A Rare Case of Adrenocorticotrophic Hormone (ACTH)-independent Macroadrenal Hyperplasia Showing Ectopic Production of ACTH

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Abstract

A 57-year-old Japanese man presented with drug-resistant hypertension without Cushingoid features. Endocrinological tests revealed autonomous secretion of cortisol with suppressed plasma adrenocorticotrophic hormone (ACTH). Imaging examinations showed multiple macronodules in the bilateral adrenal gland. These findings were consistent with subclinical Cushing's syndrome caused by ACTH-independent macronodular adrenal hyperplasia (AIMAH). Left adrenalectomy was performed and the resected adrenal lesion was consistent with the pathological diagnosis of AIMAH. Furthermore, in resected tissue, we demonstrated intraadrenal production of ACTH by immunohistochemical analysis and RIA. This is a very rare case of AIMAH showing ectopic production of ACTH, which may be associated with autonomous cortisol secretion.

Key words: ACTH-independent macroadrenal hyperplasia, ectopic ACTH production, autocrine/paracrine regulatory mechanism

(Intern Med 51: 2181-2187, 2012)

(DOI: 10.2169/internalmedicine.51.7547)

Introduction

Adrenocorticotrophic hormone (ACTH)-independent macronodular adrenal hyperplasia (AIMAH) is a very rare cause of Cushing's syndrome (CS), accounting for less than 1% of adrenal CS. In AIMAH associated with subclinical and/or overt Cushing's syndrome, cortisol production may generally be controlled by multiple illegitimate membrane receptors such as eutopic overexpression of vasopressin or serotonin 5-hydroxytryptamine 4 (5-HT₄) receptors and ectopic expression of receptors for gastric inhibitory polypeptide (GIP), or luteinizing hormone (LH) (1). However, in

rare cases, the existence of some other paracrine regulatory mechanisms was proposed to be associated with cortisol production, after the demonstration of increased adrenocortical expression of proopiomelanocortin (POMC)/ACTH, serotonin, vasopressin or glucagon in some affected adrenal tissues (1). Therefore, the exact mechanism of maintenance of cortisol production in AIMAH in the absence of plasma corticotropin is not yet fully understood. To the best of our knowledge, only a few cases of AIMAH showing intraadrenal ACTH production have been reported (2, 3).

We herein report a very rare case of AIMAH showing ectopic production of ACTH as confirmed by immunohistochemical analysis and radioimmunoassay (RIA) in the re-

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Received for publication February 15, 2012; Accepted for publication May 16, 2012

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Table 1. Endocrinological Data before and after Surgery

		Before surgery	After surgery	(Reference range)
Plasma ACTH	(pg/mL)	<5	12	(7-56)
Serum cortisol	(μg/dL)	12.6	9	(5.6-21.3)
Plasma renin activity	(ng/mL/hr)	5.7	2.4	(0.3-2.7)
Plasma aldosterone concentration	(ng/dL)	9	8.3	(2-13)
Dehydroepiandrosterone sulfate	(ng/dL)	13	8	(53-342)
Urinary 17-hydroxycorticosteroid	(mg/day)	10	5.7	(3.4-12.0)
Free cortisol	(mg/day)	49.5	19.6	(11.2-80.3)

Table 2. Dynamic Endocrine Tests before and after Surgery

Before surgery					
Diurnal rhythm	Clock time	8:00	16:00	24:00	
	ACTH(pg/mL)	<5	<5	<5	
	Cortisol (μg/dL)	16.4	18.5	9.7	
Dexamethasone suppression test	Dexamethasone(mg)	0	1	8	
	ACTH(pg/mL)	<5	<5	<5	
	Cortisol (μg/dL)	12.6	13.1	10.7	
CRH stimulation test	Time(min)	0	30	60	120
	ACTH(pg/mL)	<5	7	6	<5
	Cortisol (μg/dL)	14.6	26.7	23	19.4
After surgery					
Diurnal rhythm	Clock time	8:00	16:00	24:00	
	ACTH(pg/mL)	5	<5	<5	
	Cortisol (μg/dL)	9.3	6.9	6.2	
Dexamethasone suppression test	Dexamethasone(mg)	0	1	8	
	ACTH(pg/mL)	5	<5	<5	
	Cortisol (μg/dL)	8.9	4.5	4.2	
CRH stimulation test	Time(min)	0	30	60	120
	ACTH(pg/mL)	12	47	36	14
	Cortisol (μg/dL)	9	17.3	17.6	14.4
ACTH stimulation test	Time(min)	0	30	60	
	Cortisol (μg/dL)	10.3	24.5	27.3	

sected adrenal specimen.

