Adrenal Insufficiency after Incomplete Resection of Pituitary Macrocorticotropinoma of Cushing’s Disease: Role of High Molecular Weight ACTH

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Abstract. A 15-year-old girl with Cushing’s disease exhibited adrenal insufficiency following incomplete transsphenoidal resection of a large pituitary corticotropinoma, approximately 35 mm in diameter. Within two weeks following surgery, her plasma ACTH level decreased from 42 to 13 pmol/l, while, her plasma cortisol levels and urinary excretion of free cortisol decreased from 607 nmol/l and 1112 nmol/day to 94 nmol/l and 55 nmol/day, respectively. Immunoreactive ACTH was characterized in plasma using Sephadex G-75 column chromatography and measuring ACTH with immunoradiometric assay (IRMA) and radioimmunoassay (RIA) to determine additional peaks, other than the one demonstrated for 1–39 ACTH. In particular, when measured with RIA, a broad peak including the high molecular weight ACTH was detected as well as 1–39 ACTH. The bioactivity of the high molecular weight ACTH in patient plasma was lower than the reference range of 1–39 ACTH, which is determined by the ability of dispersed rat adrenocortical cells to secrete corticosterone. The large pituitary corticotropinoma found in this patient secreted not only 1–39 ACTH but also high molecular weight proopiomelanocortin (POMC)-derived peptides, which could be detected by measuring with IRMA and RIA for ACTH. Based on the results of biological activity and molecular ratios, no positive evidence could be found to support the hypothesis that the high molecular weight ACTH induced any postoperative adrenal insufficiency in this patient. However, based on this study, the possibility of adrenal insufficiency should be carefully monitored, even when post-operative remnant tumor tissue is clearly present in patients with Cushing’s disease, accompanied by macrocorticotropinoma.

Key words: Transsphenoidal hypophysectomy, Macrocorticotropinoma, Silent corticotropinoma

The ACTH-producing pituitary tumor, which leads to Cushing’s disease, is usually less than 10 mm in diameter, or what is known as a microadenoma. According to the numerous reports on large populations of patients with Cushing’s disease [1–11] including children and adolescents [10, 11], pituitary macroadenomas larger than 10 mm in diameter could be found in 261 of a total of 1621 patients (16.1%). However, among children and adolescents, the percentage of macroadenoma was found to be much less than this series for adults [10–12]. Conversely, the size of a silent corticotropinoma, exhibiting no signs

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of Cushing's syndrome, can typically be large [13]. This type of corticotropinoma secretes biologically inactive ACTH, which is generally discerned as a high molecular weight ACTH [14-16]. It has been reported that a large pituitary adenoma in Cushing's disease also secretes high molecular weight ACTH as well as 1-39 ACTH [15]. In this study, we report on a girl suffering from Cushing's disease owing to a large corticotropinoma that exhibited adrenal insufficiency in spite of an incomplete resection of the adenoma and exhibited a high molecular weight form of ACTH in the plasma.

Materials and Methods

Clinical examinations and hormone measurement

All sampling and loading tests were performed as described previously elsewhere [16]. Plasma immunoactivity of β-endorphin was measured by modified radioimmunoassay (RIA) as reported by Tomoo et al. [17], and the reference range was less than 2.9 pmol/l. Cross reactivity with β-lipotropin (LPH) was 3.3%. The value for other proopiomelanocortin (POMC)-related peptides, 1-39 ACTH, 1-24 ACTH, 1-10 ACTH, 1-39 ACTH, α-endorphin, Leu-enkephalin, and Met-enkephalin was less than 0.01% [18]. All other hormone levels in the plasma or serum were measured using commercially available kits. Plasma ACTH was measured by immunoradiometric assay (IRMA), Allegro HS-ACTHTM (Nichols Institute, San Juan Capistrano, CA, USA). The reference range was 2.0-11.5 pmol/l. Cross reactivity with β-LPH was 0.01%, and was far less than 0.01% with other POMC-related peptides, 1-24 ACTH, 11-24 ACTH, 18-39 ACTH, 1-10 ACTH, α-MSH, β-MSH and β-endorphin [19]. Plasma cortisol was measured by a Gammacoat cortisol kitTM (Incstar Co., Stillwater, MN, USA), and the reference range was 110-505 nmol/l.

