High Molecular Weight Form Insulin-like Growth Factor II-producing Mesenteric Sarcoma Causing Hypoglycemia

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Abstract

An 81-year-old woman presented with frequent episodes of hypoglycemia. Her serum level of insulin was normal, but her serum insulin-like growth factor (IGF)-II level was high. She was found to have a spindle cell sarcoma originated from the mesentery of the sigmoid colon, which was completely resected. Postoperatively, hypoglycemia ameliorated with concomitant reduction in serum IGF-II levels. Immunohistochemical study revealed positive immunostaining for IGF-II in tumor cells, and the abundant expression of IGF-II mRNA was demonstrated by RT-PCR. The presence of high molecular weight (HMW) form IGF-II in patient’s serum was confirmed by immunoblotting. This is the first report of a patient with HMW form IGF-II-producing mesenteric sarcoma causing hypoglycemia.

(key words: non-islet cell tumor hypoglycemia (NICTH), hypoglycemia, insulin-like growth factor (IGF)-II, mesenteric sarcoma)

Introduction

The non-islet cell tumor hypoglycemia (NICTH) syndrome is usually associated with the presence of slow-growing massive tumors of mesenchymal origin including sarcomas, fibromas, mesotheliomas, histiocytomas, hemangiopericytomas, as well as carcinomas of the liver, stomach, kidney and adrenal (1, 2). The hypoglycemia induced by these tumors can be reversed by tumor resection, but it relapses with recurrence of the tumor. Most of the non-islet cell tumors causing such hypoglycemic symptoms have been found to produce and secrete insulin-like growth factor-II (IGF-II) (1–8).

We herein describe a case of a patient with NICTH caused by a primary sarcoma derived from the mesentery of the sigmoid colon. The tumor, which was quite large, was found to express abundant IGF-II mRNA as measured by RT-PCR, to produce IGF-II-like immunoreactivity as revealed by immunohistochemistry, and to secrete a high molecular weight (HMW) form IGF-II as determined by Western blot analysis. The patient’s hypoglycemia completely disappeared following tumor resection.

Methods

Measurement of IGF levels

Serum levels of IGF-I and IGF-II were determined by specific RIAs after acid-ethanol extraction as previously described (9, 10). In these assays, the normal values for serum IGF-I and IGF-II in adults ranged from 88 to 240 ng/ml and from 374 to 804 ng/ml, respectively.

Western blot analysis

Acid-ethanol extracted serum samples were electrophoresed on a 16% SDS-polyacrylamide gel under nonreducing conditions, and the size-fractionated proteins were then electroblotted onto a nitrocellulose sheet. The sheet was blocked with 5% (wt/vol) skim milk, and then incubated with a mouse anti-IGF-II monoclonal antibody (Amano Pharmaceutical Co., Nagoya, Japan). After extensive washing, the sheet was incubated with horseradish peroxidase-conjugated anti-mouse IgG, and the complexes were detected using an enhanced chemiluminescence (ECL) sys-

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Real-time quantitative PCR
The Light Cycler (Roche Molecular Biochemicals, Mannheim, Germany) PCR protocol, in which fluorescence emission due to binding of SYBR Green I dye to amplified products is detected and measured, was used to quantify IGF-II mRNA. Total RNA was extracted from the surgically resected tumor and adjacent normal tissues using RNA zol (GIBCO/BRL, Carlsbad, CA), and cDNA synthesized using a First-Strand cDNA Synthesis Kit (Amersham Pharmacia Biotech Inc., Piscataway, NJ) according to the manufacturer’s instructions. Human IGF-II mRNA was amplified using a forward primer 5′-ACGATGACATCGAGGTGGAGAG-3′ and reverse primer 5′-GCATTATGGTGAGCCCGTTT-3′. The PCR products were examined by 1.5% agarose gel electrophoresis and were confirmed to contain 309 bp.

