Octreotide-Sensitive Ectopic ACTH Production by Islet Cell Carcinoma with Multiple Liver Metastases

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Abstract. We report a 21-year-old woman with ectopic ACTH syndrome due to islet cell carcinoma with multiple liver metastases. On admission, she showed Cushingoid appearance (moon face, central obesity etc.) and had acute respiratory distress syndrome due to pneumocystis carinii pneumonia. Laboratory examination revealed marked elevations of plasma ACTH (735 pg/ml) and cortisol (145 μg/dl) with a profound hypokalemia (2.0 mEq/l). She was found to have multiple masses in the liver and a solid mass in the tail of pancreas by abdominal computerized tomography scanning. Treatment with octreotide successfully reduced elevated plasma ACTH and cortisol levels, and she received frequent transhepatic arterial embolization and chemotherapy. The primary pancreatic tumor was surgically removed, revealing islet cell carcinoma which contained high content of ACTH (100 μg/g wet weight) and abundantly expressed proopiomelanocortin and somatostatin receptor subtype-2 mRNAs as determined by Northern blot analysis. Postoperatively, she was free from symptoms for almost one year. However, progressive enlargement of multiple liver metastases refractory to chemotherapy led her to decide on total hepatectomy and liver transplantation from her father. After liver transplantation, she remained almost free from symptoms for almost one year. However, metastases developed to the mediastinal and paraaortic lymph nodes as detected by 111In-pentetreotide scintigraphy. Eleven months after liver transplantation, she was again treated with octreotide and, 16 months after, with metyrapone, both of which were effective in reducing ACTH and cortisol levels, respectively, until she died of acute respiratory failure. This case of a young female patient with ectopic ACTH-producing islet cell carcinoma of the pancreas was quite unique in that she survived for 5 years despite the acute onset and rapid progression of the multiple liver metastases at least in part due to the long-lasting favorable response to octreotide and living-related liver transplantation.

Keywords: Ectopic ACTH syndrome, Islet cell carcinoma, Living-related liver transplantation, Octreotide, Octreotide scintigraphy

ECTOPIC ACTH syndrome, which makes up 10 to 20% of Cushing’s syndrome, is caused by ectopic secretion of ACTH and proopiomelanocortin (POMC)-derived peptides from neoplasms [1]. Approximately half of the patients with ectopic ACTH syndrome have small cell lung carcinoma, and about 20% have more indolent tumors, such as bronchial, thymic and pancreatic carcinoid tumors or medullary carcinoma of the thyroid. Neuroendocrine tumors of the gastrointestinal-pancreatic system are typically characterized by a wide spectrum in terms of anatomic localization, morphologic cell type, production of hormones, clinical presentation and functional activity, and by relatively slow tumor progression rate [2]. One exception is ACTH-producing islet cell tumors of the pancreas which tend to show metastases by the time Cushing’s syndrome develops and a rapidly progressive clinical course [3]. Sixty percent of patients are dead within 2 years, and 5-year survival is reported to be 16% [4].
The aims of the treatment for metastatic neuroendocrine tumors with excessive hormone production are twofold; one is to control excessive hormone secretion to alleviate the associated hormonal symptoms, and the other to either regress the tumors or to prevent their further growth. In ectopic ACTH syndrome, suppression of ACTH hypersecretion is therapeutically critical, because the persistent hypercortisolism causes fatal outcome when associated with opportunistic infection or hemorrhage. For liver metastases of endocrine tumors, various medical treatment modalities have been performed, such as hepatic arterial chemoembolization [5], chemotherapy [6], somatostatin analogues [7, 8], and interferon [9], but recently surgical approach by the total hepatectomy and liver transplantation has been introduced [10, 11]. However, none of these therapeutic approaches has yet to achieve satisfactory outcome.

We present here a patient with ectopic ACTH syndrome due to islet cell carcinoma with multiple liver metastases. The patient survived for 5 years with an intensive multidisciplinary treatment consisting of hormone therapy, chemotherapy, chemoembolization, and a combination of total hepatectomy and living-related liver transplantation. The clinical course of the patient is reported and the value and limitations of the currently available therapeutic modalities are discussed.

