A, Barkan A, Molitch M, Grossman AB, Kleinberg D, Clemmons D, Chanson P, Laws E, Schlechte J, Vance ML, Ho K, Giustina A (2009) Guidelines for acromegaly management: an update. The Journal of clinical endocrinology and metabolism 94 (5):1509-1517
6. Trainer PJ, Drake WM, Katznelson L, Freda PU, Herman-Bonert V, van der Lely AJ, Dimaraki EV, Stewart PM, Friend KE, Vance ML, Besser GM, Scarlett JA, Thorner MO, Parkinson C, Klibanski A, Powell JS, Barkan AL, Sheppard MC, Malsonado M, Rose DR, Clemmons DR, Johannsson G, Bengtsson BA, Stavrou S, Kleinberg DL, Cook DM, Phillips LS, Bidlingmaier M, Strasburger CJ, Hackett S, Zib K, Bennett WF, Davis RJ (2000) Treatment of acromegaly with the growth hormone-receptor antagonist pegvisomant. The New England journal of medicine 342 (16):1171-1177
7. van der Lely AJ, Hutson RK, Trainer PJ, Besser GM, Barkan AL, Katznelson L, Klibanski A, Herman-Bonert V, Melmed S, Vance ML, Freda PU, Stewart PM, Friend KE, Clemmons DR, Johannsson G, Stavrou S, Cook DM, Phillips LS, Strasburger CJ, Hackett S, Zib KA, Davis RJ, Scarlett JA, Thorner MO (2001) Long-term treatment of acromegaly with pegvisomant, a growth hormone receptor antagonist. Lancet 358 (9295):1754-1759
8. Holdaway IM (2004) Treatment of acromegaly. Hormone research 62 Suppl 3:79-92
9. Abosch A, Tyrrell JB, Lamborn KR, Hannegan LT, Applebury CB, Wilson CB (1998) Transsphenoidal microsurgery for growth hormone-secreting pituitary adenomas: initial outcome and long-term results. The Journal of clinical endocrinology and metabolism 83 (10):3411-3418
10. Barker FG, 2nd, Klibanski A, Swearingen B (2003) Transsphenoidal surgery for pituitary tumors in the United States, 1996-2000: mortality, morbidity, and the effects of hospital and surgeon volume. The Journal of clinical endocrinology and metabolism 88 (10):4709-4719
11. Kreutzer J, Vance ML, Lopes MB, Laws ER, Jr. (2001) Surgical management of GH-secreting pituitary adenomas: an outcome study using modern remission criteria. The Journal of clinical endocrinology and metabolism 86 (9):4072-4077
12. Swearingen B, Barker FG, 2nd, Katznelson L, Biller BM, Grinspoon S, Klibanski A, Moayeri N, Black PM, Zervas NT (1998) Long-term mortality after transsphenoidal surgery and adjunctive therapy for acromegaly. The Journal of clinical endocrinology and metabolism 83 (10):3419-3426
13. Bates PR, Carson MN, Trainer PJ, Wass JA, Group UKNARS (2008) Wide variation in surgical outcomes for acromegaly in the UK. Clinical endocrinology 68 (1):136-142
14. Neggers SJ, de Herder WW, Janssen JA, Feelders RA, van der Lely AJ (2009) Combined treatment for acromegaly with long-acting somatostatin analogs and pegvisomant: long-term safety for up

to 4.5 years (median 2.2 years) of follow-up in 86 patients. European journal of endocrinology / European Federation of Endocrine Societies 160 (4):529-533