Analysis of molecular size of ACTH in plasma

The blood sample showing the peak value after administration of CRH was used for analysis. The fractionating procedure for the measurement of ACTH with IRMA was performed as described previously [16, 20]. Briefly, an acidified plasma sample was chromatographed on a Sephadex G-75 column (superfine; 100 × 1.5 cm) equilibrated with 1% formic acid. A 2.5 ml plasma sample was directly pumped onto the column and 2 ml fractions were collected. The fractions were freeze-dried and a 250 μl standard solution of the IRMA-kit was added. The ACTH concentration of each fraction was measured with IRMA, Allegro HS-ACTHTM. The recovery rate using 1-39 ACTH was 74%.

For the measurement of ACTH with conventional RIA-systems, on the other hand, a Sephadex G-75 column (50 × 1.0 cm) was used for the fractionation. After freeze-drying a 2 ml plasma sample and adding a 0.5 ml elution buffer (0.1 M sodium phosphate buffer and 0.1% bovine serum albumin (BSA)), the solution was applied onto the column and 1 ml fractions were collected and freeze-dried. In the RIA for ACTH [21], anti-1-24 ACTH rabbit serum was used as a primary antibody, and 125I-1-39 ACTH was used as a tracer. The antiserum to ACTH of the RIA system did not crossreact either with α-MSH or β-endorphin. The minimum detection limit of human 1-39 ACTH was 0.22 fmol/assay tube. Intra- and inter-assay coefficients of variation were 6.8 and 8.3%, respectively. The recovery rate using 1-39 ACTH was 78%. The fraction position of 1-39 ACTH, β-LPH, and β-endorphin were confirmed beforehand using radiolabelled peptides.

Analysis of ACTH bioactivity of patient plasma

The adrenal cells were obtained from male Sprague-Dawley rats (250-300 g), which had been housed in our facilities under controlled temperature (24°C) and humidity with artificial lighting turned on daily between 0700h and 1900h, and dispersed with 0.3% collagenase (Type II, Worthington Biochemical Corp., Freehold, NJ) and 40 μg/ml DNase (Type I, Worthington). After dispersion, 5 × 10^4 cells in a 0.25 ml culture medium (Medium 199 supplemented with 10 mM HEPES, 10 mM NaHCO₃, 0.1% BSA, 20 U/ml penicillin, 20 U/ml streptomycin, 0.05 μg/ml amphotericin B, 60 μg/ml ascorbic acid and 10% FCS, pH 7.4) were plated in the wells of 96-well tissue culture cluster dishes (Costar, Cambridge, MA). Cells were maintained in a static monolayer culture for 3 days in a humidified incubator under a 5% CO₂-95% air atmosphere at 37°C. On the third day, adrenal cells were washed three times with a
0.25 ml medium for 10 min at 37°C. Preincubated media were aspirated and the cells were incubated at 37°C with 0.25 ml serum-free culture medium containing synthetic 1-39 ACTH (Sigma Co., St. Louis, MO) as a reference standard or diluted samples, which had been lyophilized from the ACTH peak fraction (fraction No. 14 in Fig. 2b) determined by gel-chromatography, for 4 h to measure corticosterone release. After incubation, the medium was aspirated and centrifuged at 500 g at 4°C for 5 min, and the supernatant was stored at -20°C until corticosterone determination. Corticosterone concentration was measured by RIA using anti-corticosterone-3CMO-BSA rabbit serum as a primary antibody and 125I-corticosterone was used as a tracer. The assay did not crossreact either with cortisol, aldosterone or deoxycorticosterone. The minimum detection level was 0.058 pmol/l assay tube. The intra- and inter-assay coefficients of variation were 3.6 and 6.4%, respectively.

The intra- and inter-assay coefficients of variation of this bioassay system were 11% and 18%, respectively. The minimum detection limit of this system was 1.1 pmol/l of 1-39 ACTH.

Histological examination

The pituitary tumor specimen was examined light-microscopically, immunohistochemically and electron-microscopically. The method of immunohistochemical examination and ultrastructural study was similar to a previous study [16].

Case report

A 15-year-old girl was referred to Division of Endocrinology and Metabolism of Matsunami General Hospital because of a large pituitary tumor discovered by computed axial tomography (CT). She had been diagnosed as schizophrenic and had been treated at a psychiatric clinic for two years. On admission, she was 155 cm in height, weighed 90 kg, and complained of sleep disturbance and auditory hallucinations. A buffalo hump and cutis striae on the abdomen were observed. Blood pressure was 152/110 mmHg. Ophthalmologic examination revealed bilateral temporal quadrantanopsia, although she complained of no visual disturbance.