Methods

For measurement of ACTH, the tumor tissues boiled in distilled water were homogenized in a glass-teslon homogenizer and centrifuged, and the supernatant was used. ACTH concentrations were determined by immunoradiometric assay kit (IRMA kit, Mitsubishi, Tokyo, Japan). The ACTH assay crossreacted 100% with ACTH(1–39), but none with any ACTH fragments [(1–13), (1–24), (11–24), (18–39)], β-lipotropin or β-endorphin; the sensitivity was 5 pg/ml.

For immunohistochemical study, sections of resected primary and metastasized tumor specimens were coated onto gelatin-coated slides and immunostained with rabbit polyclonal anti-ACTH antibody (Nihirei, Japan), using biotin-labeled rabbit IgG antibody and peroxidase-labeled streptavidin method.

For Northern blot analysis, poly(A)+ RNAs from primary pancreatic and metastatic liver tumors were extracted by the guanidinium thiocyanate method, and hybridized with cDNA probes for human POMC and somatostatin receptor subtype-2 (SSTR-2) genes labeled with a [32P]-dCTP using the random-priming method. Human POMC cDNA (272 bp) and SSTR-2 cDNA (284 bp) PCR-clones using primers (POMC forward: 5'-CTGCTGGAAGATGCGGAGA-3', complement: 5'-GAA TCGGTCCCAGCGGAAGF-3', SSTR-2 forward: 5'-TGACAGTCGTGAGATCGAG-3', complement: 5'-GCAAGAGACATCATGGTGA-3') were subcloned into plasmid vector pGEM-3ZF(-) (Promega, Madison, WI) amplified and subjected to Northern blot analysis. For RT-PCR, first-strand cDNA from each mRNA was generated by reverse transcription method using First-Strand cDNA Synthesis Kit (Amersham Pharmacia Biotech, Piscataway, NJ). Human POMC mRNA was amplified using synthetic oligomer (forward: 5'-CTGCTGGAAGATGCGGAGA-3', complement: 5'-GAAATCCGTCCAGCGGAAGF-3') and human SSTR-2 mRNA using synthetic oligomer (forward: 5'-TGACAGTCGTGAGATCGAG-3', complement: 5'-GCAAGAGACATCATGGTGA-3') as a probe. Amplification mixture contained 50 nM template cDNA and 500 nM primer DNA in DNA amplification kit (DNA Master SYBR Green I: Roche Diagnostics). Denaturation was done at 95°C, annealing at 55°C for POMC and at 53°C for SSTR-2, and extension at 72°C.

Case Report

A 21-year-old woman was well until November 1995, when she noticed malaise, diarrhea, vomiting and personality change. She was found to have multiple masses in the liver and a solid mass in the tail of pancreas by computerized tomography (CT) of the abdomen in January 1996 (Fig. 1). Laboratory examination revealed marked elevations of plasma ACTH (735 pg/ml) and cortisol (145 µg/dl) with a profound hypokalemia (2.0 mEq/l). She was referred to the endocrine/metabolism unit of our University Hospital in March 1996.

Physical examination on admission showed typical Cushingoid appearance with moon face, facial hirsutism, muscle weakness, skin pigmentation, and schizophrenic symptoms with impaired memory and emotional lability. She had hypertension (162/128 mmHg) and sinus tachycardia (124 beats/min). She had acute respiratory distress syndrome (ARDS) due
to pneumocystis carinii pneumonia, which necessitated respirator-assisted control in intensive care unit (ICU), and for which she was treated with sulfamethoxazole-trimethoprim. Metrapone (3 g/day) was given to reduce high cortisol levels. A week later, she successfully recovered from ARDS and was discharged from ICU.

