NOTE

Thymic Hyperplasia as a Source of Ectopic ACTH Production

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Abstract. A 26 year-old man with suspected Cushing's disease underwent transsphenoidal exploration of the pituitary without any evidence of microadenoma or hyperplasia. Progressive hypercortisolism necessitated bilateral adrenalectomy. Postoperatively, skin pigmentation gradually developed with a marked elevation of plasma ACTH levels, and CT scanning uncovered a thymic mass. Following removal of the thymic mass, skin pigmentation disappeared and plasma ACTH levels fell to normal. The excised mass was found to be a benign thymic hyperplasia without epithelial or carcinoid tumor cells. However, gel chromatography showed that the thymic tissue extract contained high ACTH content comparable to that of ectopic ACTH-producing tumors with a major component corresponding to ACTH(1–39). Northern blot analysis and in situ hybridization revealed the expression of pro-opiomelanocortin transcripts in lymphocytes of thymic hyperplasia. This report suggests that lymphocytes in thymic hyperplasia are the most likely site of deregulated ACTH expression causing ectopic ACTH syndrome.

Key words: Ectopic ACTH syndrome, Thymic hyperplasia, in situ hybridization, Northern blot

(*Endocrine Journal* 47: 487–492, 2000)

ECTOPIC ACTH syndrome is recognized with increasing frequency, but still poses diagnostic and therapeutic problems. Although the treatment of choice is surgical removal of the ACTH-producing tumor, the source of ectopic ACTH production often remains undetermined in patients with occult tumor [1, 2]. In such a situation, the approach is to control hypercortisolism by medical or surgical adrenalectomy until the ACTH-producing tumor becomes evident and resectable. However, the tumor may become nonresectable at the time of diagnosis, thus concerted effort to determine the site of ectopic ACTH production is warranted. Since the majority of these tumors arise from the thorax (lung and

mediastinum) in which the mediastinal tumor comprises either a thymoma or thymic carcinoid [1, 3], the presence of a thymic mass in suspected ectopic ACTH syndrome is a matter of great concern. However, benign thymic hyperplasia has been reported to occur after correction of hypercortisolism in patients with ectopic ACTH syndrome [4–6].

Pro-opiomelanocortin (POMC), a precursor of ACTH and β -endorphin, is primarily synthesized in the pituitary gland, but its transcripts and ACTH-related peptides are detected in some tumors not associated with ectopic ACTH syndrome and even in normal tissues as well [7]. Lymphocytes derived from the peripheral blood and spleen have been demonstrated to express POMC gene and produce a limited amount of ACTH and β -endorphin [8–10]. However, no report is yet available as to whether lymphocytes can serve as a source of ectopic ACTH production and cause excess hypercortisolism. We describe herein a patient with Cushing's syndrome in whom lymphocytes in thymic hyperplasia were con-

Received: October 29, 1999

Accepted: June 7, 2000

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sidered to be the site of deregulated POMC expression and excessive ACTH production following partial hypophysectomy and total adrenalectomy.

Case Report

A 26 year-old man was admitted to our hospital in October 1990 with progressive weakness, edema of lower extremities, body weight gain and hypogonadism of five months duration. He had physiognomic signs of Cushing's syndrome including centripetal obesity, moon face, and hirsutism. Blood pressure was 130/70 mmHg and a pulse rate of 74/min. Serum sodium was 146 mEq/L, potassium 3.1 mEq/L, total cholesterol 320 mg/dl, and bicarbonate 31.4 mEq/L. He had impaired glucose tolerance with glycosuria (2.4 g/day). Endocrine data revealed elevated morning cortisol level (30.3 µg/dl) and normal ACTH level (45 pg/ml) without diurnal rhythm, and increased urinary excretion of 17-KS (27.5 mg/day), 17-OHCS (45.3 mg/day) and free cortisol (3225 µg/day). Provocation of pituitary hormone secretion following combined stimulation with LH-RH, TRH and insulin revealed impaired response of GH and TSH, but normal response of LH, FSH and PRL (Table 1). Although oral administration of high-dose (8 mg) dexamethasone failed to suppress urinary 17-OHCS