Peripheral blood examination revealed that leukocyte, erythrocyte, hemoglobin and hematocrit levels were 7.8 x 10^9/l, 5.2 x 10^12/l, 164 g/l and 0.51, respectively. A fasting biochemical examination revealed that glycohemoglobin (5.9%), serum total cholesterol (6.2 mmol/l) and transaminases (asparate aminotransferase, 0.85 µkat/l; alanine aminotransferase, 1.18 µkat/l; γ-glutamyltransferase, 3.43 µkat/l) levels were slightly increased, but other items including serum total protein (77 g/l), albumin (46 g/l), serum sodium (140 mmol/l), potassium (4.3 mmol/l), serum urate (297 µmol/l) and urea nitrogen (3.6 mmol/l) were within normal limits. Creatinine clearance was 1.45 ml/s. The fasting plasma glucose was 6.0 mmol/l and increased to 12.0 and 10.1 mmol/l 60 and 120 min after ingestion of 75 g glucose, respectively. The peak level of immunoreactive insulin was 1220 pmol/l at 120 min.

Pituitary magnetic resolution imaging (MRI) exhibited a large pituitary tumor with a suprasellar extension, approximately 3.5 cm in diameter (Fig. 1). Abdominal CT revealed a fatty liver, but no adrenal tumor.

Endocrine examination (Tables 1 and 2)

As shown in Table 1, the basal plasma ACTH and cortisol levels were found to be similarly high as the levels found at 2200h. The plasma ACTH and cortisol levels decreased insufficiently after overnight suppression with administration of 2 and 8 mg of dexamethasone. After administration of 100 µg CRH, the plasma ACTH, β-endorphin and cortisol levels markedly increased (Table 2). Urinary excretion of free cortisol, 17-OHCS, and 17-KS were found to have increased. Plasma TSH, LH, FSH and GH responses to their respective hypothalamic stimulating hormones were slightly blunted. Plasma PRL level and its response to TRH were found to be normal. Plasma ADH and urinary osmolarity after overnight water restriction were found to be normal (Table 2).

Based on these results, a diagnosis of Cushing’s disease with macrocorticotropinoma was made, and transsphenoidal hypophysectomy was performed. However, a part of the tumor adhered too tightly to the hypothalamus and could not be resected completely because of the danger of hemorrhage.

Postoperative glucocorticoid substitution therapy
was not performed. On the third day following surgery, the patient complained of general fatigue, nausea, appetite loss, general arthralgia, and low grade fever. These symptoms transiently ameliorated, but appeared again and gradually deteriorated from the 10th day following surgery. On the 13th day, she appeared to be clear and conscious, but her body temperature had risen to 37.8°C. In addition, the serum C-reactive protein level was 18 mg/l, serum electrolyte level was 131 mmol/l in sodium and 5.1 mmol/l in potassium, but the fasting plasma glucose level was found to be normal, at 4.4 mmol/l. As shown in Table 1, plasma ACTH and cortisol levels were 14 pmol/l and 220 nmol/l on the fifth day following surgery. Plasma ACTH levels did not change significantly, but cortisol levels and urinary cortisol excretion decreased further to 94 nmol/l and 55 nmol/day on the 14th day, respectively (Table 2). On the 14th day, CRH administration positively increased plasma ACTH from 13 to 59 pmol/l, but plasma cortisol only increased from 94 to 182 nmol/l. A remnant tumor was clearly demonstrated by MRI (Fig. 1). Administration of 2 and 8 mg of dexamethasone at 2300h on the 14th and 15th day dramatically ameliorated her condition and decreased plasma cortisol levels to less than 28 nmol/l, but plasma ACTH decreased only by 4 and 2 pmol/l, respectively.

Prednisolone (20 mg/day) was subsequently administered, then gradually reduced, and finally withdrawn three months later. However, no symptoms of adrenal insufficiency appeared. Endocrine examinations were performed one month after the withdrawal of prednisolone. The

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Table 1. Change of plasma ACTH and cortisol levels and urinary excretion of free cortisol, 17-OHCS and 17-KS before, immediately after and 4 months after operation.

<table>
<thead>
<tr>
<th></th>
<th>Reference range</th>
<th>Before operation</th>
<th>Immediately after operation</th>
<th>4 months after operation</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0800h</td>
<td>2200h</td>
<td>D2</td>
</tr>
<tr>
<td>ACTH (pmol/l)</td>
<td>2–11</td>
<td>42</td>
<td>42</td>
<td>31</td>
</tr>
<tr>
<td>cortisol (nmol/l)</td>
<td>110–505</td>
<td>607</td>
<td>455</td>
<td>395</td>
</tr>
<tr>
<td>Urine</td>
<td>Free cortisol (nmol/day)</td>
<td>83–276</td>
<td>1112</td>
<td>1197</td>
</tr>
<tr>
<td></td>
<td>17-OHCS (mg/day)</td>
<td>2.2–7.3</td>
<td>23</td>
<td>26</td>
</tr>
<tr>
<td></td>
<td>17-KS (mg/day)</td>
<td>2.4–11.0</td>
<td>7.1</td>
<td>7.6</td>
</tr>
</tbody>
</table>

D2 and D8, values after 2 and 8 mg dexamethasone administration, respectively; 2nd, 5th, 10th, 12th, and 14th days, the days following surgery; parenthesis with asterisk, mean.