Initial endocrine studies revealed extremely elevated plasma levels of ACTH (735 pg/ml) and cortisol (119 µg/dl) levels, lack of circadian rhythm, and non-suppressibility to high-dose (8 mg) dexamethasone, consistent with the clinical diagnosis of ectopic ACTH syndrome. Pathological examination of tumor specimens obtained from liver biopsy revealed a metastastic neuroendocrine carcinoma: ACTH-positive tumor cells were confirmed by immunohistochemistry. Thus, the diagnosis of an ectopic ACTH-producing pancreatic tumor with multiple metastases to the liver was established.

To determine whether somatostatin is effective in suppressing ACTH secretion from the tumor, octreotide (100 µg) was administered by subcutaneous injection. Plasma ACTH level markedly decreased from 320 pg/ml to 190 pg/ml after 4 h. Therefore, treatment with high-dose octreotide (300 µg/day) was started, which successfully reduced plasma ACTH and cortisol levels to 13 pg/ml and 1.9 µg/dl, respectively. A daily injection of smaller dose (100 µg) of octreotide suppressed plasma ACTH (<10 pg/ml), and successfully controlled her hypercortisolism (Fig. 2). She was then treated extensively with chemotherapy consisting of eight courses of systemic chemotherapy, three courses of intrahepatic chemotherapy (adriamycin plus dacarbazine, streptozotocin plus fluorouracil), and five courses of transhepatic arterial embolization (TAE) during 9 months, leading to only a small shrinkage of hepatic multiple metastases and the primary pancreatic tumor as evaluated by abdominal CT scan. Due to the partial response to the intensive chemotherapy, she decided to have surgical treatment for the primary pancreatic tumor.

The primary tumor in the tail of the pancreas was surgically resected in November 1996. The tumor (5 × 3.5 × 2 cm) showed a cordlike arrangement and rosette formation characteristic of islet cell carcinoma (Fig. 3). Biochemical analysis of the tumor specimens revealed high content of ACTH (100 µg/g wet tissue) as measured by IRMA and abundant expression of POMC and SSTR-2 mRNAs as revealed by Northern blotting (Fig. 4). These data indicated that the pancreatic islet cell tumor is the major source of ectopic ACTH production with concomitant expression of SSTR-2.

Postoperatively, plasma levels of ACTH and cortisol dramatically decreased to the normal range, but not to undetectable levels, suggesting the residual ACTH secretion from the metastatic lesions. She was completely free from octreotide treatment. She received several courses of systemic chemotherapy, TAE and transhepatic arterial injection (TAI) for liver metastases. After 8 months, her plasma ACTH and cortisol levels gradually increased and octreotide was again started. In July 1998, octreotide (300 µg/day) failed to completely suppress ACTH secretion from the tumor.
Fig. 2. The entire clinical course and therapeutic modalities. ○ ACTH, ● cortisol, ■ octreotide,  / methyrapone, □ chemotherapy, ▲ hepatic arterial chemoembolization, □ transhepatic arterial injection, ■ interferon-α, and LTx: liver transplantation.

Fig. 3. Primary islet cell carcinoma of the pancreas. (A): Macroscopic appearance of the pancreatic tumor. (B): Microscopic section showing cordlike arrangement and rosette formation consistent with islet cell carcinoma.

The patient and her parents were given detailed information about total hepatectomy and living-related transplantation as an alternative therapeutic choice as well as the possible extrahepatic metastasis and the possible recurrence after surgery. Due to the failure of chemotherapy and octreotide to decrease tumor growth and ACTH hypersecretion, the patient decided to receive liver transplantation from her father after informed consent was obtained from the patient and her parents. In October 1998, the operation was performed by the team led by Dr. K. Tanaka at Department of Transplantation and Immunology, Kyoto University.
Fig. 4. Expression of ACTH in primary islet cell carcinoma of pancreas. (A): Immunohistochemistry of ACTH by anti-ACTH polyclonal antibody. (B): Northern blot analysis of POMC and SSTR-2 mRNAs. Arrows indicate the positions of a band corresponding to the size POMC (1.2 kb) and SSTR-2 mRNA (2.5 kb). C: Control tissues, T: Tumor tissues.

