Clinical and pathophysiological studies in acromegaly

Doctoral theses

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Budapest
2009

Introduction

Acromegaly is a severe, insidiously developing systemic disease that is caused, in most cases, by a growth hormone (GH) secreting adenoma of the anterior lobe of the pituitary.

Hypersecretion of GH and excessive production of IGF-1 affects virtually all organs and tissues of the body, causing widespread morphologic, endocrine and metabolic disturbances. Growth of a pituitary adenoma may also compress local structures.

Acromegalic patients demonstrate a two- to fourfold increase in mortality rate as compared to the general population, mainly due to cardiovascular and respiratory complications.

The aim of therapy in patients with acromegaly include effective reduction of GH and IGF-1 levels in order to prevent clinical complications of hormone overproduction and abolition of compression symptoms, possibly without compromising pituitary function.

If long-term restoration of hormone levels to the ’safe’ range (GH <2,5 ng/ml, IGF-1 below the upper limit of age and sex adjusted normal range) can be achieved by treatment, quality of life significantly improves, and mortality and life expectancy is no longer different from that of the general population.

Treatment options for acromegaly include neurosurgical, medical and irradiation (radiotherapy/radiosurgery) methods. Any of these therapeutic modalities could have – beside the definite advantages – considerable side effects, as well.

The 11β-hydroxysteroid dehydrogenase (11β-HSD) isoenzymes catalyse the interconversion of cortisol (F) and cortisone (E). The type 1 isoform (11β-HSD1) acts predominantly as a reductase converting E to active F. Type 2 enzyme (11β-HSD2) is a pure dehydrogenase, and is responsible for the inactivation of F.

Earlier studies demonstrated an increased 11β-HSD1 activity in patients with insulin resistance and obesity. GH has also been considered as an important regulator of 11β-HSD1 activity causing a shift in the set-point of the conversion of E to F towards the direction of E. Inhibition of 11β-HSD1, as evidenced by a decreased F metabolites / E metabolites ratio has been documented in patients with acromegaly, which could be reversed by successful treatment.

Acromegaly is often associated with type 2 diabetes mellitus or insulin resistance which may be expected to result in further modulation of 11β-HSD1 activity. However, F metabolism or overall F to E conversion has not been investigated and compared in active acromegalic patients with and without type 2 diabetes mellitus or impaired glucose tolerance.

The neuroprotective effects of the GH-IGF-1 system have become apparent by investigations of the pathophysiology of neurodegenerative diseases such as amyotrophic lateralsclerosis (ALS).

ALS is a progressive, neurodegenerative disorder characterised by the degeneration of lower and upper motor neurons. IGF-1 has been found to exert a protective effect against motor neuron degeneration both *in vitro* and *in vivo*, and in one clinical trial human recombinant

IGF-1 delayed the progression of functional impairment and decline of health-related quality of life in patients with ALS.

Aims

In my work I evaluated the outcome of somatostatin analogue (SSA) treatment, and compared the results of conventional radiotherapy and radiosurgery by means of retrospective analysis of hormonal values and pituitary MRI scans of acromegalic patients treated at the 2nd Department of Medicine, Semmelweis University. I investigated the relationship between GH overproduction, type 2 diabetes mellitus and impaired glucose tolerance often associated with acromegaly, and activity of the 11β-HSD1 enzyme. To study the neuroprotective effect of the GH-IGF-1 system, I assessed the relationship between therapeutic interventions influencing the activity of the GH-IGF-1 system and the course of the neurological disease in a patient with co-existing acromegaly and ALS. My aims are formulated in the following points:

1. In order to investigate the efficacy of the broadening treatment modalities (neurosurgical intervention, conventional radiotherapy and radiosurgery, medical treatment with dopamine-agonists, somatostatin analogues and GH-antagonists) for acromegaly in Hungarian patients, I set an aim to assess the outcome of the newly available somatostatin analogue treatment by means of analysis of clinical data, hormone laboratory values and imaging examinations of acromegalic patients

nd Department of Medicine, Semmelweis University. I wanted to explore whether any difference could exist in therapeutic effectiveness between cases treated primarily with somatostatin analogues and those who underwent surgical or surgical and irradiation procedure prior to somatostatin analogue treatment.