Table 2. Preoperative responses of pituitary hormones and cortisol to the respective stimulating factors.

<table>
<thead>
<tr>
<th></th>
<th>Basal</th>
<th>Peak</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACTH (100 µg CRH)* (pmol/l)</td>
<td>42</td>
<td>73</td>
</tr>
<tr>
<td>β-endorphin (100 µg CRH)* (pmol/l)</td>
<td>5.2</td>
<td>9.3</td>
</tr>
<tr>
<td>cortisol (100 µg CRH)* (nmol/l)</td>
<td>607</td>
<td>828</td>
</tr>
<tr>
<td>LH (100 µg LHRH)* (IU/l)</td>
<td>&lt;0.5</td>
<td>0.8</td>
</tr>
<tr>
<td>FSH (100 µg LHRH)* (IU/l)</td>
<td>0.6</td>
<td>0.9</td>
</tr>
<tr>
<td>TSH (500 µg TRH)* (mIU/l)</td>
<td>1.0</td>
<td>8.3</td>
</tr>
<tr>
<td>PRL (500 µg TRH)* (µg/l)</td>
<td>14</td>
<td>22</td>
</tr>
<tr>
<td>GH (100 µg GRH)* (µg/l)</td>
<td>0.47</td>
<td>4.1</td>
</tr>
<tr>
<td>ADH (overnight water restriction)* (pmol/l)</td>
<td>2.0 (1800h)</td>
<td>2.7 (0700h)</td>
</tr>
<tr>
<td>Urine osmolality (overnight water restriction)* (mmol/kg)</td>
<td>294 (1800h)</td>
<td>634 (0700h)</td>
</tr>
</tbody>
</table>

Parenthesis with asterisk, stimulation factors intravenously administered or overnight water restriction from 1800h on previous day to 0700h on the next day.
body weight had decreased to 75 kg and the blood pressure was normalized at 128/82 mmHg. Plasma ACTH was high-normal, but plasma cortisol levels were rather low-normal (Table 1). Urinary excretion of free cortisol, 17-OHCS and 17-KS also decreased (Table 1). Plasma cortisol was completely suppressed to less than 28 nmol/l after administration of 2 mg of dexamethasone. However, plasma ACTH was still found to be 1.5 pmol/l even after administration of 8 mg dexamethasone. Plasma ACTH still markedly increased from 11 to 62 pmol/l after administration of 100 μg CRH. However, the response of plasma cortisol to CRH was normal, increasing from 157 to 513 nmol/l. CRH-stimulation, following 2 mg dexamethasone administration in four divided doses (starting at 1200h) for 2 days, increased plasma ACTH and cortisol levels from 3 to 12 pmol/l and less than 28 to 149 nmol/l, respectively. Intravenous administration of 4 μg desmopressin increased plasma ACTH and cortisol levels from 8 to 12 pmol/l and 143 to 303 nmol/l, respectively. These results were still consistent with Cushing’s disease.

**Chromatographic characterization of plasma ACTH**

The elution profile from the plasma sample measured with IRMA of ACTH showed two peaks, one of which was eluted at the same position as 1-39 ACTH and the other before the 1-39 ACTH position, indicating a high molecular weight form of ACTH.

**Fig. 1.** Magnetic resolution imaging (MRI) of pituitary corticotropinoma. Large pituitary tumor (arrow and large asterisk) 35 mm in diameter with suprasellar extension was revealed before operation. a) Sagittal section, b) Coronal section. Postoperative MRI revealed remnant tumor tissue (small arrows and small asterisks). c) Sagittal section, d) Coronal section.
When calculated with the area under the curve, the molecular ratio of the high molecular weight ACTH from fractions No. 14 to No. 17 was found to be 11% of all measurable ACTH. When measured with RIA, a peak including the 1-39 ACTH-position and two peaks positioned before 1-39 ACTH could be detected (Fig. 2b). The molecular ratio of the high molecular weight ACTH from fractions No. 14 to No. 17 was found to be 16.5%.

These results indicated the presence of high molecular weight immunoreactive ACTH other than 1-39 ACTH in the plasma.

