Successful reduction of ACTH secretion in a case of intractable Cushing’s disease with pituitary Crooke’s cell adenoma by combined modality therapy including temozolomide

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Abstract. Crooke’s cell adenoma (CCA) is an aggressive subtype of corticotroph adenoma; however, CCA is associated with a high incidence of low expression of methyl guanine methyl transferase (MGMT), suggesting that temozolomide (TMZ) treatment might be effective for this tumor type. The case of a 56-year-old woman with Cushing’s disease caused by a pituitary CCA is presented. At the age of 38 years, the patient presented to our hospital with polyuria and a visual field defect. MRI and laboratory studies showed a 4.5-cm-diameter pituitary tumor with plasma adrenocorticotropic hormone (ACTH) and serum cortisol levels of more than 500 pg/mL and 40 μg/dL, respectively. At 39 years of age, the patient underwent a craniotomy, and her plasma ACTH and cortisol levels decreased to less than 200 pg/mL and 10 μg/dL, respectively; however, these hormone levels increased gradually to 3,940 pg/mL and 70 μg/dL, respectively, by the time the patient was 56 years old. Histopathological re-examination of the previously resected specimen showed that the pituitary tumor was MGMT-negative CCA. TMZ treatment after the second operation decreased the plasma ACTH levels from 600–800 pg/mL to 70–300 pg/mL. No signs of recurrence were observed in the seven years following these treatments with added prophylactic radiation therapy. These clinical findings suggest that TMZ treatment to patients with CCA accompanied with elevated ACTH may be a good indication to induce lowering ACTH levels and tumor shrinkage.

Key words: Cushing disease, Crooke’s cell adenoma, Methyl guanine methyl transferase (MGMT), Temozolomide

CROOKE’S CELL ADENOMA (CCA) is an aggressive subtype of corticotroph adenoma; it is usually large, frequently invades surrounding tissues, and is resistant to both surgery and radiation therapy [1]. However, CCA has reportedly been associated with a high incidence of low expression of methyl guanine methyl transferase (MGMT), a DNA mismatch repair gene [2], suggesting that temozolomide (TMZ) is a potentially effective agent for this tumor type. A case of Cushing’s disease caused by a massive pituitary CCA that was successfully treated by TMZ administration after transsphenoidal surgery was performed as the second operation, followed by prophylactic radiation therapy is presented.

Case Report

A 56-year-old woman was admitted to Shimane University Hospital complaining of general fatigue due to hypertension and leg edema. At 39 years of age, she presented to the hospital with the chief complaint of year-long continuous weight gain. Pigmentation was observed on her nails and skin of the extensor surfaces of her fingers, elbows, and knees. She suffered from polyuria and delirium, as well as a right visual field loss due to a 4.5-
A 50-year-old woman presented with a 2–3 cm-diameter pituitary tumor that displaced the right optic nerve and invaded the right cavernous sinus. She was diagnosed as having Cushing’s disease by the plasma ACTH level (500 pg/mL) and serum cortisol level (40 μg/dL), and it was associated with growth hormone (GH) deficiency, central hypothyroidism, central hypogonadism, and diabetes insipidus, which had been diagnosed on the previous admission. Her pituitary tumor was partially resected by craniotomy, resulting in a decrease of her plasma ACTH and serum cortisol levels (200 pg/mL and 10 μg/dL, respectively). However, these hormone levels increased gradually along with the enlargement of the residual pituitary tumor. Despite treatment with cabergoline (2 mg/three times a week), octreotide LAR (20 mg/month), and the subsequent additions of metyrapone (3,750 mg) and mitotane (1,500 mg), her plasma ACTH and serum cortisol levels increased to 3,940 pg/mL and 70 μg/dL, respectively, by 56 years of age. She was admitted to the hospital to control her hypercortisolemia, the cause of her chief complaint.

High blood pressure (172/95 mmHg) and sinus tachycardia (103/min) were observed. Physical examination showed central obesity (BMI 30.5 kg/m²), a moon-shaped face, and a buffalo hump. The skin was thin and free of purpura. Gray pigmentation was noted on the nails and extensor joint regions of the fingers, elbows, and knees. Hirsutism was absent. Right pupil dilation was accompanied by ipsilateral blepharoptosis. Muscle weakness and severe edema were noted in bilateral lower extremities.

