CASE REPORT

Effect of cabergoline treatment on Cushing's disease caused by aberrant adrenocorticotropin-secreting macroadenoma

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ABSTRACT. The present case involves a 47-yr-old woman with Cushing's disease due to pituitary macroadenoma. The patient had suffered from hypertension and obesity for two yr. Her serum cortisol levels were moderately elevated throughout the observation period, and dexamethasone failed to suppress the cortisol secretion. Plasma ACTH levels were markedly high (>100 pg/ml) and did not respond to CRH provocation. Gel filtration analysis of the patient's plasma detected the existence of big ACTH molecules, which eluted with a peak of authentic 1-39 ACTH. Cranial magnetic resonance imaging (MRI) revealed a 3 cm pituitary tumor occupying the sellar region and right cavernous sinus with diffuse enhancement by gadolinium. The pituitary mass was removed by transsphenoidal surgery, and was pathologically identified as compatible to ACTH-producing pituitary adenoma by immunohistochemistry. RT-PCR analysis of total cellular RNA extracted from the resected adenoma revealed a relatively high expression level of dopamine D2 receptor (D2R) mRNA. Therefore, a long-acting D2R agonist, cabergoline (0.25 to 0.5 mg/week), was administered for the remnant adenoma, which gradually reduced ACTH levels in 90 days. In addition, cranial MRI exhibited shrinkage of the remnant pituitary mass after a 6-month treatment with cabergoline. This case demonstrates the efficacy of cabergoline to treat Cushing's disease caused by pituitary macroadenoma secreting aberrant ACTH molecules.
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INTRODUCTION

Cushing's disease is caused by hypercoltisolism due to chronic overproduction of ACTH from pituitary corticotropes. The majority of corticotrope tumors are microadenomas with diameters <10 mm (1). In contrast, ACTH-secreting macroadenomas are much less frequent, and are occasionally accompanied by visual field defects and impairment of the secretion of other pituitary hormones (2). Recent studies have shown that macroadenomas secrete more ACTH than microadenomas, and frequently are less responsive than microadenomas to glucocorticoid suppression of ACTH using dexamethasone (3), suggesting that macroadenomas exhibit greater autonomous ACTH secretion and more pronounced hypercorticism (2).

Cushing's disease is currently treated with transsphenoidal removal of the ACTH-producing adenoma. The cure rate of this surgical procedure is 80 to 90% for the microadenomas (4), whereas it is at most 50% for the macroadenomas (5). When the surgical procedure alone fails to successfully cure Cushing's disease, pituitary irradiation and/or medication regimens need to be administered in an attempt to achieve biochemical remission (4, 5).

In the present case, based on studies that have reported dopamine D2 receptor (D2R) expression in

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resected adenoma tissue, we have used a new long-acting dopamine agonist, cabergoline, to treat a Cushing macroadenoma secreting aberrant ACTH.

CASE REPORT

A 47-yr-old Japanese woman who presented with hypertension, obesity and facial edema was referred to our hospital with suspicion of Cushing's syndrome. A pituitary tumor in the sella turcica was detected by pituitary magnetic resonance imaging (MRI), and hypercortisolemia with increased plasma ACTH levels was also uncovered. Her past medical history was negative with the exception of a history of hypertension and menopause occurring at the relatively young age of 45. She had gradually gained 10 kg body weight over the previous 7 yr, and upon admission she was 160.3 cm tall and weighed 68 kg (body mass index, 26.5 kg/m^2). Her blood pressure was 122/80 mmHg and pulse rate was 80 bpm under the medication. Typical Cushingoid features, including central/truncal obesity, buffalo hump, striae cutis, skin atrophy, muscle weakness and hirsutism, were not seen. Laboratory examinations, including 75 g oral glucose tolerance test, revealed moderate glucose intolerance (252 mg/dl at 2 h) and hyperuricemia (7.2 mg/dl, normal, 2.4-5.7). Cranial MRI revealed a mass lesion (~30 mm in diameter) in the sella turcica, showing low intensity on T1-images with diffuse enhancement by gadolinium (Fig. 1). Endocrine examinations revealed that plasma ACTH (60 to 109.7 pg/ml; normal, 9-52) was markedly elevated and did not exhibit normal circadian fluctuations. Cortisol levels (20.5 to 30.4 μg/dl; normal 8-25) were only slightly above normal. Dexamethasone (1 to 4 mg) failed to suppress the serum cortisol level, and CRH stimulation (100 μg iv) evoked no changes in the levels of ACTH (basal level to peak: 84.8 and 99.0 pg/ml) or cortisol (25.5 to 26.7 μg/dl). Analysis of the other pituitary hormones revealed that the levels of PRL, TSH and vasopressin were within the normal ranges but GH and gonadotropin levels were low. Upon confirming the diagnosis of Cushing's disease, transsphenoidal surgery was performed (Fig .1). The resected tumor was pathologically diagnosed as an ACTH-producing pituitary adenoma by immunohistochemistry (Fig. 2). Post-operative levels of ACTH (48.7 pg/ml) and cortisol (15.9 μg/dl) were reduced by 50%, and the responsiveness of ACTH (basal level to peak: 48.7 to 185.1 pg/ml) and cortisol (15.9 to 37.2 μg/dl) to exogenous CRH was restored. Acute effects of bromocriptine (2.5 mg po) and octreotide (100 μg sc) were evaluated after surgery, resulting poor suppression of plasma ACTH by both drugs (10 and 16% reduction by bromocriptine and octreotide, respectively). Subsequently a long-acting dopamine agonist, cabergoline, was administered in an attempt to normalize the plasma ACTH level. As shown in Figure 3, cabergoline treatment (0.25 to 0.5 mg/week) for 3 months gradually suppressed ACTH and cortisol levels and, moreover, the treatment for 6 months also diminished the size of remnant adenoma (Fig. 1).