**Biological activity of immunoreactive ACTH in patient plasma**

As shown in Fig. 3, the patient plasma, whose ACTH concentration in the incubation medium was 10.6 pmol/l, secreted corticosterone at a rate of only 0.81 μmol/l from rat adrenocortical cells. The ability of the high molecular weight ACTH (fraction No. 14) in the patient’s plasma to secrete corticosterone was 28% of the mean of 1-39 ACTH.

**Histopathological findings**

Chromophobic cells had a diffuse architecture and a partly sinusoidal one. Few mitoses were seen. Immunohistochemically, anti-ACTH antibodies stained weakly. No cells could be found to be stained with anti-FSH, anti-PRL, anti-GH, anti-LH or anti-TSH antibodies. Electron microscopically, tumor cells were angular and had ovoid nuclei. The cytoplasm contained well-developed rough endoplasmic reticulum, free ribosomes, Golgi complexes and secretory granules. Secretory granules varied from 140-270 nm in diameter with variability in electron density. The bundles of intermediate filaments located around the nucleus.

**Discussion**

Silent corticotropinoma, which exhibits no Cushing’s syndrome, secretes biologically inactive ACTH, but not active 1-39 ACTH [14-16]. The size of a silent corticotropinoma is typically large when discovered, and the tumor exhibits a high incidence of postoperative recurrence [22, 23]. Recently, it has
been reported that unusual POMC derivatives are secreted not only from silent corticotropinomas [14-16], but also from macrocorticotropinomas exhibiting Cushing's syndrome [15, 24, 25]. The concentration of POMC in patients with Cushing's disease due to pituitary microadenoma ranges approximately from the equimolar level to a slightly higher level than found in ACTH [26]. Unusual processing of POMC in the tumor may cause this phenomenon [14-16]. Even though this secreted ACTH is not as biologically active as 1-39 ACTH and/or may inhibit the biological activity of 1-39 ACTH, the total biological activity of a large amount of ACTH produced from the corticotropinoma may sufficiently induce Cushing's syndrome. It has been hypothesized that the macrocorticotropinoma causing Cushing's disease is a relatively inefficient producer of ACTH, but that it causes Cushing's syndrome by virtue of its mass [27]. Macroadenoma of Cushing's disease may not belong in the same category as microadenoma in Cushing's disease, but rather it may fit into the same category as silent corticotropinoma.

Postoperative hypoadrenocorticism could consistently be observed in most patients with clinical remission [28-31]. A variety of criteria have been used to assess the cure after transsphenoidal surgery [1, 6, 32]. Immediately following complete resection of the pituitary corticotropinoma of Cushing's disease, adrenocortical steroidogenesis is suppressed because of the suppression of the normal CRH-ACTH axis by the preoperative cortisol excess. According to a recent consensus [33], the best criteria of the cure of Cushing's disease following surgery should be based on undetectable plasma cortisol (<28 to 56 nmol/l) and ACTH (<1 to 2 pmol/l) levels, depending upon assay sensitivity. Accordingly, after complete resection of the corticotropinoma of Cushing's disease, acute adrenal insufficiency should occur without any glucocorticoid substitution. However, when a part of the tumor tissue still remains to be resected, it is conceivable that no adrenal insufficiency occurs as a matter of course. Plasma cortisol level was only 94 nmol/l in spite of a 13 pmol/l of plasma ACTH level in our patient. Adrenal insufficiency may have occurred under conditions where plasma cortisol levels could be detected, possibly because of a down-regulated cortisol receptor number immediately following surgery.
The high molecular weight POMC-derived peptides, which were measured with IRMA, could be detected in the plasma after separation with column chromatography. Its biological activity was only 28% of the mean activity of 1-39 ACTH, but its molecular ratio in all of the IRMA-measurable ACTH was 11%. Unfortunately, no examination was performed regarding the competition of the binding capacity of the high molecular weight ACTH with that of 1-39 ACTH to the ACTH receptor. The high molecular weight ACTH, which is not only in the peak fraction No. 14 but also in the peak fractions other than 1-39 ACTH, hypothetically, may have competed with 1-39 ACTH in binding to the receptor in our patient. The high molecular weight ACTH secreted from the remnant tumor may have played a role in the onset of adrenal insufficiency in our patient. Furthermore, no assessment was made about the amino acid sequence of ACTH which resided in the elution position of 1-39 ACTH. It is unknown whether its amino acid-sequence and biological activity were the same as 1-39 ACTH or not.

When a large amount of biologically insufficient ACTH is secreted from a macrotocorticotropinoma of Cushing’s disease, careful observation for acute adrenocortical insufficiency is required even after incomplete resection of the tumor.

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