15. Neggers SJ, van Aken MO, Janssen JA, Feelders RA, de Herder WW, van der Lely AJ (2007) Long-term efficacy and safety of combined treatment of somatostatin analogs and pegvisomant in acromegaly. The Journal of clinical endocrinology and metabolism 92 (12):4598-4601
16. Trainer PJ (2009) ACROSTUDY: the first 5 years. European journal of endocrinology / European Federation of Endocrine Societies 161 Suppl 1:S19-24. doi:EJE-09-0322 [pii] 10.1530/EJE-09-0322
17. Neggers SJ, van der Lely AJ (2009) Somatostatin analog and pegvisomant combination therapy for acromegaly. Nature reviews 5 (10):546-552
18. Jorgensen JO, Feldt-Rasmussen U, Frystyk J, Chen JW, Kristensen LO, Hagen C, Orskov H (2005) Co-treatment of acromegaly with a somatostatin analog and a growth hormone receptor antagonist. The Journal of clinical endocrinology and metabolism 90 (10):5627-5631
19. Wurzburger MI, Prelevic GM, Sonksen PH, Balint-Peric LA, Wheeler M (1993) The effect of recombinant human growth hormone on regulation of growth hormone secretion and blood glucose in insulin-dependent diabetes. The Journal of clinical endocrinology and metabolism 77 (1): 267-272
20. Shishko PI, Dreval AV, Abugova IA, Zajarny IU, Goncharov VC (1994) Insulin-like growth factors and binding proteins in patients with recent-onset type 1 (insulin-dependent) diabetes mellitus: influence of diabetes control and intraportal insulin infusion. Diabetes research and clinical practice 25 (1):1-12
21. Leung KC, Doyle N, Ballesteros M, Waters MJ, Ho KK (2000) Insulin regulation of human hepatic growth hormone receptors: divergent effects on biosynthesis and surface translocation. The Journal of clinical endocrinology and metabolism 85 (12):4712-4720
22. Murray RD, Kim K, Ren SG, Chelly M, Umehara Y, Melmed S (2004) Central and peripheral actions of somatostatin on the growth hormone-IGF-I axis. The Journal of clinical investigation 114 (3): 349-356
23. Shi YF, Zhu XF, Harris AG, Zhang JX, Dai Q (1993) Prospective study of the long-term effects of somatostatin analog (octreotide) on gallbladder function and gallstone formation in Chinese acromegalic patients. The Journal of clinical endocrinology and metabolism 76 (1):32-37
24. Schreiber I, Buchfelder M, Droste M, Forssmann K, Mann K, Saller B, Strasburger CJ, German Pegvisomant I (2007) Treatment of acromegaly with the GH receptor antagonist pegvisomant in clinical practice: safety and efficacy evaluation from the German Pegvisomant Observational Study. European journal of endocrinology / European Federation of Endocrine Societies 156 (1):75-82
25. Van der Lely AJ, Bernabeu I, Cap J, Caron P, Colao AA, Marek J, Neggers SJ, Birman P (2010) Coadministration of lanreotide Autogel(R) and pegvisomant normalizes IGF1 levels and is well tolerated in patients with acromegaly partially controlled by somatostatin analogs alone. European journal of endocrinology / European Federation of Endocrine Societies. doi:EJE-10-0867 [pii] 10.1530/EJE-10-0867
26. Neggers SJ, van Aken MO, de Herder WW, Feelders RA, Janssen JA, Badia X, Webb SM, van der Lely AJ (2008) Quality of life in acromegalic patients during long-term somatostatin analog treatment with and without pegvisomant. The Journal of clinical endocrinology and metabolism 93 (10): 3853-3859
27. Feenstra J, van Aken MO, de Herder WW, Feelders RA, van der Lely AJ (2006) Drug-induced hepatitis in an acromegalic patient during combined treatment with pegvisomant and octreotide long-acting repeatable attributed to the use of pegvisomant. European journal of endocrinology / European Federation of Endocrine Societies 154 (6):805-806

28. Hodish I, Barkan A, Medscape (2008) Long-term effects of pegvisomant in patients with acromegaly. Nature clinical practice 4 (6):324-332
29. Bernabeu I, Marazuela M, Lucas T, Loidi L, Alvarez-Escola C, Luque-Ramirez M, Fernandez-Rodriguez E, Paniagua AE, Quinteiro C, Casanueva FF (2010) Pegvisomant-induced liver injury is related to the UGT1A1*28 polymorphism of Gilbert's syndrome. The Journal of clinical endocrinology and metabolism 95 (5):2147-2154. doi:jc.2009-2547 [pii] 10.1210/jc.2009-2547
30. Buchfelder M, Weigel D, Droste M, Mann K, Saller B, Brubach K, Stalla GK, Bidlingmaier M, Strasburger CJ (2009) Pituitary tumor size in acromegaly during pegvisomant treatment: experience from MR re-evaluations of the German Pegvisomant Observational Study. European journal of endocrinology / European Federation of Endocrine Societies 161 (1):27-35. doi:EJE-08-0910 [pii] 10.1530/EJE-08-0910
31. Bonert VS, Kennedy L, Petersenn S, Barkan A, Carmichael J, Melmed S (2008) Lipodystrophy in patients with acromegaly receiving pegvisomant. The Journal of clinical endocrinology and metabolism 93 (9):3515-3518
32. Maffei P, Martini C, Pagano C, Sicolo N, Corbetti F (2006) Lipohypertrophy in acromegaly induced by the new growth hormone receptor antagonist pegvisomant. Annals of internal medicine 145 (4):310-312
33. Parkinson C, Drake WM, Roberts ME, Meeran K, Besser GM, Trainer PJ (2002) A comparison of the effects of pegvisomant and octreotide on glucose, insulin, gastrin, cholecystokinin, and pancreatic polypeptide responses to oral glucose and a standard mixed meal. The Journal of clinical endocrinology and metabolism 87 (4):1797-1804
34. De Marinis L, Bianchi A, Fusco A, Cimino V, Mormando M, Tilaro L, Mazziotti G, Pontecorvi A, Giustina A (2007) Long-term effects of the combination of pegvisomant with somatostatin analogs (SSA) on glucose homeostasis in non-diabetic patients with active acromegaly partially resistant to SSA. Pituitary 10 (3):227-232
35. Biochemical assessment and long-term monitoring in patients with acromegaly: statement from a joint consensus conference of the Growth Hormone Research Society and the Pituitary Society (2004). The Journal of clinical endocrinology and metabolism 89 (7):3099-3102
36. Giustina A, Barkan A, Casanueva FF, Cavagnini F, Frohman L, Ho K, Veldhuis J, Wass J, Von Werder K, Melmed S (2000) Criteria for cure of acromegaly: a consensus statement. The Journal of clinical endocrinology and metabolism 85 (2):526-529
37. Biermasz NR, Dekker FW, Pereira AM, van Thiel SW, Schutte PJ, van Dulken H, Romijn JA, Roelfsema F (2004) Determinants of survival in treated acromegaly in a single center: predictive value of serial insulin-like growth factor I measurements. The Journal of clinical endocrinology and metabolism 89 (6):2789-2796
38. Biermasz NR, Pereira AM, Smit JW, Romijn JA, Roelfsema F (2005) Morbidity after long-term remission for acromegaly: persisting joint-related complaints cause reduced quality of life. The Journal of clinical endocrinology and metabolism 90 (5):2731-2739
39. Bonapart IE, van Domburg R, ten Have SM, de Herder WW, Erdman RA, Janssen JA, van der Lely AJ (2005) The 'bio-assay' quality of life might be a better marker of disease activity in acromegalic patients than serum total IGF-I concentrations. European journal of endocrinology / European Federation of Endocrine Societies 152 (2):217-224
40. Hua SC, Yan YH, Chang TC (2006) Associations of remission status and lanreotide treatment with quality of life in patients with treated acromegaly. European journal of endocrinology / European Federation of Endocrine Societies 155 (6):831-837

