Combined acromegaly and subclinical Cushing disease related to high-molecular-weight adrenocorticotropic hormone

Case report

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A 36-year-old man with a 1-year history of diabetes mellitus was referred to the authors’ hospital for further endocrinological evaluation of acromegaly. On physical examination, typical acromegalic features but no typical cushingoid features were observed. The clinical diagnosis of growth hormone (GH)–producing pituitary adenoma was confirmed by MR imaging findings, nonsuppression of serum GH levels during a 75-g oral glucose tolerance test (trough GH 6.33 ng/ml), and elevated serum insulin-like growth factor—1 levels (1361.3 ng/ml). Moreover, autonomous adrenocorticotropic hormone (ACTH) secretion was suspected, based on inadequate suppression of ACTH or cortisol levels by an 0.5-mg overnight dexamethasone suppression test. Analysis of the patient’s plasma by using the gel filtration method revealed the presence of a high-molecular-weight (HMW) form of ACTH known to exhibit low biological activity. Transsphenoidal adenomectomy was performed for the pituitary tumor. Immunohistochemical investigation of the resected specimen showed strong and diffuse immunoreactivity to GH and focal immunoreactivity to ACTH. Although there have been a few cases of pituitary adenoma that produced GH and ACTH concomitantly, this is the first report of the detection of HMW ACTH in patients with GH- and ACTH-producing adenomas. Furthermore, the previous cases also did not exhibit typical cushingoid features. It is suggested that the secretion of ACTH in patients with concurrent GH- and ACTH-secreting adenomas might consist of the HMW form and that the HMW ACTH is consequently associated with a subclinical Cushing state. (DOI: 10.3171/2008.8.JNS08154)

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Growth hormone–producing adenomas, which are known to cause acromegaly, often co-secrete other anterior pituitary hormones.¹,¹¹ Because ~25% of GH-producing adenomas concomitantly produce PRL, this combination is well known.¹¹ However, other combinations, such as concomitant GH- and ACTH-producing adenomas, are extremely rare.⁵,¹¹

In general, ACTH overproduction results in oversecretion of cortisol, which leads to cushingoid features, such as moon face, central obesity, buffalo hump, and striae. Despite the presence of a pituitary tumor with ACTH production, a few cases with absence of typically clinical features of Cushing syndrome have been reported.⁷,¹⁰,¹²,¹⁷ Recently, this situation has been referred to as subclinical CD, similar to adrenal subclinical Cushing syndrome.

A pituitary macroadenoma rarely produces low biologically active forms of ACTH, such as pro-ACTH and POMC.⁵,¹⁴ It is known that ACTH is synthesized as a part of POMC, which is cleaved by PC1 at the COOH terminus of POMC to produce β-LPH and at the NOOH terminus of POMC to produce pro-ACTH, and then at the

Abbreviations used in this paper: ACTH = adrenocorticotropic hormone; β-LPH = β-lipotropin; CD = Cushing disease; CRH = corticotropin-releasing hormone; FSH = follicle-stimulating hormone; GH = growth hormone; HMW = high-molecular-weight; LHRH = luteinizing hormone–releasing hormone; PC = prohormone convertase; POMC = proopiomelanocortin; PRL = prolactin; RIA = radioimmunoassay; TRH = thyrotropin-releasing hormone; TSH = thyroid-stimulating hormone.

This article contains some figures that are displayed in color online but in black and white in the print edition.
COOH terminus of pro-ACTH to produce ACTH. The gel chromatography technique can detect pro-ACTH or POMC as HMW ACTH.

Taken together, HMW ACTH is pro-ACTH or POMC, and is known as a low biologically active form of ACTH.

We report an interesting case of acromegaly with elevated plasma levels of the HMW form of ACTH that exhibited typical acromegalic features and no typical cushingoid features.

Case Report

History and Examination. This 36-year-old man was referred to our hospital for further endocrinological evaluation of acromegaly. He had received a diagnosis of Type 2 diabetes mellitus 1 year prior to admission. No endocrine diseases or malignant tumors had been noticed in his family. On physical examination, he was 189 cm tall and weighed 80.1 kg. His blood pressure was 136/60 mm Hg. He had facial features that were slightly acromegalic, with moderately deep nasolabial furrows, thick lips, and a supraorbital ridge. No cushingoid features, including moon face, central obesity, buffalo hump, or striae, were evident.