2. To study the results of new radiotherapeutic opportunities available in acromegaly, I set the comparison of the results and side effects of the treatments as an aim in acromegalic patients who received conventional radiotherapy and were followed up at the 2nd Department of Medicine of Semmelweis University and in acromegalic patients in care of a foreign institute who underwent radiosurgical procedure carried out by Hungarian specialists.

3. For evaluation of the effect of GH overproduction on 11β-HSD1 activity I set the measurement and comparison of serum F, E and F/E ratio as an aim in active and by treatment cured acromegalic patients. I was interested whether type 2 diabetes mellitus or impaired glucose tolerance often associated with acromegaly could influence F/E ratio indicative of 11β-HSD1 activity.

4. By exploration of the literature about the neuroprotective effects of IGF-1, I looked for the answer in a patient with acromegaly and co-existing ALS whether any relationship could exist between the cessation of GH and IGF-1 values following the cure of acromegaly by

Patients and methods

Patients included in the efficacy analysis of somatostatin analogue treatment

- 32 acromegalic patients (26 women, 6 men) receiving somatostatin analogue (Octreotid LAR, Lanreotid autogel or Lanreotid SR) treatment for at least one year within the past 10 years at the 2nd Department of Medicine, Semmelweis University.

Patients included in the comparative analysis of efficacy of conventional radiotherapy and radiosurgery

- 40 acromegalic patients followed up at the 2nd Department of Medicine, Semmelweis University who were treated with conventional radiotherapy.
- 26 acromegalic patients in care of a foreign institute who underwent gamma knife stereotactic radiosurgery carried out by Hungarian gamma knife specialists.

Patients included in the study of 11β-HSD1 activity

- 76 active acromegalic patients (28 men and 48 women; 49 patients with normal carbohydrate metabolism, 26 patients suffering from type 2 diabetes mellitus or impaired glucose

tolerance; one patient with type 1 diabetes mellitus was excluded from the second part of the study),

- 68 acromegalic patients cured by treatment (26 men and 42 women).

The case proposing the neuroprotective role of IGF-1

66-year-old woman presented at the 2nd Department of Medicine, Semmelweis University in July 2003 because of headache, speaking difficulty and typical acromegalic appearance.

Neurological examination: moderate dysarthria, absent soft palate reflex

MRI of the brain: 0.5 cm intrasellar pituitary tumour, dislocation of pituitary stalk to the left

Serum GH: 3.4 ng/ml; 3.2 ng/ml during OGTT; Serum IGF-1: 535 ng/ml (238%)

Dg: Acromegaly due to a GH-producing pituitary microadenoma

Hormone measurements for the evaluation of pituitary function

Measurement of serum GH and IGF-1 in fasting blood samples taken between 08:00 and 09:00 h (ICMA and IRMA methods)

OGTT combined with GH determination

Before treatment, and at 1-3 months intervals during treatment in efficacy follow-up studies

Evaluation of cortisol/cortisone ratio indicative of 11β-HSD1 activity

Measurement of plasma F and E in fasting blood samples taken between 08:00 and 09:00 h (by RIA method), at 1-3 months intervals

Analysis of plasma F, E, and F/E: descriptive statistics, 2 sample T-test, Wilcoxon-test

Radiological examinations

Determination of pituitary tumour size in acromegalic patients: pituitary MRI, rarely CT scan

Pituitary MRI scan at 6-12 months intervals during somatostatin analogue treatment

Pituitary MRI or CT scan following external radiotherapy or stereotactic radiosurgery: at 6-12 months intervals up to 2 or 4 years; every 2 years afterwards.