41. Rowles SV, Prieto L, Badia X, Shalet SM, Webb SM, Trainer PJ (2005) Quality of life (QOL) in patients with acromegaly is severely impaired: use of a novel measure of QOL: acromegaly quality of life questionnaire. The Journal of clinical endocrinology and metabolism 90 (6):3337-3341
42. Webb SM, Prieto L, Badia X, Albareda M, Catala M, Gaztambide S, Lucas T, Paramo C, Pico A, Lucas A, Halperin I, Obiols G, Astorga R (2002) Acromegaly Quality of Life Questionnaire (ACROQOL) a new health-related quality of life questionnaire for patients with acromegaly: development and psychometric properties. Clinical endocrinology 57 (2):251-258
43. Badia X, Webb SM, Prieto L, Lara N (2004) Acromegaly Quality of Life Questionnaire (AcroQoL). Health Qual Life Outcomes 2:13. doi:10.1186/1477-7525-2-13 1477-7525-2-13 [pii]
44. Webb SM (2006) Quality of life in acromegaly. Neuroendocrinology 83 (3-4):224-229
45. Segev Y, Landau D, Rasch R, Flyvbjerg A, Phillip M (1999) Growth hormone receptor antagonism prevents early renal changes in nonobese diabetic mice. J Am Soc Nephrol 10 (11):2374-2381
46. Keenan AM, McKenna SP, Doward LC, Conaghan PG, Emery P, Tennant A (2008) Development and validation of a needs-based quality of life instrument for osteoarthritis. Arthritis and rheumatism 59 (6):841-848
47. Linde L, Sorensen J, Ostergaard M, Horslev-Petersen K, Hetland ML (2008) Health-related quality of life: validity, reliability, and responsiveness of SF-36, 15D, EQ-5D [corrected] RAQoL, and HAQ in patients with rheumatoid arthritis. The Journal of rheumatology 35 (8):1528-1537
48. Rabinowitz D, Merimee TJ, Burgess JA, Riggs L (1966) Growth hormone and insulin release after arginine: indifference to hyperglycemia and epinephrine. The Journal of clinical endocrinology and metabolism 26 (10):1170-1172
49. Neggers SJ, Kopchick JJ, Jorgensen JO, van der Lely AJ (2011) Hypothesis: Extra-hepatic acromegaly: a new paradigm? European journal of endocrinology / European Federation of Endocrine Societies 164 (1):11-16. doi:EJE-10-0969 [pii] 10.1530/EJE-10-0969
50. Flyvbjerg A, Bennett WF, Rasch R, Kopchick JJ, Scarlett JA (1999) Inhibitory effect of a growth hormone receptor antagonist (G120K-PEG) on renal enlargement, glomerular hypertrophy, and urinary albumin excretion in experimental diabetes in mice. Diabetes 48 (2):377-382
51. Koop BL, Harris AG, Ezzat S (1994) Effect of octreotide on glucose tolerance in acromegaly. European journal of endocrinology / European Federation of Endocrine Societies 130 (6):581-586
52. Rubeck KZ, Madsen M, Andreasen CM, Fisker S, Frystyk J, Jorgensen JO (2010) Conventional and novel biomarkers of treatment outcome in patients with acromegaly: discordant results after somatostatin analog treatment compared with surgery. European journal of endocrinology / European Federation of Endocrine Societies 163 (5):717-726. doi:EJE-10-0640 [pii] 10.1530/EJE-10-0640
53. Pokrajac A, Frystyk J, Flyvbjerg A, Trainer PJ (2009) Pituitary-independent effect of octreotide on IGF1 generation. European journal of endocrinology / European Federation of Endocrine Societies 160 (4):543-548. doi:EJE-08-0822 [pii] 10.1530/EJE-08-0822
54. Rico-Bautista E, Flores-Morales A, Fernandez-Perez L (2006) Suppressor of cytokine signaling (SOCS) 2, a protein with multiple functions. Cytokine Growth Factor Rev 17 (6):431-439. doi: S1359-6101(06)00057-8 [pii] 10.1016/j.cytogfr.2006.09.008
55. Horvat S, Medrano JF (2001) Lack of Socs2 expression causes the high-growth phenotype in mice. Genomics 72 (2):209-212. doi:10.1006/geno.2000.6441 S0888-7543(00)96441-3 [pii]
56. Rico-Bautista E, Greenhalgh CJ, Tollet-Egnell P, Hilton DJ, Alexander WS, Norstedt G, Flores-Morales A (2005) Suppressor of cytokine signaling-2 deficiency induces molecular and metabolic changes that partially overlap with growth hormone-dependent effects. Mol Endocrinol 19 (3): 781-793. doi:me.2004-0040 [pii] 10.1210/me.2004-0040