Case Report

A 57-year-old Japanese man was admitted to our hospital for further evaluation of bilateral adrenal masses detected incidentally on abdominal computed tomography (CT). He presented with a history of weight gain and hypertension resistant to treatment with antihypertensive agents. He was 1.69 m tall and weighed 79.7 kg. Physical examination revealed no characteristic signs of Cushing's syndrome, such as central obesity, moon face, buffalo hump, purplish abdominal striae, facial acne or muscle weakness. His resting pulse was 68 beats/min, and his blood pressure was 128/88 mmHg under daily treatment with 40 mg nifedipine, 30 mg derapril hydrochloride and 1 mg indapamide. Laboratory examination showed elevated LDL-cholesterol levels (156 mg/dL), fasting plasma glucose (118 mg/dL), and glycosylated hemoglobin levels (6.6%) (according to the National Glyco-

hemoglobin Standardization Program [NGSP]). An oral glucose tolerance test (75 g) revealed diabetic-range glucose intolerance.

Endocrine tests revealed normal basal cortisol levels, suppressed plasma ACTH level and normal 24-hour-urinary excretion of free cortisol (Table 1). Dynamic endocrine tests showed altered circadian rhythm of cortisol with complete suppression of plasma ACTH, non-suppressibility of cortisol by low-dose (1 mg) and high-dose (8 mg) dexamethasone suppression test (DST), and no response of ACTH to corticotropin-releasing hormone (CRH) stimulation (Table 2). These data are consistent with the diagnosis of subclinical CS (SCS) as proposed from the Committee on "the Disorders of Adrenal Hormones" by the Ministry of Health and Welfare of Japan, 1996. Abdominal CT and magnetic resonance imaging (MRI) revealed bilateral macronodular adrenal hyperplasia with nodules measuring up to 2.5 cm on the right and 3.5 cm on the left (Fig. 1A). Adrenal scintigraphic imaging with ¹³¹I-adosterol revealed uptake of the iso-

Figure 1. Abdominal CT and adrenal scintigram on admission. (A) Abdominal CT. Enlarged adrenal glands with multiple nodules. (B) Adrenal scintigram. Increased uptake of radioisotopes was noted.

Table 3. Screening Tests for Abnormal Cortisol Response

Screening tests	Receptor	Plasma cortisol ($\mu\text{g/dL}$)		
		Basal	Peak	% of basal
Standard mixed meal	GIP	16.4	17.0	104
LHRH 100 μg i.v.	LH, FSH, GnRH	13.6	14.2	104
TRH 100 μg i.v.	TSH, TRH, prolactin	15.4	14.2	92
Pitressin (AVP) 10IU i.m.	V ₁₋₃ AVP	8.9	28.3	318
Mosapride	5-HT ₄	10.8	19.4	180

Basal and maximum plasma cortisol values observed in the clinical tests are represented; maximum plasma cortisol variations are expressed as a percentage of the basal levels. The cortisol response was considered as a positive response when it was above 150% of the basal levels.

tope in the area corresponding to both adrenal glands, with the degree of uptake being greater in the left gland than that in the right gland (Fig. 1B). These findings were consistent with Cushing's syndrome caused by AIMAH. To screen for ectopic or increased eutopic hormone receptor expression with all medication discontinued 4 days before the tests, the following tests were performed: a standard mixed meal (116 g carbohydrate, 27 g protein, and 14 g fat), luteinizing hormone-releasing hormone (LHRH) (100 μg i.v.), thyrotropin-releasing hormone (TRH) (100 μg i.v.), arginine vasopressin (AVP) (10 IU i.m.) and mosapride citrate (15 mg orally). A 50% increase in the plasma cortisol levels was considered as a positive response, as defined by Lacroix et al (4). Administration of mosapride and AVP, which were the 5-HT₄ receptor agonist and the vasopressin receptor agonist, respectively, increased the plasma cortisol levels with undetectable plasma ACTH levels (Table 3). However, no significant cortisol responses were found after an intake of standard mixed meal and administration of LHRH and TRH (Table 3).