Evaluation of treatment results

Evaluation of the efficacy of somatostatin analogue treatment, and conventional radiotherapy or stereotactic radiosurgery: based on control radiological findings and hormonal values

Treatment effective if: 1. serum GH decreases to the 'safe' value (<2,5 ng/ml); 2. IGF-1 level normalises; 3. no progression on the radiological image

Results

1. Efficacy analysis of somatostatin analogue treatment in acromegalic patients

After a 3-month treatment with somatostatin analogues, both serum GH and IGF-1 levels decreased significantly and they remained around the same decreased levels throughout the treatment period. Serum GH decreased from 15.7±4.9 to 5.5±1.4 ng/ml, and serum IGF-1, expressed as percentage of the upper limit of age- and sex-adjusted reference value decreased from 204±14%l to 135±12% at the end of treatment. No significant difference was found comparing GH and relative IGF-1 levels of the patient groups treated only with somatostatin analogue or by surgical or surgical and irradiation therapies applied prior to medical treatment.

At the end of treatment 36.7% of patients had safe serum GH (<2.5 ng/ml), while serum IGF-1 returned below the upper limit of age- and sex-adjusted reference range in 41.4% of patients. No significant difference was found in the ratio of patients who reached safe hormone levels as a result of somatostatin analogue treatment between patients treated only with somatostatin analogues, and those patients who underwent surgery or surgery and pituitary irradiation prior to somatostatin analogue treatment. Pretreatment GH and relative IGF-1 levels of patients who reached safe serum GH or normal relative IGF-1 values did not differ significantly from initial GH and relative IGF-1 values of those patients who failed to achieve target hormone levels as a result of somatostatin analogue treatment.

Pituitary MRI showed regression of the adenoma in 46% of patients, whereas in 54% of patients there was no change in the size of the pituitary adenoma. Tumour progression was not detected in any of the cases.

2. Comparison of the efficacy of radiosurgery versus conventional radiotherapy in acromegalic patients

In the conventional radiotherapy group, tumor growth control during follow-up for an average of 13 years (range: 3-34 years) was achieved in all 40 patients; the tumor totally resolved in 16 patients (40.0%), the volume decreased in 15 patients (37.5%), and remained unchanged in 9 patients (22.5%). Tumour progression was not detected in any of the cases.

In the radiosurgery group, tumor growth control during follow-up (4 years in average; range: 8-124 months) was detected in all 23 evaluable patients (100%). The tumor totally resolved in 6 patients (26%), the volume decreased in 12 patients (52%), and was unchanged in 5 patients (22%). Increase in tumour size was not detected in any of the cases.

Serum GH levels were decreased below 5 ng/ml in 17 patients (42.5%) in the conventional radiotherapy group and in 13 patients (62%) in the gamma knife steraotactic radiosurgery group at 24 months during follow-up. This moderate difference in serum GH levels between the two groups of patients was also detectable 3 years after radiation therapy, as serum GH levels of 5 ng/ml or less were found in

50% and 92% of patients who received conventional radiotherapy and gamma knife stereotactic radiosurgery, respectively.

During long-term follow-up, serum GH levels decreased below 1 ng/ml in 19 of the 40 patients (47.4%) who received conventional radiotherapy. Long-term follow-up serum GH data were available in 21 patients treated with gamma knife stereotactic radiosurgery; of these patients, 8 (38%) reached serum GH levels less than 1 ng/ml. Interestingly, further data analysis of patients who reached a serum GH level of 1 ng/ml during the long term showed that in the group of patients receiving conventional radiotherapy, the time needed to reach a serum GH level below 1 ng/ml was 7.9 years in average (median, 6 years), while in the group of patients receiving gamma knife stereotactic radiosurgery, serum GH decreased below 1 ng/ml within 4.5 years in average (median. 57 months).