57. Kato H, Nomura K, Osabe D, Shinohara S, Mizumori O, Katashima R, Iwasaki S, Nishimura K, Yoshino M, Kobori M, Ichiishi E, Nakamura N, Yoshikawa T, Tanahashi T, Keshavarz P, Kunika K, Moritani M, Kudo E, Tsugawa K, Takata Y, Hamada D, Yasui N, Miyamoto T, Shiota H, Inoue H, Itakura M (2006) Association of single-nucleotide polymorphisms in the suppressor of cytokine signaling 2 (SOCS2) gene with type 2 diabetes in the Japanese. Genomics 87 (4):446-458. doi: S0888-7543(05)00332-0 [pii] 10.1016/j.ygeno.2005.11.009
58. Nyberg F (2000) Growth hormone in the brain: characteristics of specific brain targets for the hormone and their functional significance. Front Neuroendocrinol 21 (4):330-348. doi:10.1006/frne.2000.0200 S0091-3022(00)90200-0 [pii]
59. Beuschlein F, Hancke K, Petrick M, Gobel H, Honegger J, Reincke M (2005) Growth hormone receptor mRNA expression in non-functioning and somatotroph pituitary adenomas: implications for growth hormone substitution therapy? Exp Clin Endocrinol Diabetes 113 (4):214-218. doi: 10.1055/s-2005-837668
60. Veldhuis JD, Bidlingmaier M, Bailey J, Erickson D, Sandroni P (2010) A pegylated growth hormone receptor antagonist, pegvisomant, does not enter the brain in humans. The Journal of clinical endocrinology and metabolism 95 (8):3844-3847. doi:jc.2010-0538 [pii] 10.1210/jc.2010-0538
61. Brokken LJ, Scheenhart JW, Wiersinga WM, Prummel MF (2001) Suppression of serum TSH by Graves' Ig: evidence for a functional pituitary TSH receptor. The Journal of clinical endocrinology and metabolism 86 (10):4814-4817
62. Asa SL, Digiovanni R, Jiang J, Ward ML, Loesch K, Yamada S, Sano T, Yoshimoto K, Frank SJ, Ezzat S (2007) A growth hormone receptor mutation impairs growth hormone autofeedback signaling in pituitary tumors. Cancer Res 67 (15):7505-7511. doi:67/15/7505 [pii] 10.1158/0008-5472.CAN-07-0219
63. Lamberts SW, van Koetsveld P, Hofland L (1989) A close correlation between the inhibitory effects of insulin-like growth factor-I and SMS 201-995 on growth hormone release by acromegalic pituitary tumours in vitro and in vivo. Clinical endocrinology 31 (4):401-410
64. Berelowitz M, Szabo M, Frohman LA, Firestone S, Chu L, Hintz RL (1981) Somatomedin-C mediates growth hormone negative feedback by effects on both the hypothalamus and the pituitary. Science (New York, NY 212 (4500):1279-1281
65. Brazeau P, Vale W, Burgus R, Ling N, Butcher M, Rivier J, Guillemin R (1973) Hypothalamic polypeptide that inhibits the secretion of immunoreactive pituitary growth hormone. Science (New York, NY 179 (68):77-79

Chapter 9

Summary/Samenvatting

Summary

Acromegaly is a rare disease, but has the attention of many physicians, researchers and pharmaceutical companies. This could be caused by the fascination these professionals have for this rare disease. This fascination goes back for centuries. In the last four decades three new groups of medication for the treatment of acromegaly have been discovered: dopamine agonists, somatostatin analogs and a growth hormone antagonist. The last to be discovered was pegvisomant, the growth hormone receptor antagonist. Pegvisomant is the most effective (medical) treatment to date.

In **Chapter 2** the long-term efficacy and safety of combination treatment with long-acting somatostatin analogs and weekly pegvisomant was reported. Efficacy of more than 90%, in normalizing IGF1 was observed. This is of the same magnitude as pegvisomant monotherapy. However, the necessary dose to control IGF1 seems to be lower. This results in major cost reductions. In eight of the 32 subjects examined, elevated transaminases occurred during combination treatment. Two other patients had liver enzyme disturbances, which were due to bile obstruction. Diabetic acromegaly patients had 5.1 times higher risk for developing liver enzyme disturbances than non-diabetics. However, no patient had to interrupt the combined treatment. No tumor size increase was observed in any patient and in 13% of the patients a decrease in pituitary tumor size was observed.

In **Chapter 3** the long-term safety of combination treatment with long- acting somatostatin analogs and weekly pegvisomant was reported, following a safety warning by the Canadian Health Authorities. The safety warning relating to liver enzyme disturbances was based on 26 patients during combination treatment. In a cohort of 86 patients, presented in chapter 3, 13/86 patients developed liver enzyme disturbances vs. 12/229 during monotherapy pegvisomant. Diabetics did not have a higher risk for developing liver enzyme disturbances of more than 3 times the upper limit of normal. Again an efficacy rate of more than 90% in normalizing IGF1 was observed. In 19% of patients a decrease of more than 20% in pituitary tumor size was observed.

Although in the previous chapters the efficacy of combination treatment at a lower mean weekly dose of pegvisomant was discussed, no direct comparison was possible.