In this case, the bilateral macronodular adrenal hyperplasia did not secrete much hormone. However, the size of the adrenal gland was very large (greater than 7.0 cm diameter) and there has been a report of a patient with AIMAH who developed clinically overt Cushing's syndrome from pre-clinical state within a 7-year period (5), therefore, we recommended that the patient undergo surgery. However, the patient did not wish to undergo bilateral adrenalectomy, as that would have meant lifetime glucocorticoid replacement.

Therefore, unilateral adrenalectomy of the predominantly enlarged left adrenal gland was performed. Even though glucocorticoids were not substituted postoperatively, there were no manifestations of adrenal insufficiency. After operation, the plasma ACTH levels returned to normal, and simultaneously, those responses to CRH also improved. In addition, the loss of the circadian rhythm of serum cortisol secretion improved (Table 1, 2). The blood pressure was well-controlled despite discontinuation of antihypertensive medication, and the glycemic control improved. We further examined the mechanism of abnormal cortisol production in the resected AIMAH tissue. The protocol for collection of the tissues and the experimental procedures were approved by the Ethics Committee of the University of Toyama, and written informed consent was obtained from all the participants in this study.

The resected adrenal gland weighed 95 g, and contained multiple nodules ranging in size from several 35 mm to 15 mm in greatest diameter (Fig. 2A). Histological examination revealed that those nodules were composed predominantly of markedly hyperplastic large clear cells interspersed with colonies of acidophilic small compact cells (Fig. 2B). The adjacent adrenal cortex was severely atrophied. Immunohistochemical study demonstrated that P450c17 was expressed predominantly in the small compact cells, whereas 3 β -hydroxysteroid dehydrogenase (3 β -HSD) was exclusively localized in the large clear cells (Fig. 2C, D). These results were consistent with the reported features of AIMAH. Next, we examined the ectopic expression of ACTH in the AIMAH tissue. Immunohistochemical studies showed focal immunoreactivity for ACTH in the hyperplastic adrenal cortex, but not in the medulla (Fig. 2E, F). Immunohistochemical analyses for chromogranin A and serotonin were negative in the AIMAH tissue (data not shown).

Furthermore, we measured immunoreactive ACTH concentration in the AIMAH tissue extract as previously reported (6). The standard curve of ACTH-RIA and its cross reactivity with an AIMAH tissue extract are shown in Fig. 3A. Immunoreactive ACTH concentration in the AIMAH tissue extract was 49.0 ng/g wet wt. We also studied the expression of melanocortin-2 receptor (MC2R),

Figure 2. Macroscopic view, Histological examination, and immunohistochemical analysis of the left adrenal gland. (A) Macroscopic view of the left adrenal gland. The adrenal glands were enlarged with multiple nodules. (B) Hematoxylin and Eosin staining of the resected left adrenal gland. The adrenal gland was composed of relatively small compact cells and clear cells (original magnification $\times 10$). (C) (D) Immunohistochemical analysis of the resected left adrenal gland. Immunohistochemical analysis demonstrated immunoreactivity for 3 β -HSD (C) in the large clear cells and for P-450c17 (D) in the small compact cells (original magnification $\times 10$). (E) Immunohistochemical detection of ACTH in the adrenocortical tissue. Heterogeneous staining and focal distribution of positive cells are observed (original magnification $\times 40$). (F) Immunohistochemical detection of ACTH in the medulla. No detectable immunoreactive material was observed in medulla with antibody to ACTH (original magnification $\times 40$).