Fig. 5. Metastatic liver after total hepatectomy. (A) Cross section of multiple liver metastases. (B) Microscopic appearance of liver metastases consistent with islet cell carcinomas. (C) mRNAs expression of POMC and SSTR-2 by RT-PCR. C: Control tissues, T: Tumor tissues.

Hospital after its approval by the Ethical Committee of Kyoto University. Metastatic liver and regional lymph nodes at the hepatic pedicle were removed; the liver weighed 2700 g with fibrous surface and hard nodular masses composed of tumor cells identical to those of primary pancreatic tumor (Fig. 5). Biochemical analysis of the metastatic tumor specimens also showed the presence of ACTH (10 ng/g wet tissue) by IRMA and the expression of POMC and SSTR-2 mRNAs by RT-PCR (Fig. 5).

Postoperatively, plasma ACTH and cortisol levels fell dramatically to as low as 20–30 pg/ml and 2 μg/dl, respectively. Postoperative course was uneventful and she was treated with immunosuppressive drugs (tacrolimus, prednisolone, cyclophosphamide) without octreotide administration. She was discharged in
December 1998 and started to work again. She was doing well for the subsequent follow-up at outpatient clinic of our University Hospital.

Preoperative $^{111}$In-pentetreotide scintigraphy showed diffuse and multiple uptakes in the metastatic tumors in the liver and the equivocal small spots in the mediastinum (Fig. 6A). Postoperative scintigraphy (December 1998) revealed distinct scattered small hot spots in the mediastinum and the pelvis, suggestive of multiple metastases to the systemic lymph nodes (Fig. 6B), which CT and magnetic resonance image (MRI) failed to detect. Follow-up scintigraphy (May 2000) again revealed an increased and enlarged uptake by the metastatic lymph nodes in mediastinum, hilus, hepatic portal region, and pelvis (Fig. 6C).

Treatment with octreotide (300 μg/day) and metyrapone (3 g/day) was reinstituted, which managed to decrease plasma ACTH (200 pg/ml) and cortisol level (20 μg/dl). In June 2000, she complained of dyspnea; X-ray and CT scan of the chest showed scattered small nodules in both lungs with pleural effusion. She received interferon-α (5,000,000 U daily for initial two weeks and subsequently, twice a week). She had palliative treatment at home until she died from acute respiratory failure on December 30, 2000.

**Discussion**

Ectopic ACTH-secreting pancreatic tumors causing Cushing’s syndrome are relatively rare; it accounts for about 10% of the entire ectopic ACTH syndrome and about 7% of all pancreatic endocrine tumors [12, 13]. It has been reported that ACTH-producing islet cell tumors are usually malignant and the majority of cases already showed liver metastasis at the time of diagnosis [3]. The prognosis of ectopic ACTH-producing pancreatic islet cell carcinomas is poor because they usually progress more rapidly than any other neuroendocrine tumors. Our patient was also found to have multiple liver metastases at the initial visit to our hospital when she presented with signs of Cushing’s syndrome. In the present case, the diagnosis of ectopic ACTH-producing pancreatic islet cell carcinoma was established not only by the clinical and biochemical data, but also by the demonstration of immunoreactive ACTH and POMC mRNA in the primary as well as in the metastatic tumors. In contrast to the exceedingly high content of ACTH in the primary pancreatic tumor, its content in the metastatic tumors in the liver was low. Given the massive bulk of the metastatic tumor tissue, it is reasonable to assume that the rapidly
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Growing metastatic tumor was responsible for the major ectopic source of ACTH secretion causing the recurrence of Cushing’s syndrome after resecting the primary tumor.