In **Chapter 4**, two patients controlled with high dose pegvisomant were converted to combined treatment. The dose of pegvisomant was greatly reduced by 80-150 mg a week. This resulted in a cost reduction of between €16,000 and 40,000 annually. However, the addition of a somatostatin analog to the treatment regimen increased fasting glucose and glycosylated hemoglobin levels, necessitating the addition of glucose lowering medication.

From the patient's perspective, an important parameter of disease control is the quality of life. Since this is difficult to assess in day-to-day practice, physicians tend to be focused more on growth hormone and IGF1 serum concentrations. To date normalization of the biochemical markers in acromegaly is facile; physicians should endeavor for a higher goal and start to focus more on quality of life.

In the study described in **Chapter 5** a low dose of weekly pegvisomant was added to the treatment of acromegalics with normal IGF1 levels receiving monotherapy with somatostatin analogs, to assess a change in quality of life. This was assessed in a prospective, investigator-initiated, double blind, placebo-controlled, crossover study. The addition of pegvisomant increased quality of life assessed by Acromegaly Quality of Life Questionnaire (AcroQoL) and Patient-Assessed Acromegaly Symptom Questionnaire. The magnitude of the improvement in AcroQoL was equal to the improvement which can be observed when naïve acromegaly patients achieve normalization of IGF1. The typical growth hormone related symptoms such as perspiration, soft tissue swelling, and the AcroQoL physical can be improved without a change in IGF1 levels. This suggests that, although IGF1 levels are normal, peripheral growth hormone action is still too high. Therefore, it is questionable if it is correct to use the step-up approach in which patients are treated only with pegvisomant when somatostatin analog monotherapy is unable to normalize IGF-I levels.

Pegvisomant treatment is highly effective in controlling IGF1, but during treatment growth hormone levels increase. The exact mechanism behind this remains unclear.

In **Chapter 6** a possible mechanism for the increase in growth hormone was assessed. An ultra-short feedback loop via GH receptors in the anterior pituitary gland, as shown for another pituitary hormone thyroid stimulating hormone, could explain this. In 32 somatotroph adenomas and 4 samples of human liver tissue after RNA extraction, mRNA expression of the full length growth hormone receptor (GHR) and d3GHR variant were assessed by real time PCR. The mRNA expression of both GHRs at the pituitary adenoma level is low. After 24 and 72 hours of incubation with different concentrations (10^{-6}-10^{-9}M) of pegvisomant, growth hormone secretion was assessed in three primary pituitary adenoma cell cultures. The addition of pegvisomant had no effect on growth hormone secretion by these cells.

Therefore, the increase in growth hormone levels during pegvisomant treatment in acromegaly patients seems not to be mediated by interference of an ultra-short feedback loop via GHR in the somatotroph adenoma, but rather via reduced feedback due to lowered circulating IGF-I levels.

Medical treatment of acromegaly with long-acting somatostatin analogs and the growth hormone receptor antagonist, pegvisomant, has made it possible to achieve normal serum IGF1 concentrations in a majority of patients with acromegaly. These two compounds, however, impact upon the growth hormone–IGF1 axis in different ways, challenging the traditional biochemical assessment of the therapeutic response.

In **Chapter 7** a new hypothesis "extra-hepatic acromegaly" is clarified with animal and human data. Pegvisomant monotherapy in acromegaly leads to a blockade of peripheral GH action without decreasing IGF1, which we could call "hepatic acromegaly". Normalization of IGF1 with pegvisomant treatment could cause peripheral tissues to become growth hormone deficient. However, somatostatin analogs have the opposite effect. Somatostatin analogs reduce growth hormone and thereby IGF1. Additionally, they also decrease insulin and glucagon. This can result in a worsening of glycemic control during long-term somatostatin analog treatment. The lower portal insulin level selectively results in hepatic growth hormone resistance, by decreasing the number of growth hormone receptors in the liver. This GH resistance ensues as a result of the reduction in circulating IGF1. IGF1 suppression by SRIF can be growth hormone independent in humans. Therefore, normal IGF1 levels in acromegaly during somatostatin analog treatment does not necessarily imply disease control in peripheral tissues. Since somatostatin analogs make the liver growth hormone resistant, but do not control the acromegaly in peripheral tissues, patients still have a decreased quality of life and continuing symptoms. This we could call extra-hepatic acromegaly. Somatostatin analogs and pegvisomant exert complementary suppressive effects on the growth hormone-IGF1 axis making combination treatment an interesting option. This is especially the case, since the addition of pegvisomant to somatostatin analog controlled acromegaly patients results in an increased quality of life. However, novel biomarkers are necessary to assess disease activity in peripheral tissues and the liver.

Samenvatting

Acromegalie is een zeldzame aandoening, maar op een of andere manier zijn vele artsen, onderzoekers en farmaceutische bedrijven geïnteresseerd. Dit kan verklaard worden door de eeuwenlange fascinatie voor deze zeldzame ziekte. In de laatste 4 decennia zijn 3 nieuwe groepen medicijnen ontdekt voor de behandeling van acromegalie, namelijk dopamine agonisten, somatostatine analoga en een groeihormoon antagonist. De meest recente ontdekking was pegvisomant, een groeihormoon receptor antagonist. Pegvisomant is het meest effectieve geneesmiddel op dit moment.