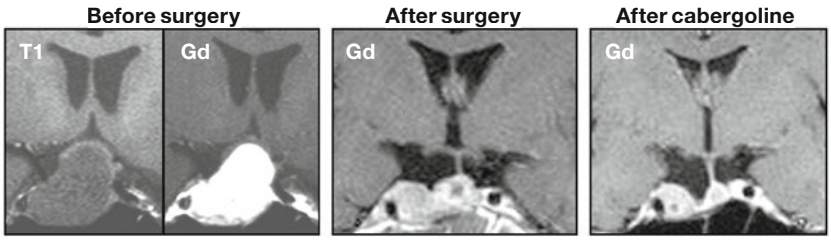

Fig. 1 - *Magnetic resonance imaging (MRI) findings of pituitary tumor. Coronal T1-images with gadolinium (Gd) enhancement are shown before transsphenoidal surgery, after surgery and 6 months after cabergoline therapy.*

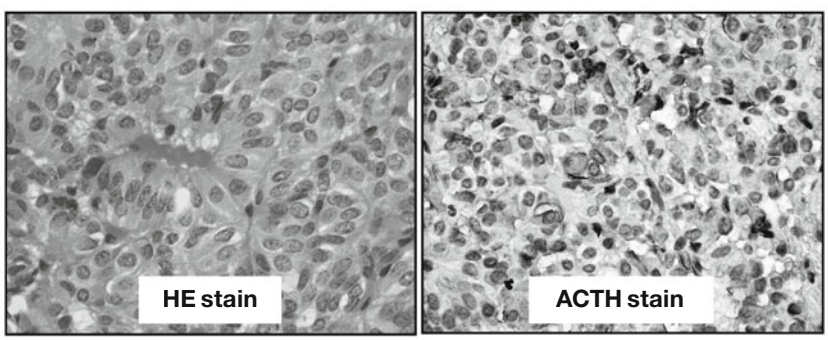

Fig. 2 - *Pathological findings of the resected tumor. Hematoxilin-eosin (HE) staining, x 400; and immunohistochemisty using anti-ACTH antibody, x 400.*