Diagnostic Test Results. On laboratory examination, a complete blood count, serum electrolytes, and the results of renal and liver tests were all normal. Glycosylated hemoglobin and fasting plasma glucose were 5.8% and 140 mg/dl, respectively. Serum levels of free triiodothyronine (4 pg/ml), free thyroxine (1.1 ng/dl), and TSH (2.2 µU/ml) were within normal limits. The diagnosis of a GH-secreting adenoma was confirmed by MR imaging (Fig. 1), nonsuppression of GH serum levels during a 75-g oral glucose tolerance test (trough GH 6.33 ng/ml) (Fig. 2A), and elevated serum insulin-like growth factor-I levels (1361.3 ng/ml; normal range 67–318 ng/ml). Serum GH levels were paradoxically increased after stimulation with LHRH, TRH, and CRH (Figs. 2B, 2C, and 3B), and decreased after oral administration of bromocriptine and subcutaneous injection of octreotide, respectively (data not shown). In addition, the plasma ACTH levels at 7:00 a.m. (91.7–228.8 pg/ml) were markedly elevated, whereas the plasma cortisol levels at 7:00 a.m. (13.4–16.4 µg/dl) and the urinary free cortisol level (76 µg/day) were within normal range. Plasma ACTH and cortisol revealed normal circadian variation (Fig. 3A). Both plasma ACTH and cortisol concentrations were partially suppressed by the 0.5-mg overnight dexamethasone suppression test, whereas both were inhibited with a dosage of 8 mg dexamethasone (Fig. 3A). The plasma levels of ACTH and cortisol were increased in response to the administration of human CRH (Fig. 3B). Therefore, the tumor appeared to secrete ACTH concomitantly.

Operation and Postoperative Course. The patient underwent transsphenoidal adenomectomy for a pituitary tumor. Histologically, the tumor exhibited diffuse growth
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of polygonal cells with mild anisonucleosis and abundant acidophilic cytoplasm arranged in solid nests (Fig. 4A); the findings were reinforced by reticulin stain (Fig. 4A, inset). In the tumor, immunohistochemical investigation showed strong immunoreactivity to GH (Fig. 4B) and focal immunoreactivity to ACTH (Fig. 4C) and TSH (Fig. 4D). No immunoreactivity to PRL, FSH, or LH was detected. After surgery, ACTH (53.4 pg/ml) as well as the basal levels of GH (0.36 ng/ml) and insulin-like growth factor–I (201 ng/ml) were decreased markedly. The response of ACTH to human CRH became almost normal (data not shown).

Analysis of the ACTH Molecular Size in Plasma. The plasma collected from the patient before surgery was used for the analysis of the molecular size of ACTH and β-endorphin. The fractionating procedure for the measurement of ACTH and β-endorphin with RIA was performed as described previously. In brief, a 1-ml plasma sample was lyophilized and reconstituted with 0.5 ml of elution buffer (63 mM Na2HPO4, 13 mM ethylenediaminetetraacetic acid–2Na, 0.05% NaN3, 0.1% bovine serum albumin, pH 7.4), and was applied onto a 50-cm Sephadex G75 superfine column (Amersham Biosciences Corp.) eluted at a rate of 6 ml/hour. One-milliliter fractions were collected and subjected to RIA. The ACTH RIA was performed using rabbit polyclonal antiserum against ACTH1–24. The ACTH RIA cross-reacts equally with ACTH1–39, and POMC, but not with β-endorphin or β-LPH. The β-endorphin RIA was performed using rabbit polyclonal

![Fig. 3. Charts showing results of laboratory tests. Left: Daily changes of ACTH and cortisol, and low- (0.5-mg) and high- (8-mg) dexamethasone (Dex) suppression test. Right: Results of the CRH stimulation test. Arrows indicate initiation of stimulation. Symbols are defined on the y axes.](image)

![Fig. 4. Photomicrographs showing histopathological features and immunoprofile of a pituitary adenoma. Immunohistochemical staining was used to mark GH in the tumor; GH immunoreactivity, demonstrated as a brown reaction product of diaminobenzidine, was detected. Tissue prepared with H & E (A) and reticulin (inset) stain, showing solid tumor nest. Immunohistochemically, the tumor cells show diffuse cytoplasmic reactivity to anti-GH antibody (B) and focal reactivity to anti-ACTH (C) and anti-TSH (D) antibody. Original magnification × 200.](image)
antiserum against human β-endorphin. The β-endorphin RIA cross-reacts equally with β-endorphin, β-LPH, and POMC, but not ACTH_{1-24} or ACTH_{1-39}.