In **hoofdstuk 2** worden de lange termijn effectiviteits- en veiligheidsgegevens van de combinatiebehandeling van somatostatine analoga met wekelijkse pegvisomant beschreven. De effectiviteit van de combinatie behandeling om het IGF1 te normaliseren is meer dan 90%. Dit komt overeen met de pegvisomant monotherapie, echter is de dosis om dit te bereiken lager. Het gevolg hiervan is dat combinatietherapie goedkoper is dan monotherapie pegvisomant. In acht van de 32 acromegalie patiënten werden verhoogde transaminasen gevonden tijdens combinatiebehandeling. Twee andere patiënten hadden cholestatische leverenzym afwijkingen. Patiënten met diabetes mellitus en acromegalie hadden een 5.1 hoger risico op het ontwikkelen van leverenzymstoornissen. Ondanks de leverenzymstoornissen heeft niemand de therapie hoeven te onderbreken. In geen van de patiënten werd tumorgroei van het hypofyse adenoom gezien, maar in 13% van de patiënten werd afname van het tumorvolume gezien.

In **hoofdstuk 3** worden de lange termijn veiligheidsgegevens van de combinatie behandeling van somatostatine analoga met wekelijkse pegvisomant beschreven naar aanleiding van een veiligheidswaarschuwing van de Canadese gezondheidsautoriteiten. De waarschuwing was gebaseerd op een studie van 26 patiënten waarin een deel leverenzymstoornissen ontwikkelden. In een groep van 86 patiënten, welke beschreven worden in hoofdstuk 3, ontwikkelden 13 patiënten leverenzymstoornissen. Tijdens monotherapie pegvisomant waren dat 12 van de 229 patiënten. Diabetici met acromegalie hadden nu geen hoger risico meer op het ontwikkelen van leverenzymstoornissen van meer dan 3 maal de boven limiet. De effectiviteit om IGF1 te normaliseren was wederom meer dan 90%. Tumorvolume afname, van meer dan 20%, werd in meer dan 19% van de patiënten gevonden.

Ondanks dat in de voorgaande hoofdstukken beschreven wordt dat de combinatiebehandeling net zo effectief is als de monotherapie pegvisomant, alleen met een gemiddeld lagere wekelijkse dosis, is er geen direct vergelijk tussen monotherapie pegvisomant en combinatie therapie mogelijk.

In **hoofdstuk 4**, twee patiënten die normale IGF1 waarden hadden met hoge dosis pegvisomant monotherapie werden geconverteerd naar combinatietherapie. De dosis reductie die behaald werd, verschilde enorm van 80-150 mg per week tussen beide patiënten. Dit levert een kostenbesparing op van €16.000 en €40.000 jaarlijks. Echter de toevoeging van somatostatine analoog leidde tot een verhoogde nuchtere glucose en HbA1c. Door glucos verlagende geneesmiddel verbeterden deze waarden weer.

Vanuit het perspectief van de patiënt gezien is de kwaliteit van leven misschien wel de meest belangrijke parameter. Echter is dit moeilijk meetbaar in de dagelijkse praktijk, misschien dat daarom de meeste artsen zich met name richten op IGF1 en groeihormoon. Tegenwoordig is het normaliseren van de biochemische waarden in het plasma geen probleem. Daarom zouden artsen naar een nieuwe uitdaging moeten zoeken namelijk kwaliteit van leven.

In **hoofdstuk 5** werd er aan de behandeling van acromegalie patiënten die met somatostatine analoga normale IGF1 waarden hadden, een wekelijks lage dosis pegvisomant toegevoegd om te bestuderen of de kwaliteit van leven zou toenemen. Dit werd in een "prospective, investigator-initiated, double blind, placebo-controlled, crossover" studie verricht. Door de toevoeging van pegvisomant nam de kwaliteit van leven toe, welke werd gemeten met de Acromegaly Quality of Life Questionnaire (AcroQoL) en de Patient-Assessed Acromegaly Symptom Questionnaire. De toename in kwaliteit van leven was gelijk aan de toename die men kan waarnemen als een acromegalie patiënt van verhoogde IGF1 daalt naar een normale waarde. De typische groeihormoon gerelateerde symptomen zoals zweten, weke delen zwelling en de fysieke dimensie van de AcroQoL verbeterde, zonder dat het IGF1 daalde. Dit suggereert, dat er ondanks de normale IGF1 waarden in perifere weefsel nog steeds een overschot aan groeihormoon te zien is. Het is daarom de vraag of combinatie therapie gereserveerd moet zijn voor die patiënten die een verhoogd IGF1 hebben.

Pegvisomant behandeling is erg effectief in het normaliseren van IGF1, maar dit leidt tot toename in groeihormoon waarden. De verklaring hiervoor is onduidelijk. In **hoofdstuk 6** wordt een mogelijk mechanisme voor de toename in groeihormoon die tijdens de behandeling met pegvisomant optreedt onderzocht. Een mogelijke verklaring zou een ultra-short feedback loop via de groeihormoon receptoren op de hypofyse kunnen zijn. Bij TSH is een soortgelijk systeem bekend. In 32 somatotrophe hypofyse adenomen en 4 stukjes leverweefsel werd er na RNA extractie, mRNA gemeten voor groeihormoon receptor (GHR) en de GHR variant door middel van een real time PCR. De mRNA expressie van beide hypofyse GHRs was laag. Na een incubatie van 24 en 72 uur met pegvisomant, in oplopende concentraties (10^{-6}-10^{-9}M), werd de groeihormoon concentratie gemeten in drie hypofyse adenomen. De toevoeging van pegvisomant leidde niet tot een toename van groeihormoon secretie in de hypofyse adenomen media.