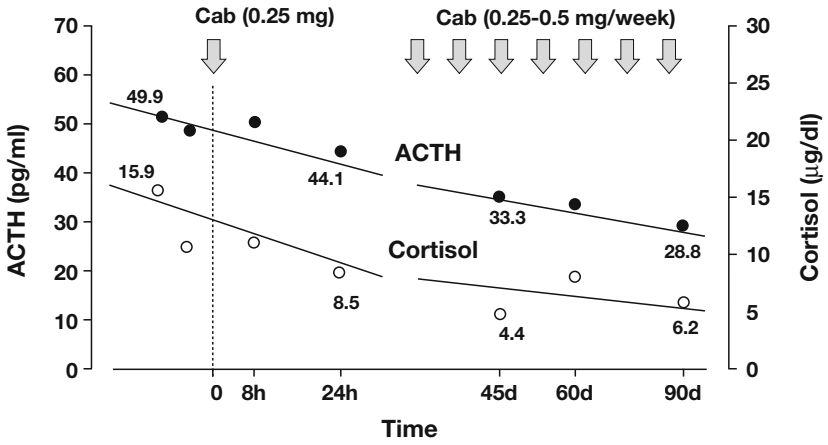

Fig. 3 - *Changes in ACTH and cortisol level during cabergoline treatment.*

Gel chromatography analysis of ACTH molecules

Molecular size analysis of circulating immunoreactive (IR)-ACTH was performed by gel chromatography as previously reported (6). Briefly, the plasma sample stored at –40 C was extracted using SEP-PAK C_{18} cartridge (Waters, Milford, MA). The plasma elutant (3 ml) was acidified and applied to a Sephadex G-75 column (1× 60 cm; Amersham-Pharmacia Biotech) and eluted with 1% formic acid. After each 2 ml-fraction was fractionated and lyophilized, the ACTH concentration of each fraction was determined by two different immunoradiometric assays (IRMA). As a result, ACTH IRMA "Yuka" kit (using antibodies against 1-24 ACTH and 18-39 ACTH; Mitsubishi Chemical, Tokyo, Japan) detected only a main peak of 1-39 ACTH, whereas Nichols Allegro ACTH kit (using antibodies against 1-17 ACTH and 34-39 ACTH; Nihon Medi-Physics, Tokyo, Japan) detected bigger ACTH molecules in addition to the main molecule of 1-39 ACTH (Fig. 4).

RT-PCR analysis of total cellular RNA extracted from pituitary adenomas

Total cellular RNA was extracted from freshly resected tissues using TRIzol® (Invitrogen Corp., Carlsbad, CA). The expression of D2R was detected by RT-PCR analysis as previously reported (7). The RNAs extracted from a different ACTH-producing adenoma (bromocriptine-insensitive), a PRL-producing adenoma, a FSH-producing adenoma and a non-functioning adenoma were also evaluated by RT-PCR as control study. The primer pairs for PCR were selected from different exons of the corresponding genes to discriminate PCR products that might arise from possible chromosome DNA contaminants. Specifically, they were derived from the cDNA clones at the following nucleotide numbers: 800-820 and 1261-1281 for D2R (GenBank accession number, NM_000795); and 401-420 and 571-590 for L19 (NM_000981). The extracted RNA (500 ng) was subjected to a RT reaction using First-Strand cDNA Synthesis System® (Invitrogen Corp., Carlsbad, CA) at 42 C for 50 min and 70 C for 10 min. Subsequently, 38 cycles of PCR were performed using Taq DNA polymerase (Invitrogen) under the conditions of 96 C for 30 sec, 60 C for 30 sec and 72 C for 30 sec. Aliquots of PCR products were electrophoresed on 1.5% agarose gels and visualized after ethidium bromide staining. As shown in Figure 5, D2R was clearly expressed in the present ACTH adenoma, in which two splicing variant forms – D2Rs (395 bp) and D2R*l* (482 bp) – were detected, whereas the expression of D2R was not detected in the total RNA extracted from a different case of ACTH adenoma lacking in bromocriptine effectiveness. A PRL adenoma exhibited a strong signal of D2R*l*, while a FSH adenoma and a non-functioning adenoma had very weak signals of D2R*l*. In all the RNAs examined, internal control L19 (195 bp) expression was almost equally detected.

DISCUSSION

Cabergoline is a new dopamine D2R agonist with a higher affinity and longer half-life than bromocriptine (8). The effectiveness and tolerance have been well recognized in the treatment of PRL- and GH-producing adenomas (9-11). However, only a few cases of ACTH adenomas successfully treated with cabergoline have been reported. In a recent trial, a patient following bilateral adrenalectomy showed complete remission of Nelson's syndrome after 1-yr cabergoline therapy (12). Shrinkage of an ACTH silent adenoma was also demonstrated 4 months after the commencement of cabergoline (0.5 mg