After conventional radiotherapy, 26 of the 40 patients (65%) developed pituitary hypofunction over the follow-up period. However, 11 of 26 patients had some decrease of pituitary function prior to radiotherapy due to tumoural compression or as a result of the neurosurgical procedure. Several years after conventional radiotherapy, 3 patients showed memory impairment, 3 patients developed psychiatric disorder, 1 patient had internal hydrocephalus, and 1 patient had a brainstem vascular lesion.

In the radiosurgery group pituitary hypofunction was absent before and during follow-up. One patient developed diplopia following radiosurgery, but other complications were absent.

3. Investigation of 11β-HSD1 activity in acromegalic patients

Plasma F concentrations were similar in patients with active acromegaly and in those who were cured with pituitary surgery, irradiation and/or medical therapy (mean±SE, 12.4±0.3 and 12.7±0.4 μg/dl, respectively). However, plasma E levels were significantly higher in patients with active compared to those with cured acromegaly (2.8±0.1 and 2.2±0.1 μg/dl, respectively; $p<0.001$), resulting in a lower F/E ratio in patients with active disease (4.6±0.1 vs. 5.9±0.2 in the cured group of patients, $p<0.001$).

There was a significant positive correlation between BMI and F ($p<0.002$) and a significant negative correlation between BMI and F/E ratios ($p<0.029$) in patients with cured acromegaly. However, BMI failed to show correlations with F/E ratios, or with F and E concentrations in patients with active disease.

When the effect of altered carbohydrate homeostasis on plasma F and E was analysed, the results indicated significantly lower plasma E levels and higher plasma F/E ratios in active acromegalic patients with type 2 diabetes mellitus or impaired glucose tolerance compared to those with normal carbohydrate metabolism (E, 2.5±0.1 and 3.0±0.1 μg/dl, respectively; F/E, 5.1±0.2 and 4.4±0.1; $p<0.001$), whereas plasma F concentrations were similar in these two groups (12.1±0.4 and 12.6±0.3 μg/dl, respectively). The severity of glucose metabolism impairment failed to exert a significant impact on E and F

concentrations or F/E ratios, as these values were similar in patients with type 2 diabetes and in those with impaired glucose tolerance.

4. Rapid progression of amyotrophic lateral sclerosis in an acromegalic patient after surgical resection of a growth hormone producing pituitary adenoma

After establishment of the diagnosis of the GH-producing pituitary microadenoma in our patient suffering from dysarthria and absent soft palate reflex beside other clinical signs and symptoms of acromegaly, octreotide LAR treatment was administered from August, 2003 until July, 2004 (initially 20 mg every 28 days, then 30 mg every 28 days as im. injections). Although the treatment resulted in a biochemical response (serum GH 0.8-1.7 ng/ml; serum IGF-1 297-334 ng/ml), clinical improvement did not follow, the patient continued to complain of headache and speaking difficulty. Repeat neurological examination disclosed only an absence of the soft palate reflex without other signs or symptoms of bulbar or spinal palsy, muscle atrophy or sensory disturbance. Pituitary MRI performed in July, 2004 revealed that the size of the pituitary microadenoma was unchanged.

In order to stop hormone overproduction, in September 2004, the patient underwent transsphenoidal pituitary surgery. Histology and immunohistochemistry confirmed a GH-producing pituitary adenoma. After pituitary surgery serum GH was 1.8 ng/ml (mean of five samples), and its nadir during OGTT was 0.1 ng/ml. Serum IGF-I was also decreased (293 ng/ml) compared to pretreatment value. Due to

development of a mild secondary hypothyroidism, the patient was treated with a daily dose of 50 μg l-thyroxin postoperatively. Plasma F, ACTH and prolactin levels were normal. Four months after pituitary surgery serum IGF-I returned to normal (207 ng/ml).

After pituitary surgery the patient's condition progressively deteriorated. Within four months she developed a progressive bulbar palsy with severe dysarthria, dysphagia, sialorrhea, atrophy and fasciculation of the tongue, and weakness in both hands along with fatigue. The patient had very brisk reflexes. Neurological investigations, including electromyography confirmed the diagnosis of ALS with predominantly bulbar signs and symptoms of the disease. Despite riluzole treatment, there was a continuous progression of neurological symptoms with severe bulbar and upper limb weakness and muscle wasting.