Daarom is de toename van groeihormoon spiegels, gedurende pegvisomant therapie niet te verklaren door een ultra-short feedback loop, maar door de daling van het IGF1 in het serum van patiënten.

Behandeling van acromegalie met somatostatine analoga en een groeihormoon receptor antagonist, pegvisomant, heeft het eenvoudig gemaakt om normale IGF1 waarden te bereiken in de meerderheid van de acromegalie patiënten. Toch hebben deze beide middelen een totaal verschillend effect op de groeihormoon-IGF1 as. Dit heeft invloed op de wijzen waarop er naar de bekende biochemische parameters wordt gekeken.

In **hoofdstuk 7** wordt een nieuwe hypothese "extra hepatische acromegalie" beschreven. Deze hypothese wordt onderbouwd door gegevens die afkomstig zijn uit onderzoek in dier en mensen. De behandeling met alleen pegvisomant leidt in eerste instantie tot een blokkade van de perifere groeihormoon receptoren zonder een verlaging van het serum IGF1. Dit noemen we "hepatische acromegalie". Om met pegvisomant een verlaging van het IGF1 te bereiken zal de dosis zo hoog zijn dat de perifere weefsels waarschijnlijk groeihormoon deficiënt worden. Bij somatostatine analoga is dat precies andersom. Somatostatine analoga verlagen groeihormoon en daardoor IGF1. Daar bovenop verlagen ze insuline en glucagon. Dit leidt op de langere termijn tot een verslechtering van de glucose homeostase, bij het gebruik van somatostatine analoga. De lagere concentratie insuline in de poortader leidt tot een afname van groeihormoon receptoren op de lever, wat op zijn beurt weer leidt tot een afname van IGF1 concentratie. Maar somatostatine analoga kunnen ook direct de productie van IGF1 door de lever verminderen. Daarom is een normaal IGF1 bij acromegalie patiënten die behandeld worden met een somatostatine analoog geen garantie dat het groeihormoon ook normaal is. Omdat de somatostatine analoga de lever groeihormoon resistent maken, maar de perifere weefsels nog steeds te veel groeihormoon laten zien. Dit noemen we "extra hepatische acromegalie". Deze extra hepatische acromegalie leidt tot symptomen en een afgenomen kwaliteit van leven bij acromegalie patiënten. Kortom pegvisomant en somatostatine analoga hebben in zekere zin een tegengestelde werking op de groeihormoon-IGF1 as en dit maakt een combinatie van beide middelen erg aantrekkelijk. Zeker omdat de toevoeging van pegvisomant aan somatostatine analoga in "gecontroleerde" patiënten een toename in kwaliteit van leven gaf. Maar in de toekomst moet er gezocht worden naar betere bio-markers voor ziekte activiteit op perifeer en lever niveau.

List of abbreviations

AcroQoL	Acromegaly quality of life questionnaire
ALS	Acid-labile-subunit
ALT	Alanine aminotransferase
AST	Aminotransaminase
DM	Diabetes mellitus
EKG	Electrocardiography
GH	Growth hormone
GHR	Growth hormone receptor
GHRH	GH-releasing hormone
GHS-RIa	Ghrelin secretagogue receptor type Ia
GNAS	Guanine nucleotide-binding protein
HbA1c	Glycosylated hemoglobin
IGF1	Insulin-like growth factor 1
IGF1 BP3	IGF1 binding protein 3
INN	Glibenclamide
LAN	Lanreotide autosolution
LFT	Liver function tests
MRCP	Magnetic resonance cholangiopancreatography
MRI	Magnetic Resonance Imaging
MT	Metformin
N/A	Not applicable
OD	Once daily
OW	Once weekly
PASQ	Patient-Assessed Acromegaly Symptom Questionnaire
PEG-V	Pegvisomant
QoL	Quality of life
RT	Radiotherapy
SD	Standard deviation
SMR	Standardized mortality rate
SOCS	Suppressor of cytokine signalling
SOM230	Pasireotide
SRIF	Somatostatin
SST	Somatostatin receptor
STAT	Signal transducers and activators of transcription
TC	Total cholesterol

TD	Twice weekly
TLET	Transient elevated liver enzyme tests
TrD	Trice daily
TSH	Thyroid stimulating hormone
TSS	Transsphenoidal surgery
TW	Twice weekly
ULN	Upper limit of normal
WAT	White adipose tissue
WKS	Weeks
YRS	Years
γ-GT	γ-Glutamyltranspeptidase

Chapter 10

Publications

Neggers SJCMM , van der Meulen J. A Clinical Prediction Rule for Renal Artery Stenosis. Annals of Internal Medicine 1999;131(3): 228.

Verbrugge SJC, Vazquez de Anda G, GommersD, Neggers SJCMM et al. Exogenous surfactant preserves lung function and reduces alveolar Evans blue dye influx in a rat model of ventilation induced lung injury. Anaesthesiology 1998; 89: 467-474.