which was the receptor for ACTH, by RT-PCR as previously reported (7). The expression of MC2R in resected AIMAH tissue was about half of that in normal adrenal gland, which was obtained from patient undergoing nephrectomy for kidney cancer (Fig. 3B). Furthermore, to confirm the positive cortisol responses to the 5-HT₄ receptor (5-HT₄-R) agonist and the vasopressin receptor agonist, we examined the expression of 5-HT₄-R and vasopressin receptor (V1-V3) by RT-PCR in resected AIMAH tissue. The expression of 5-HT₄-R was about 2,000-fold of that in normal adrenal gland (Fig. 3C), and the expression of V1 receptor was slightly more than that in normal adrenal gland (Fig. 3D). However, we were not able to detect the V2 and V3 receptor (data not shown).

Discussion

In AIMAH associated with subclinical and/or overt Cushing's syndrome, cortisol production may generally be controlled by multiple illegitimate membrane receptors for circulating and/or locally produced bioactive signals, however, in rare cases, paracrine regulatory mechanisms, namely, increased adrenocortical expression of POMC/ACTH, serotonin, vasopressin or glucagon, may also be involved (1). In the present case, cortisol secretion was stimulated by AVP and a 5-HT₄ receptor agonist and we confirmed the eutopic overexpression of their respective receptors in resected AIMAH tissue. In addition, we also demonstrated increased production of ACTH by immunohistochemical analysis and RIA and also detected the expression of MC2R by RT-PCR

Figure 3. Analysis of tissue concentration of ACTH and expression of MC2R, 5-HT4 receptor and V1 receptor. (A) Standard curve of ACTH-RIA and its cross-reactivity with a tissue extract sample from a case of AIMAH. ●: standard curve (rACTH 1-39), □: tissue extract from this AIMAH case. Antiserum against ACTH1-24 was used at a final dilution of 1:75,000. ACTH-RIA cross-reacted with 1-24 ACTH and 1-39 ACTH and POMC, but not with alpha-MSH, beta-endorphin or CLIP. (B)-(D) Expression of MC2R (B), 5-HT4 receptor (C) and V1 receptor (D) in AIMAH tissue and normal adrenal cortex. Sample from AIMAH tissue fragments was used for separate RT-PCR reactions and compared to normal adrenal cortex, which was obtained from a patient undergoing nephrectomy for kidney cancer. We performed quantitative RT-PCR as previously reported (7). The primers for MC2R, 5-HT4 receptor, V1 receptor, and β -actin are shown in Table 4. The expression of each mRNA sample was normalized to the values for β -actin mRNA.

Table 4. The Primers for MC2R and β -actin

Primer name	sequence
MC2R (forward)	5'-TCA CAC TGA CCA TCC TGC TC -3'
MC2R (reverse)	5'-TTT GAA TGC GTC CCT GAG CT -3'
5-HT4 receptor(forward)	5'-TCA CCT GTG CTG CAT TTC TC-3'
5-HT4 receptor(reverse)	5'-ATT CCA GCC TTG CAT TAT AG-3'
V1 receptor (forward)	5'-GAT CGT GAC GGC TTA CAT CG -3'
V1 receptor (reverse)	5'-GCA GTG ATG GTG ATG GTA GG-3'
β -actin (forward)	5'-AAA TCT GGC ACC ACA CCT TC -3'
β -actin (reverse)	5'-TAG CAC AGC CTG GAT AGC AA -3'

in resected AIMAH tissue. From these findings, we concluded that in the present patient with AIMAH, in addition to aberrant expression serotonin and vasopressin receptors, ectopic production of ACTH in the AIMAH tissue was also involved in the autonomous cortisol secretion by autocrine mechanism.