The optimal treatment and the final goal for patients with ectopic ACTH syndrome are the complete surgical excision of the tumors and the correction of hormonal abnormality. However, in the pancreatic islet tumors with multiple liver metastases, such as in our case, hepatic artery occlusion or TAE combined with chemotherapy consisting of alternating two-drug regimens (doxorubicin plus dacarbazine and streptozocin plus fluorouracil) is useful to regress unresectable hepatic metastasis prior to surgery [5, 6]. The effectiveness of chemotherapy combined with interferon-α or octreotide on the regression of neuroendocrine tumors has also been reported [9]. In our patient, we repeatedly performed TAE before surgical excision of the primary pancreatic tumor, although the therapy became no longer feasible due to the stenosis of hepatic artery and the progression of collateral circulation. At the same time, we performed several courses of systemic and intrahepatic chemotherapy to reduce the tumor volume. Nevertheless, these treatments proved to be transient and only produced partial response to suppress tumor growth. Administration of interferon-α in combination with octreotide used in the later stage of this case failed to affect her clinical course.

It has been reported that octreotide treatment stabilized tumor growth in 36.5% of the metastatic endocrine gastroenteropancreatic tumors for at least 12 months without causing tumor regression [7]. Another retrospective study reported that octreotide treatment decreased tumor volume in 13% of neuroendocrine tumors [8]. Octreotide treatment, however, had no suppressive effect on the tumor growth, although it consistently controlled ACTH hypersecretion in the present case.

For patients with neuroendocrine carcinoma associated with multiple and unresectable liver metastases, treatment with total hepatectomy and liver transplantation has recently been considered as an alternative therapeutic choice [10]. Meta-analysis of 103 patients with total hepatectomy and liver transplantation for neuroendocrine carcinoma revealed that the overall 2- and 5-year-survival rates were 60% and 47%, respectively, and the recurrence-free rate was 24% [11]. In the present case, total hepatectomy with living-related liver transplantation from her father was performed, which led to normalization of plasma ACTH levels for about one year, and survival for 26 months thereafter. Generally, neuroendocrine tumors are indolent, but even in patients with such tumors already metastasized to the liver, the benefit accrued from liver transplantation should be considered as an alternative therapy.

Octreotide, a somatostatin analogue, has been shown to decrease hormone secretion from a variety of endocrine tumors, such as pituitary adenomas, islet cell tumors and carcinoids [14]. Since octreotide therapy often results in rapid improvement of symptoms caused by excessive hormone secretion, it is widely used in patients with these hormone-producing tumors. Such inhibitory effects are mediated through somatostatin receptor subtype, SSTR-2 and/or SSTR-5 [14]; more than 80% of islet cell tumors express SSTR-5 [15], mainly SSTR-2, -1 and/or -3 [16]. In the present case, administration of octreotide successfully reduced ACTH hypersecretion and the resected primary and metastatic tumor expressed abundant SSTR-2 mRNAs by Northern blot and RT-PCR. Short-term effectiveness of octreotide has been reported in reducing ectopic ACTH secretion from the metastatic islet cell carcinoma [17, 18]. It should be noted that the present case showed exceptionally long-term effectiveness of the octreotide therapy to control ACTH hypersecretion throughout the entire clinical course of more than 5 years without apparent desensitization.

111In pentetreotide scintigraphy is useful for the visualization of the gastroenteropancreatic neuroendocrine tumors with positive somatostatin receptor [19]. 201Tl scintigraphy, although not specific, has also been reported to be useful in diagnosing ectopic ACTH syndrome due to bronchial carcinoid [20]. In this case, we performed 111In pentetreotide scintigraphy before and after liver transplantation to follow-up the metastatic spread of the tumor. The preoperative faint spots in the mediastinum later became more distinct and enlarged, suggestive of lymph node metastases. Retrospectively, these equivocal hot spots detectable at the early stage even when diagnostic imaging of CT and MRI failed to detect them, were considered as metastatic lesions, although it is apparently difficult to distinguish them from the nonspecific inflammatory lesions [21]. Despite the comparable results of the overall utility between the conventional imaging methods and the 111In pentetreotide scintigraphy in localizing ectopic ACTH-secreting tumors [22, 23], the scintigraphy appears sensitive enough to
detect the tumors when octreotide is effective in reducing ACTH secretion from the tumors with the abundant expression of SSTR-2 receptors such as in our case.

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References