excretion more than 50% of the baseline levels, intravenous infusion of dexamethasone (5 mg) suppressed plasma cortisol levels from 39.4 to 19.0 µg/dl. Oral administration of metyrapone (3 g) increased urinary 17-OHCS excretion from 52.2 to 98.7 mg/day. Intravenous administration of human corticotropin-releasing hormone (100 µg) caused elevation of plasma levels of ACTH and cortisol (Table 1). CT scan and magnetic resonance imaging (MRI) of the brain gave no evidence of pituitary tumor. Neither CT scan nor ultrasonography revealed any abnormal mass in the abdomen, lung or mediastinum, except for bilateral adrenal enlargement. All efforts to determine the possible source of ectopic ACTH production were nondiagnostic. Inferior petrosus sinus sampling was not performed due to unskillful technique. Because of the most probable diagnosis of Cushing's disease, the patient had surgical exploration of the pituitary gland via transphenoidal approach in November, 1990; neither microadenoma nor corticotroph hyperplasia was identified, but Crooke cells were present in the adenohypophysis. Despite partial hypophysectomy, his hypercortisolism persisted which could not be alleviated with medical treatment with metyrapone (2–3 g/day) and mitotane (3 g/day). Bilateral adrenalectomy was performed in July 1991, which resulted in complete remission of Cushing's syndrome. He was on maintenance dose of hydrocorti-

Table 1. Evaluation of anterior pituitary hormone secretion

1. Combined stimulation test ^a						
Time (min)	Glucose (mg/dL)	GH (ng/mL)	TSH (µU/mL)	LH (mU/L)	FSH (mU/L)	PRL (ng/mL)
0	88	<1	<1	1.2	4.0	8.2
15	66	1	1	9.4	5.2	25.0
30	46	<1	2	14.2	7.5	21.1
60	53	<1	2	12.6	7.8	15.8
90	73	1	1	12.5	8.2	11.5
120	74	1	1	12.8	8.0	11.4
2. CRH stimulation ^b						
Time (min)	0	15	30	60	90	120
ACTH (pg/ml)	45	40	76	112	46	27
Cortisol (µg/dl)	40.9	47.9	70.3	87.3	54.4	49.7

^a Insulin (0.2 U/kg), LH-RH (100 mg), TRH (500 mg), iv bolus.

^b CRH (100 µg), iv bolus

sone (20 mg/day). Hyperpigmentation of skin developed and plasma ACTH levels gradually increased as high as to 447 pg/ml. Repeated MRI of the brain revealed no evidence of pituitary mass suggestive of adenoma or Nelson's syndrome. In April 1992, CT scan showed an anterior mediastinal mass suggestive of thymoma (Fig. 1). A thymic mass (10×9 cm size) was resected in June 1992. Microscopical examination revealed well-encapsulated benign thymic hyperplasia with well-maintained cortico-medullary boundary; no epithelial or carcinoid tumor cells were demonstrated (Fig. 2A). Postoperative plasma ACTH levels fell to 14 pg/ml. One mg oral dexamethazone suppressed ACTH to less than the detectable limit and the patient restored normal diurnal ACTH rhythm. Combined stimulation with insulin, LH-RH and TRH showed improved peak response of

TSH, but blunted responses of GH, LH and FSH. Hyperpigmentation of skin gradually disappeared. During 8 year-follow-up interval, he has been in good health and repeated ACTH determinations were normal (10–20 pg/ml).

Fig. 1. CT of the thorax before and after bilateral adrenalectomy. (A) No thymic mass visible within the anterior mediastinum. (B) A distinct anterior mediastinal mass 9 months after bilateral adrenalectomy.

Fig. 2. Microscopical examination of the excised thymic mass. (A) Thymic tissue showing decreased involution and occasional lymphoid follicle formation in the parenchyma (Hematoxylin-eosin stain). (B) In situ hybridization of POMC mRNA in thymic tissue. Note that most thymocytes are positive while Hassall's corpuscle is negative. (C) Negative mRNA signals after RNase treatment.

Methods

In situ hybridization

In situ hybridization was performed using a digoxigenin-UTP cRNA specific to the exon 3 of POMC gene on paraffin-embedded sections (4 μ m) of the surgically removed thymic tissue.

Radioimmunoassay and gel permeation chromatography

ACTH concentration in the sample was measured by radioimmunoassay using the antibody against N-terminal fragment of ACTH [11]. In brief, 0.1 ml sample and 0.1 ml antibody (final dilution, 1 : 50,000) were preincubated at 4°C for 24 hrs, followed by the addition of 0.1 ml [¹²⁵I] ACTH (specific activity: 2000 Ci/mmol, Amersham Japan) and further incubation for 24 hrs. The bound ligands were separated from free by a double antibody method. The limit of detection of ACTH was 5 pg/ml; inter- and intra-assay coefficients of variation were 10.5 and 5.0%, respectively. Gel permeation chromatography was performed using Sephadex-50 column (Pharmacia, Uppsala, Sweden) as described [12].

Northern blot analysis

We prepared poly (A)⁺ RNAs by the guanidinium thiocyanate method. We hybridized northern blots

with cDNA probes for human POMC gene (1.9 kb) labeled with α -[³²P]-dCTP using the random-priming method.