The elution profile using ACTH RIA from the plasma sample measured showed 1 peak, which eluted at fraction numbers 15 to 19 (Fig. 5), indicating an HMW form of ACTH. The authentic ACTH_{1-39} at the site of 29 to 30 was not observed. Since the elution profiles using β-endorphin RIA, which could identify POMC, were not detected at all (data not shown), most of the detected HMW ACTH would be pro-ACTH.

Discussion

This patient was an extremely rare case, with a pituitary GH- and ACTH-producing adenoma, who presented with typical acromegalic and no cushingoid features. Furthermore, gel chromatography of plasma samples revealed that most of the ACTH was the HMW form.

Only a few cases of subclinical CD have been reported. However, because immunohistochemical analysis would be the most useful method to evaluate it, the tumor positive for both GH and ACTH, which was one of the most valid procedures to assess the production, were not investigated in our case. ACTH, which was one of the most valid procedures to assess the production, were not investigated in our case. ACTH, which was one of the most valid procedures to assess the production, were not investigated in our case.

Five cases of concurrent secretion of GH and ACTH from pituitary tumor have been reported. One of these cases, which was reported by Blevins et al., exhibited 2 pituitary adenomas that each produced GH or ACTH; therefore this was different from our case. The other previous cases of pituitary tumor presented with no typical cushingoid appearance. In short, these cases, as well as ours, are likely to fulfill the criteria for subclinical CD, and the previous cases might also exhibit the HMW form of ACTH. Unfortunately, in their cases, the molecular size of plasma ACTH was not assessed. We speculate that the plasma ACTH in most of these cases with GH- and ACTH-producing adenoma could be composed of the HMW form, although accumulation of more cases is necessary to resolve this association. In addition, although obvious mechanisms of subclinical CD have not been recognized, HMW ACTH, which is known as a low-activity form, might be considered one of the possible mechanisms.

In considering the pathogenesis of our case, it might be important to note that the detected ACTH was the HMW form, particularly pro-ACTH. Tumor cells would be less differentiated than their normal counterparts, and therefore the presence of the regulated secretory pathway could vary depending on the degree of differentiation. Thus, this tumor might not be capable of processing pro-ACTH to ACTH. In addition, Matsuno et al. hypothesized that the secretion of HMW ACTH is caused by the impaired expression of transcriptional factors, such as POMC. The fact that the detected ACTH was pro-ACTH, but not POMC, might raise the possibility that some transcriptional factors apart from POMC are essential to process pro-ACTH to ACTH. Accordingly, in this case, the POMC gene and transcriptional factors that lead to pro-ACTH, but not to authentic ACTH, might be involved during proliferation or differentiation of the tumor. If we could perform immunohistochemical analyses, including POMC, ACTH, and chromogranin-A, which are useful to assess the maturation of secretory granules, these hypotheses might be proven fully.

Some patients with CD demonstrate suppressibility to 1-mg dexamethasone; use of this diagnostic criterion misclassified up to 15% of such patients as negative. Therefore, to enhance sensitivity, Japanese endocrinological experts including our coauthor (Y. Oki) have advocated requiring a lower dose (0.5 mg) of dexamethasone and alternative cutoff for suppression of the postdexamethasone serum cortisol to > 2.4 µg/dl. In fact, a preliminary study (unpublished data) by another group showed that the reference range of the postdexamethasone serum cortisol is from 0.8 to 2 µg/dl in the Japanese population without CD. Accordingly, this case possibly had insufficient suppression of ACTH and cortisol secretion by 0.5-mg dexamethasone administration.

A limitation of our case study should be taken into consideration. The mRNA expression analysis of GH and ACTH, which was one of the most valid procedures to assess the production, were not investigated in our case. However, because immunohistochemical analysis would be the most useful method to evaluate it, the tumor possibly produced GH and ACTH.

Conclusions

This is the first report of the detection of HMW ACTH, particularly pro-ACTH, in patients with GH- and ACTH-producing adenoma. It is suggested that secreted ACTH in patients with concurrent GH- and ACTH-secreting adenomas might be the HMW form, and that the HMW ACTH is consequently associated with a subclinical Cushing state.

Disclaimer

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

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