Laboratory data are summarized in Table 1. Levels of serum sodium were higher and serum potassium were lower than their reference ranges. Elevated levels of ACTH and cortisol, as well as hypophenuria with a low level of anti-diuretic hormone, were observed. Magnetic resonance imaging showed that a giant pituitary tumor (5.7 cm) invaded the right cavernous sinus and infiltrated the preoptic cistern and sphenoid sinus. The tumor was iso-intense on T1-weighed images, high to iso-intense on T2-weighed ones, and enhanced by gadolinium (Fig. 1). Abdominal CT showed enlarged bilateral adrenal glands. The plasma ACTH was examined by gel chromatography because the serum cortisol levels were low relative to the plasma ACTH levels. This analysis
showed that “big ACTH” comprised 65% of all of the ACTH immunoreactivity (Fig. 2).

An immunohistochemical re-evaluation of the tissue specimen obtained from the previous pituitary surgery was performed, and over half of the pituitary cells showed Crooke’s degeneration (Fig. 3A); this was confirmed by low-molecular-weight cytokeratin (CAM 5.2) staining (Fig. 3B). Immunohistochemical examinations demonstrated that these cells were positive for ACTH (Fig. 3C); however, the other anterior pituitary hormones, such as GH, PRL, TSH, FSH, and LH, were immunonegative (data not shown). In addition, the Ki-67 (MIB-1) labeling index was less than 1% (data not shown), and O6-methylguanine-DNA methyltransferase (MGMT) expression was not observed (Fig 3D). This enlarged pituitary tumor was resected as radically as possible in the second surgery via a transsphenoidal approach. The histological findings were similar to those of the specimens from the first operation. Additional treatment was needed to control the insufficiently reduced plasma ACTH levels (600–800 pg/mL) (Fig. 4).

TMZ treatment at an initial dose of 150 mg/m² for five days was started with the intention of controlling the excess ACTH. The maintenance dose of 100 mg/m² for five days every four weeks was administered nine times without any serious side effects, except for pancytopenia and severe nausea at the second and third courses of 150 and 200 mg/m² TMZ, respectively. During the TMZ treatment, the plasma ACTH levels gradually decreased to 70–300 pg/mL, and the patient demonstrated no response to the corticotropin-releasing hormone test at three months after the end of chemotherapy. Compared with the plasma ACTH levels, serum cortisol levels decreased to less than 10 μg/dL, which suggested that the residual pituitary tumor secreted biologically inactive ACTH. Thus, the administration of metyrapone was discontinued, and glucocorticoid replacement therapy was initiated. Hypertension, dyslipidemia, and typical Cushingoid features, such as moon-shaped face, skin pigmentation, and leg edema, disappeared. Both adrenal glands shrank in size, and mitotane treatment was stopped. The residual pituitary tumor outside the right cavernous sinus

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after the second operation (Fig. 5A) had obviously shrunk by 13 months after the discontinuation of TMZ treatment (Fig. 5B). Conventional radiation therapy (50 Gy) was then administered prophylactically. No signs of recurrence, such as elevation of plasma ACTH levels or enlargement of the pituitary tumor, were observed in the seven years following TMZ treatment (Fig. 5C).

**Molecular size analysis of plasma ACTH by gel chromatography**

Gel chromatography was performed as previously reported to ascertain the molecular size of the circulating plasma ACTH levels [3, 4]. The plasma samples stored at –40°C were extracted via solid phase extraction using an SEP-PAC C18 cartridge (Waters, Milford, MA) and acidified (pH 1.5) and eluted with 1% formic acid using a Sephadex G-75 column (Amersham Pharmacia Biotech, Piscataway, NJ). ACTH levels of the collected fractionated samples were determined by conventional immunoradiometric assay.