Verbrugge SJC, Uhlig S, Neggers SJCMM et al. Different ventilatory strategies affect lung function, but do not increase serum TNF-α and PGI_2 levels in lung-lavaged rates in vivo. Anaesthesiology 1999; 91: 1834-43.

Neggers SJCMM et al. Long term efficacy and safety of combined treatment with Somatostatin analogs and Pegvisomant in active acromegaly. J Clin Endocrinol Metab. 2007 Dec;92(12):4598-601.

Neggers SJCMM et al. Significant Improvement of quality of life in biochemically controlled acromegalic patients during long-term somatostatin analog treatment by the addition of low-dose weekly. pegvisomant. J Clin Endocrinol Metab. 2008 Oct;93(10): 3853-9.

Alwani RA & Neggers SJ, et al. Cushing's syndrome due to ectopic ACTH production by (neuroendocrine) prostate carcinoma. Pituitary. 2009;12(3):280-3.

Neggers SJCMM et, al. Combined treatment for acromegaly with long-acting somatostatin analogues and pegvisomant: Long-term safety up to 4.5 years (median 2.2 yrs) of follow-up in 86 patients. Eur J Endocrinol. 2009 Apr;160(4):529-33.

van Casteren NJ, Pieters R, Dohle GR, van Baalen M, Neggers S, et al. Cranial irradiation does not result in pituitary-gonadal axis dysfunction in very long-term male survivors of childhood acute lymphoblastic leukemia. Leukemia. 2009 Dec;23(12):2310-3.

Neggers SJCMM, van der Lely AJ. Review: Combined treatment with long-acting somatostatin analogues and Pegvisomant in acromegaly. Nat Rev Endocrinol. 2009 Oct;5(10): 546-52.

Neggers SJCMM et al. Chapter; Long-term treatment in acromegaly. Acromegaly: A handbook of history, current therapy and future prospects, 2009

Feelders RA, Hofland LJ, van Aken MO, Neggers SJ, et al. Medical therapy of acromegaly: efficacy and safety of somatostatin analogues. Drugs 2009;69(16):2207-26.

van Waas M, Neggers SJ, et al. Components of the metabolic syndrome in 500 adult long-term survivors of childhood cancer. Ann Oncol. 2010 May;21(5):1121-6.

Mulder RL, Kremer LC, van Santen HM, Ket JL, van Trotsenburg AS, Koning CC, Schouten-van Meeteren AY, Caron HN, Neggers SJ, et al. Prevalence and risk factors of radiation-induced growth hormone deficiency in childhood cancer survivors: a systematic review. Cancer Treat Rev. 2009 Nov;35(7):616-32.

Marjolein van Waas, Sebastian J. Neggers et al. The metabolic syndrome in adult survivors of childhood cancer, an overview. J Pediatr Hematol Oncol. 2010 Apr;32(3):171-9.

Neggers SJ, et al. Hypothesis: Extra-hepatic acromegaly: a new paradigm? Eur J Endocrinol. 2011 Jan;164(1):11-6.

Van der Lely AJ, Bernabeu I, Cap J, Caron P, Colao AA, Marek J, Neggers SJ, et al. Co-administration of lanreotide Autogel(R) and pegvisomant normalizes IGF1 levels and is well tolerated in patients with acromegaly partially controlled by somatostatin analogs alone. Eur J Endocrinol. 2011 Mar;164(3):325-333.

Neggers SJ, van der Lely AJ. Modulation of glucocorticoid metabolism by the GH-IGF-I axis. Endocr Dev. 2011;20:181-6.

Neggers SJ, et al. Conversion of daily pegvisomant to weekly pegvisomant combined with long-acting somatostatin analogs, in controlled acromegaly patients. Pituitary. 2011 Jan 8.

Neggers SJCMM., et al. Growth Hormone Receptor mRNA expression and GH production with and without Pegvisomant, in Somatotroph Pituitary adenomas. Submitted.

Neggers SJCMM, van der Lely AJ. Combined treatment in acromegaly. Growth Hormone & IGF Research. Invited review, submitted.

M. van Waas, SJCMM Neggers, et al. Endocrine late sequelae in very long-term survivors of childhood non-Hodgkin lymphoma. Submitted.

Neggers SJCMM, et al. What is active acromegaly and which parameters do we have? Clin Endocrinol. Invited review, submitted.

Neggers SJCMM, et al. Chapter; Physiological roles of des-acyl ghrelin. Ghrelin in Health and Disease 2011.

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Curriculum vitae

De auteur van dit proefschrift werd geboren op 26 februari 1974 te Tilburg. Na het atheneum gevolgd te hebben op de scholengemeenschap Durendael te Oisterwijk studeerde hij Geneeskunde aan de Erasmus Universiteit te Rotterdam. Het artsexamen werd afgelegd in 2000. Hierna begon hij als assistent-geneeskundige niet in opleiding inwendige geneeskunde in het Academisch Ziekenhuis Rotterdam. In 2001 startte hij met zijn opleiding Inwendige Geneeskunde in het Academisch Ziekenhuis Rotterdam (opleider Prof.Dr. H.A.P. Pols). In 2005 trad hij toe tot het aandachtsgebied Endocrinologie (opleider Prof.Dr. AJ. Van der Lelij). In deze periode startte hij met het promotieonderzoek. In 2008 trad hij toe tot de staf van de Inwendige Geneeskunde en Kindergeneeskunde in het Erasmus MC en won hij de Europese "Young Investigators Award".