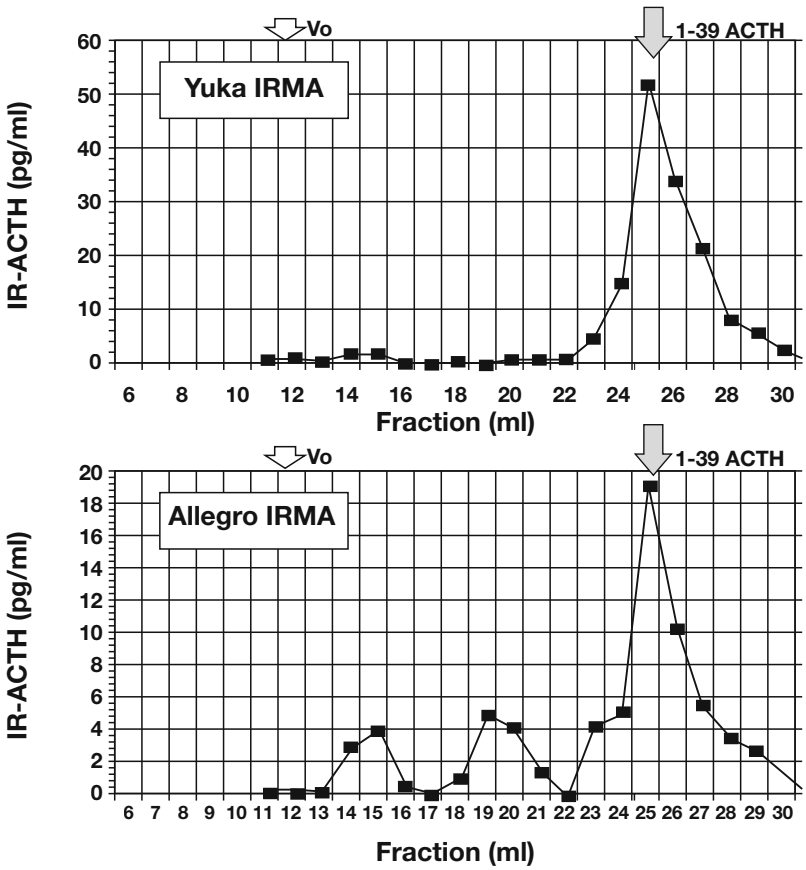

Fig. 4 - *Gel filtration analysis of preoperative plasma ACTH. IR-ACTH concentration was determined by the IRMA methods using ACTH IRMA "Yuka" kit (Mitsubishi; upper panel) and Allegro ACTH kit (Nihon Medi-Physics; lower panel). Vo, void volume.*

every 2 days), in which the number of D2R on the tumor was highlighted as a good indicator for the sensitivity to dopamine agonists, using in situ hybridization and receptor autoradiography (13).
In the anterior pituitary, the hormonal response to a dopamine agonist is related to the D2R activity (14). The D2R belongs to the family of G-protein coupled receptors, and acts through the inhibition of the adenylate cyclase. Alternative splicing of the D2R gene transcription leads to two variant forms including short and long isoform, D2R*s* and D2R*l*, respectively (14). The D2R*l* isoform predominates in the human pituitary. This is consistent with our present data showing exclusive expression of D2R*l* in various adenomas, in particular in a PRL adenoma. It is also notable that the present ACTH adenoma clearly expressed D2R*s*, although the expression level was less abundant than D2R*l* form (Fig. 5). Furthermore, a recent *in vitro* study on clinically non-functioning adenomas indicates that the cells expressing D2R*s* form are associated with the growth inhibitory effect of dopamine agonists (15). The clinical relevance of expression of D2R in the tu-

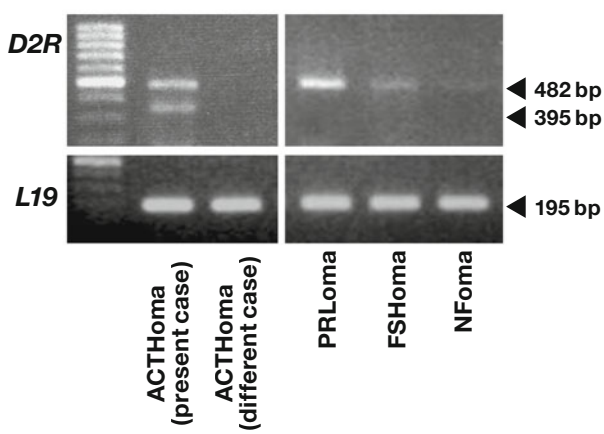

Fig. 5 - *Expression of D2 receptor (D2R) in the resected tumor. D2R expression was examined by RT-PCR using total cellular RNAs extracted from the pituitary adenomas. ACTHoma: ACTH-producing adenoma (the present case and a different case); PRLoma: PRL-producing adenoma; FSHoma: FSH-producing adenoma; NFoma: non-functioning adenoma. L19 level was shown as an internal control.*

mor to the effectiveness of bromocriptine was also presented in a case of FSH-producing adenoma (16), in which the message of D2Rs form was detected in the adenoma tissue. Based on the present data, it is also possible that the existence of D2Rs form in ACTH-producing adenomas is involved in the effectiveness to cabergoline treatment.