**Discussion**

Crooke’s cell adenoma (CCA), a histological variant form of corticotroph adenoma with hyaline degeneration, is an invasive macroadenoma that presents with Cushing’s disease or is endocrinologically silent. It is a high-risk pituitary adenoma because it frequently invades surrounding tissues and recurs after reoperation and/or radiotherapy [5]. A recent consecutive surgical case series of patients with a preoperative diagnosis of Cushing’s disease showed that 75–80% of these patients exhibit Crooke’s changes [6], suggesting that Cushing’s disease may be a potentially aggressive illness in which it is difficult to control tumor expansion and hormonal levels. Various combined, multimodal, and sequential therapies have therefore been explored. The recent guidelines for the treatment of aggressive pituitary tumors published by the European Society of Endocrinology recommend pasireotide [7], a novel somatostatin analog that binds to four of the five somatostatin receptor (SST) subtypes, with a higher affinity for SST1 and SST5 than octreotide or lanreotide [8, 9]. Pasireotide is recommended as a...
standard medication to control elevated plasma ACTH levels induced by Cushing’s disease cases that fail or are ineligible for surgical therapy [7], because corticotroph tumors express SST5 highly. However, the present patient was not able to receive this agent because it was not approved for the treatment of Cushing’s disease in Japan until March 2018. In addition, the aforementioned guidelines also recommend that repeat surgery should be considered prior to other treatment options [7]. Remission of the symptoms caused by hypercortisolism was achieved by massive tumor debulking via the transsphenoidal approach performed by expert neurosurgeons, indicating that surgical extraction is the most efficacious procedure to obtain hormonal reduction, as described in the guidelines.

Radiation therapy is also recommended for patients with clinically relevant tumor growth despite the possibility of surgery or standard medication because it may offer the possibility of long-term reduction of tumor growth [7]. However, control of cortisol excess with radiation is generally more difficult than that of tumor size [10-12]. Thus, in the present case, TMZ chemotherapy was given prior to radiation to reduce the residual excess secretion of plasma ACTH levels after the second operation.

Partial or complete volume reduction by TMZ treat-
ment was reported in 47% of cases [7]. However, the effect of TMZ on plasma ACTH levels was unclear. TMZ, which is an oral alkylating agent generally used for brain glioblastoma, is recommended as a first-line drug for aggressive pituitary tumors [13, 14]. The present clinical course showed that additional improvement of plasma ACTH levels was not observed after the radiation therapy, suggesting that endocrinological remission of hypercortisolemia due to elevated plasma ACTH levels was achieved by TMZ administration. Similar ameliorations were reported in cases of ACTH-secreting carcinoma and aggressive corticotroph adenoma [15-17]. These findings suggest that TMZ administration might be an excellent approach to address early endocrinological remission relative to radiation therapy. Five cases (two men and three women; age range, 43–61 years) of CCA showing Cushing’s syndrome treated with TMZ, including the present case, have been reported (Table 2) [18-21]. The expression of MGMT was negative in three cases and not examined in the others [19, 21], and, except for one patient for whom the Ki-67 index was not obtained [18], the Ki-67 indices were less than 2% in all of the patients, including indices equal to or less than 1% in three cases. Based on these findings, CCA presenting as Cushing’s syndrome seems to be responsive to TMZ. Indeed, partial or complete reduction of tumor size, as well as decreases in plasma ACTH, was observed in all patients, except for one case in which the laboratory data were not documented [18]. The dose of TMZ prescribed in previous reports was 150–200 mg/m$^2$ once daily, for five consecutive days, every 28 days, nine to 12 times. Hence, in comparison with prior reports, a decrease of plasma ACTH levels was achieved in the present case with a smaller dosage and minimal treatment cycles. One report showed that the pituitary tumor and plasma ACTH levels suddenly increased at the eighth cycle of TMZ treatment [21]; however, two previous cases achieved complete remission at 18 and 33 months [19, 20]. In addition, the present case demonstrated no recurrence of elevated plasma ACTH levels in the seven years following TMZ treatment. One of the unique ways in which the present case differed from the previous cases was that the present patient underwent prophylactic radiation therapy after the administration of TMZ, which might have depressed or stabilized the invasive potential of the pituitary lesion. In addition, the secreted ACTH of the present patient contained “big-ACTH.” Thus, endocrinological recurrence, such as Cushingoid symptoms due to elevated ACTH levels, might not have appeared in the present case as easily as in cases of Cushing’s disease caused by