The actions of AVP are mediated by three GPCRs: V1 receptor expressed mainly in vascular smooth muscle cells, adrenal glands, liver, platelets, brain and mesangial cells; V2 receptor in kidney; and V3 receptor in pituitary, and possibly in the kidney. In a previous report, AVP-responsive AIMAH was reported to be caused by the expression of eutopic V1 receptors or ectopic V2 and V3 receptors in the adrenal cortex (8). In this study, we detected the eutopic overexpression of only V1 receptor, but not V2 or V3 receptor compared with normal adrenal cortex. From these findings, in our AIMAH case, we speculated that the AVP-induced cortisol secretion may be via the eutopic overexpression of V1 receptor. Serotonin-responsive AIMAH has been reported to be associated with eutopic overexpression of the 5-HT4-R or more rarely, ectopic expression of the 5-HT7 receptor (9). In this study, we demonstrated that administration of mosapride, which is a second-generation 5-HT4-R agonist, induced an increase of the serum cortisol level in vivo and confirmed eutopic overexpression of the 5-HT4-R in resected AIMAH tissue. We speculated that the effect of mosapride on cortisol secretion may be via the eutopic overexpression of 5-HT4-R.

Moreover, RT-PCR analysis showed that the expression of MC2R in resected AIMAH tissue was about half of that in normal adrenal gland. Recently, Hofland et al. demonstrated that ACTH levels were associated with adrenal levels of MC2R in adrenal hyperplasia samples (10). Therefore, we speculated that the decreased expression of MC2R was attributable to the suppression of ACTH before surgery.

We also measured the tissue concentrations of ACTH by RIA in four additional AIMAH cases, however, ACTH was not detected in any of these cases (data not shown). To the best of our knowledge, only two groups, Lefebvre et al. (2) and Mazzuco et al. (3), have demonstrated an association between intraadrenal POMC/ACTH production and the etiology of AIMAH. They cultured the AIMAH cells obtained from the resected AIMAH tissue, and examined the secretion of ACTH from these cells. They reported that the concentration of ACTH was 1.5-5 pmol/L in the culture medium of the AIMAH cell, which was sufficient to exert pharmacological stimulation on melanocortin 2 receptor (MC2R), and the addition of AIMAH conditioned medium to normal cells really resulted in a marked increase of cortisol production. In the present study, as we did not culture the AIMAH cells obtained from the resected AIMAH tissue, we could not examine the secretion of ACTH from AIMAH cells. However, in the present case, the immunoreactive ACTH content in the adrenal tissue (49.0 ng/g wet wt) was much higher than that in the case reported by Lefebvre et al. (1.98 ng/g wet wt) (2). From these data, we speculated that the ACTH produced by AIMAH cells in our case may be sufficient to produce the autonomous cortisol.

In spite of the intraadrenal ACTH overproduction, the plasma ACTH levels were suppressed. In our case, the immunoreactive ACTH content in the adrenal tissue (49.0 ng/g wet wt) was approximately 1/50th of that in the pituitary tissue. From these data and discussed above, we speculated that the amount of ACTH produced in AIMAH tissue was sufficient to stimulate MC2R, but not enough to be detected in the blood.

It has been demonstrated that ACTH can be synthesized and released by adrenochromaffin cells (11-13). In this study, the ACTH in the AIMAH tissue extract that we detected, may be attributable to the distribution of medullary cells within the hyperplastic adrenal mass. In order to exclude this possibility, we performed immunohistochemical analysis of the adrenal tissue for ACTH, which revealed focal immunoreactivity to ACTH only in the hyperplastic adrenal cortex but not in the medulla, which stained positively with chromogranin A. These data indicate that the detection of corticotropin in the AIMAH tissue extract by RIA was caused by the hyperplastic adrenal cortex.

The mechanisms by which a subpopulation of steroidogenic cells in the AIMAH tissue show an aberrant ACTH production remain unknown. However, Lefebvre et al. (2) speculated that in some cases of AIMAH, the intermingling of normal adrenocortical cells with Leydig cells, which can physiologically synthesize POMC and its derived pep-

tides (14), was associated with intraadrenal ACTH production (2).

In conclusion, in the present *in vitro* study, we showed ectopic production of ACTH and expression of MC2R. These findings suggest that, in rare cases of AIMAH, intraadrenal ACTH production may play a role in the abnormal cortisol production.

The authors state that they have no Conflict of Interest (COI).

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