One year after pituitary surgery, GH deficiency was verified and in September, 2005 a trial with human recombinant GH was started. After the initial daily dose of 0.2 mg, GH treatment was continued with a dose of 0.3 mg per day for one year. During GH therapy serum IGF-I levels were in the upper limit of the normal range. Nevertheless, the treatment failed to produce any significant improvement in the patient's condition and she became completely dependent on another persons help. In October, 2006 the patient developed breathing difficulty and she died of a sudden respiratory arrest.

Neuropathological examinations confirmed the diagnosis of the motor neuron disease.

Conclusions

1. By means of retrospective analysis of hormone and pituitary MRI findings of 36 acromegalic patients who received somatostatin analogue treatment we showed that after a 3-month treatment with somatostatin analogues, both serum GH and IGF-1 levels decreased significantly and they remained around the same decreased levels throughout the treatment period. At the end of treatment 36.7% of patients had safe serum GH (<2.5 ng/ml), while serum IGF-1 returned below the upper limit of age- and sex-adjusted reference range in 41.4% of patients. Pituitary MRI showed regression of the adenoma in 46% of patients, whereas in 54 % of patients there was no change in the size of the pituitary adenoma. Tumour progression was not detected in any of the cases. Our results confirm that somatostatin analogues are effective therapeutic options for acromegalic patients when primary surgical treatment cannot be performed due to complications and associated disorders, or in patients whose acromegaly remains active after pituitary surgery or after pituitary surgery and irradiation.

2. Our findings based on long-term hormonal and imaging follow-up data of acromegalic patients treated with conventional radiotherapy or gamma knife radiosurgery indicate that gamma knife stereotactic radiosurgery results in a more rapid improvement of serum GH levels,

but in the long term – regarding the effect on GH-overproduction – there is no difference in the efficacies of the two methods. Considering the side effects of the two radiotherapeutic procedures, our results are in agreement with earlier observations showing a more favourable spectrum of side effects in patients undergoing radiosurgery compared to those receiving conventional radiotherapy. However, a shorter follow-up time of patients treated with radiosurgery remains a limitation of these earlier series and ours.

3. Based on my investigations on a considerable number of samples obtained from active and - as a result of various treatment procedures - cured acromegalic patients, I showed that plasma E level is significantly higher and plasma F/E ratio is significantly lower in patients with active acromegaly compared to the values measured in those cured by treatment. These observations confirm that disease activity may exert a significant impact on 11β-HSD1 activity. My further remarkable observation is that plasma E is significantly lower and plasma F/E is significantly higher in active acromegalic patients with type 2 diabetes mellitus or impaired glucose tolerance than in those with normal carbohydrate metabolism. I consider it possible that change of 11β-HSD1 activity, by alteration of the actual set-point of interconversion of E and F, could play a role in the development of carbohydrate metabolic disorders in acromegalic patients. Thus, it is possible that patients with active acromegaly who display a weaker inhibition of 11β-HSD1 activity are more likely to develop an impaired

glucose metabolism compared to those who have a greater GH- and IGF-1-induced inhibition of this enzyme.

4. We observed for the first time in the literature that cessation of the high GH and IGF-1 levels by pituitary surgery in an acromegalic patient leads to rapid progression of the co-existing ALS. I attributed the relationship between the cure of acromegaly and the rapid progression of ALS to the neuroprotective effect of IGF-1, that is supported by earlier *in vitro* and *in vivo* investigations. As replacement therapy with human recombinant GH for one year failed to produce significant improvement or a delay in the progression of ALS, we suppose that GH treatment caused insufficient increase of IGF-1 level in the neighbourhood of motor neurons and, therefore, was unable to compensate for the loss of the beneficial, neuroprotective effect of high endogenous GH and IGF-1 concentrations prior to pituitary surgery.

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