Results

In situ hybridization confirmed the expression of pro-opiomelanocortin (POMC) transcripts in normal lymphocytes of the excised thymus (Fig. 2B), which disappeared after RNase treatment (Fig. 2C). The excised thymic tissue extracts contained high concentration of ACTH-like immunoreactivity (98 μ g/g tissue) as determined by radioimmunoassay. Gel chromatographic analysis of the thymic tissue extract revealed a major peak corresponding to human ACTH(1-39) and a yet unspecified minor peak (M.W. 2700) (Fig. 3). Northern hybridization of poly (A)⁺ RNA extracted from thymic tissue using human POMC cDNA as a probe revealed a band corresponding to the size (1.2 kb) of POMC mRNA (Fig. 4).

Discussion

Dramatic enlargement of the thymus is reported to occur as early as 3-4 weeks following remission of hypercortisolism after removal of the tumors or treatment with steroidogenesis inhibitors [4, 6], which spontaneously regressed or persisted for as long as

Fig. 3. Gel chromatography using Sephadex G50 column (1 $\times$ 50 cm) of thymic tissue extract. A major component of ACTH was eluted at the position corresponding to the size of human ACTH (1-39).

Fig. 4. Northern blot analysis of POMC mRNA of the thymic tissue. Poly (A)⁺ RNA extracted from thymic tissue was hybridized with POMC cDNA as a probe. A band corresponding to the size (1.2 kb) of POMC mRNA was evident. Arrow indicates the positions of 18S and 28S ribosomal RNAs.

14 months [5]. Thoracotomy in such cases with "thymic pseudo-tumors" revealed only a hyperplastic thymus without demonstration of ACTH and CRF, and did not relieve hypercortisolism [2, 5, 6]. The precise mechanism of thymic hyperplasia remains unknown, but a 'rebound' phenomenon is plausible. It has been demonstrated that glucocorticoids induce a striking reduction in size of the thymus [13], and the glucocorticoid-induced thymic atrophy can be followed by regrowth of the thymus to a larger size ('rebound' enlargement) than the original after drug withdrawal [14]. These observations were consistent with our case since thymic enlargement occurred only after a dramatic decline in cortisol levels by total adrenalectomy. On the basis of these reports, physicians have been alerted to avert a diagnostic thymectomy especially when thymic appearance suggests benign hyperplasia.

In the present patient, however, benign thymic hyperplasia was most likely to be the source of ectopic ACTH production by five lines of evidence: (1) complete remission clinically (disappearance of skin hyperpigmentation) and biochemically (normaliza-

tion of plasma ACTH levels) following removal of the hyperplastic thymus, (2) high concentration of immunoreactive ACTH in the resected thymic tissue extract comparable to that of most tumors causing ectopic ACTH syndrome, (3) presence of major component of ACTH corresponding to the size of ACTH(1-39) by gel chromatography, (4) expression of POMC mRNA in thymic tissue by Northern blot analysis, and (5) *in situ* detection of POMC transcripts in thymic lymphocytes. To our knowledge, this is the first reported case with the ectopic ACTH syndrome in whom lymphocytes in thymic hyperplasia are most likely to be the site of deregulated POMC expression and excessive ACTH production.

POMC, a precursor of ACTH and β -endorphin, is primarily synthesized in the pituitary gland, but trace amounts of its transcripts and ACTH-related peptides are detected in some tumors not associated with ectopic ACTH syndrome and even in normal tissues as well [7]. Ectopic ACTH production by leukocytes in patients with leukemia and lymphoma has been only reported very rarely [15], as has been that by plasmacytoma [3] and by inflammatory 'mass' [16]. Expression of POMC transcripts and presence of immunoreactive ACTH have also been reported in a variety of normal human tissues, including lymphocytes derived from the peripheral blood, spleen, and thymus [8-10, 17]. This should provide a rationale for normal thymic lymphocytes as a possible site of ectopic ACTH production in the event of deregulated transcriptional states. The thymic lymphocytes of the present patient showed a distinct band of POMC mRNA which had a similar size (1.2 kb) to that of the pituitary transcripts as revealed by Northern blotting, while gel chromatographic analysis revealed the presence of a small molecular weight form (M.W. 2700) in addition to a major peak corresponding to human ACTH(1-39). These results suggest a possible altered processing of POMC transcripts in the patient's thymic tissue.

This case reinforces the difficulties involved in diagnosis of occult ectopic ACTH-producing tumors, and demonstrates that hyperplastic, but apparently normal, thymic tissue could be the source of ectopic ACTH production. Physicians must also be aware of this other possibility to "rebound" thymic hyperplasia [4] whenever diagnostic thymectomy is indicated.

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