Table 2  Review of the literature on Crooke’s cell adenomas showing Cushing’s syndrome treated with TMZ

<table>
<thead>
<tr>
<th>Authors</th>
<th>Age</th>
<th>Sex</th>
<th>Tumor size</th>
<th>MGMT</th>
<th>Ki-67 (%)</th>
<th>Previous therapies</th>
<th>TMZ treatment schedule</th>
<th>Number of TMZ courses</th>
<th>Tumor regression (observation period)</th>
<th>Endocrinological remission (GC replacement)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mohammed et al. [18]</td>
<td>43</td>
<td>M</td>
<td>Macro</td>
<td>Negative</td>
<td>≤1</td>
<td>TSS (twice), craniotomy, gamma knife</td>
<td>150–200 mg/m$^2$ × 5/28 days</td>
<td>12</td>
<td>Yes (16 months)</td>
<td>N/A</td>
</tr>
<tr>
<td>Asimakopoulou et al. [19]</td>
<td>55</td>
<td>F</td>
<td>Macro 22 mm</td>
<td>N/A</td>
<td>≤1</td>
<td>TSS, conventional radiotherapy, gamma knife</td>
<td>150–200 mg/m$^2$ × 5/28 days</td>
<td>10</td>
<td>Yes (18 months)</td>
<td>Yes (Yes)</td>
</tr>
<tr>
<td>Kurowaska et al. [20]</td>
<td>54</td>
<td>M</td>
<td>Macro 39 mm</td>
<td>Negative</td>
<td>≤1</td>
<td>TSS</td>
<td>150–200 mg/m$^2$ × 5/28 days</td>
<td>9</td>
<td>Yes (33 months)</td>
<td>Yes (No)</td>
</tr>
<tr>
<td>Gilis-Januszewska et al. [21]</td>
<td>61</td>
<td>M</td>
<td>Macro 33 mm</td>
<td>N/A</td>
<td>≤2</td>
<td>TSS (4 times), bilateral adrenalectomy, stereotactic radiotherapy, gamma knife</td>
<td>150–200 mg/m$^2$ × 5/28 days</td>
<td>9</td>
<td>Yes, relapse at 8th TMZ course</td>
<td>Temporary, maximally 52% decrease of baseline ACTH</td>
</tr>
<tr>
<td>Our case</td>
<td>56</td>
<td>F</td>
<td>Macro 57 mm</td>
<td>Negative</td>
<td>≤1</td>
<td>craniotomy, TSS</td>
<td>100–200 mg/m$^2$ × 5/28 days</td>
<td>9</td>
<td>Yes, added preventative radiation (7 years)</td>
<td>Yes (Yes)</td>
</tr>
</tbody>
</table>

TMZ, temozolomide; Macro, macro adenomas; MGMT, methyl guanine methyl transferase; N/A, not available; TSS, transsphenoidal surgery; GC, glucocorticoid
“authentic” ACTH, because 65% of the plasma ACTH levels were biologically inactive in this case. Considering these observations, CCA accompanied by elevated ACTH levels, potentially caused by “big-ACTH”, may be a good indication for TMZ treatment at 150–200 mg/m² once daily, for five consecutive days, every 28 days, at least nine times, in order to achieve biochemical remission via the decrease in plasma ACTH levels and shrinkage in tumor size. However, further studies are needed to determine the optimal dose and cycles of TMZ treatment and clarify whether TMZ can re-rescue recurrent cases of CCA accompanied with elevated ACTH levels, because this agent might cause secondary hematological malignancies [22, 23] and seems not to be effective in a second course of treatment for aggressive ACTH pituitary tumors [24].

**Conclusion**

A case of Cushing’s disease caused by CCA was described. The pituitary mass and plasma ACTH levels were successfully controlled with TMZ administration after transsphenoidal surgery, which was performed as the second operation, followed by prophylactic radiation therapy. CCA with elevated ACTH, potentially caused by “big-ACTH”, may be a good indication for TMZ treatment to induce biochemical remission via a decrease in plasma ACTH levels and tumor size.

**Disclosure**

None of the authors has any potential conflicts interest associated with this research.

**References**

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