In the present case, the molecular form of circulating ACTH was found to be 1-39 ACTH; however, higher molecular-weight ACTH molecules composed of two peaks were concomitantly detected by Alergo IRMA method using Nichols antibody. The presence of D2R in ACTH adenomas could be directly involved in the POMC processing and the subsequent ACTH production. Experimentally, D2R-deficient mice were shown to have increased POMC expression with intermediate lobe hypertrophy (17). In these mice, the processing of POMC is also enhanced due to the overexpression of prohormone convertase-1 (PC1) in the intermediate lobe, leading to increase in circulating ACTH, which ultimately results in a Cushingoid phenotype (17). Thus, the D2 action is a key factor in controlling POMC expression, as well as in processing POMC-derived peptides in the pituitary. It is therefore possible that higher D2R expression in the present Cushing's adenoma may be associated with impairment of the POMC processing through less activation of the PC1, causing the secretion of aberrant ACTH molecules from the pituitary adenoma.

In summary, we presented a case of Cushing's disease that was successfully treated with cabergoline. To examine the D2R expression in the resected tumors is a conventional method in order to evaluate the efficacy of dopamine agonist on the treatment of Cushing's disease. This case also raised a clinical relevance between the aberrant processing of ACTH precurcer and dopamine D2 action in Cushing's macroadenomas.

REFERENCES

1. Bevan JS, Webster J, Burke CW, Scanlon MF. Dopamine agonists and pituitary tumor shrinkage. Endocr Rev 1992, 13: 220-40.
2. Selvais P, Donckier J, Buysschaert M, Maiter D. Cushing's disease: a comparison of pituitary corticotroph microadenomas and macroadenomas. Eur J Endocrinol 1998, 138: 153-9.
3. Katznelson L, Bogan JS, Trob JR, et al. Biochemical assessment of Cushing's disease in patients with corticotroph macroadenomas. J Clin Endocrinol Metab 1998, 83: 1619-23.
4. Mampalam TJ, Tyrrell JB, Wilson CB. Transsphenoidal microsurgery for Cushing disease. A report of 216 cases. Ann Intern Med 1988, 109: 487-93.
5. Shimon I, Melmed S. Management of pituitary tumors. Ann Intern Med 1998, 129: 472-83.
6. Hashimoto K, Kaneda T, Nagano I, Asaba K, Takeda K, Takao T. Pituitary adenoma showing intermittent secretion of high molecular weight adrenocorticotropin without evidenice of Cushing's disease. Horm Res 1999, 52: 39-44.
7. Takeda M, Otsuka F, Suzuki J, et al. Involvement of activin/BMP system in development of human pituitary gonadotropinomas and nonfunctioning adenomas. Biochem Biophys Res Commun 2003, 306: 812-8.
8. Webster J, Piscitelli G, Polli A, Ferrari CI, Ismail I, Scanlon MF. A comparison of cabergoline and bromocriptine in the treatment of hyperprolactinemic amenorrhea. Cabergoline Comparative Study Group. N Engl J Med 1994, 331: 904-9.
9. Verhelst J, Abs R, Maiter D, et al. Cabergoline in the treatment of hyperprolactinemia: a study in 455 patients. J Clin Endocrinol Metab 1999, 84: 2518-22.
10. Abs R, Verhelst J, Maiter D, et al. Cabergoline in the treatment of acromegaly: a study in 64 patients. J Clin Endocrinol Metab 1998, 83: 374-8.
11. T'Sjoen G, Defeyter I, Van De Saffele J, Rubens R, Vandeweghe M. Macroprolactinoma associated with Cushing's disease, successfully treated with cabergoline. J Endocrinol Invest 2002, 25: 172-5.
12. Pivonello R, Faggiano A, Di Salle F, Filippella M, Lombardi G, Colao A. Complete remission of Nelson's syndrome after 1-year treatment with cabergoline. J Endocrinol Invest 1999, 22: 860-5.
13. Petrossians P, Ronci N, Valdes Socin H, et al. ACTH silent adenoma shrinking under cabergoline. Eur J Endocrinol 2001, 144: 51-7.
14. Ben-Jonathan N, Hnasko R. Dopamine as a prolactin (PRL) inhibitor. Endocr Rev 2001, 22: 724-63.
15. Renner U, Arzberger T, Pagotto U, et al. Heterogeneous dopamine D2 receptor subtype messenger ribonucleic acid expression in clinically nonfunctioning pituitary adenomas. J Clin Endocrinol Metab 1998, 83: 1368-75.
16. Murata Y, Ando H, Nagasaka T, et al. Successful pregnancy after bromocriptine therapy in an anovulatory woman complicated with ovarian hyperstimulation caused by follicle-stimulating hormone-producing plurihormonal pituitary microadenoma. J Clin Endocrinol Metab 2003, 88: 1988-93.
17. Saiardi A, Borrelli E. Absence of dopaminergic control on melanotrophs leads to Cushing's-like syndrome in mice. Mol Endocrinol 1998, 12: 1133-9.