Immunohistochemistry
Tissue sections were exposed to 5% normal goat serum for 20 minutes, followed by anti-IGF-II monoclonal antibody (Amano; 1: 500 dilution) which had been proven to be suitable for immunohistochemical study (11), then rinsed in PBS and incubated for 30 minutes with the streptavidin-peroxidase complex reagent. After rinsing in PBS, the sections were exposed to 3-amino-9-ethylcarbazole for 5 minutes.

Case Report
An 81-year-old woman was brought to the emergency room of Tokyo Metropolitan Fuchu Hospital because of impaired consciousness (GCS 12) and hypoglycemia (34 mg/dl) on May 1999. Three years earlier, her mental activity began to decrease, and progressive amnesia developed in 1998. She experienced muscle spasms in the extremities whenever she was hungry since April 1999.

On admission, physical examination revealed a large solid mass, approximately 5 cm in diameter, in the lower abdominal region. Laboratory tests revealed a low plasma glucose level (35 mg/dl), which lasted over the subsequent days (40–60 mg/dl) despite the continuous intravenous infusion of glucose (300 g/day).

Laboratory and endocrine data on admission are shown in Table 1. Serum level of immunoreactive insulin (IRI) was normal (2.0 μU/ml) and undetectable after overnight fasting. Oral glucose tolerance testing (75 g) revealed normal IRI response, reaching a peak at 30 minutes, followed by undetectable levels by 2 hours. Low level of IGF-I (54.1 ng/ml) and elevated level of IGF-II (1,030 ng/ml) with high IGF-II/IGF-I ratio (19.1) were noted. Except for the low level of GH, those of other hormones (ACTH, TSH, prolactin, gonadotropins, cortisol, thyroid hormones, glucagon) were all within the normal ranges. These endocrine data were incompatible with the diagnosis of insulinoma, pituitary and adrenal insufficiency as a cause of her severe hypoglycemia.

A pelvic contrast computed tomography (CT) scan revealed the presence of a heterogenous large mass, 16×13.5×12 cm in size, extending from the pelvis to the umbilicus, with a peripheral contrast effect (Fig. 1). The pelvic mass derived from the mesentery of the sigmoid colon was surgically and completely resected; the resected solid, lobular mass was 21×12×11 cm in size, and weighed 1.95 kg (Fig. 2A). Histologically, the tumor cells showed atypical appearance with increased number of spindle-shaped cells consistent with the pathological diagnosis of spindle cell sarcoma (Fig. 2B).

Postoperative course was uneventful, and plasma glucose levels increased to 506 mg/dl just after operation under continuous intravenous glucose infusion, but remained between

| Case Report |

Table 1. Laboratory and Endocrine Data on Admission

<table>
<thead>
<tr>
<th>1. CBC and Biochemistry</th>
<th>2. Hormones</th>
</tr>
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<tbody>
<tr>
<td>WBC 7,300/mm³</td>
<td>GH 0.1 ng/ml</td>
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<tr>
<td>RBC 395/mm³</td>
<td>PRL 8.1 ng/ml</td>
</tr>
<tr>
<td>Pt 34.9/mm³</td>
<td>LH 12.7 mIU/ml</td>
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<td>TP 6.4 g/dl</td>
<td>FSH 50.1 mIU/ml</td>
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<td>BUN 6.6 mg/dl</td>
<td>ACTH 42.5 pg/ml</td>
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<tr>
<td>Cr 0.5 mg/dl</td>
<td>TSH 2.4 μU/ml</td>
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<tr>
<td>Na 143 mEq/l</td>
<td>Cortisol 20.3 μg/dl</td>
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<tr>
<td>K 3.9 mEq/l</td>
<td>ΣT3 3.0 μg/ml</td>
</tr>
<tr>
<td>Ca 9.7 mEq/l</td>
<td>ΣT4 1.5 μg/ml</td>
</tr>
<tr>
<td>AST 17 U/l</td>
<td>Glucagon 116 μg/ml</td>
</tr>
<tr>
<td>ALT 13 U/l</td>
<td>IRI 2.0 μU/ml</td>
</tr>
<tr>
<td>ALP 568 U/l</td>
<td>C-peptide 0.1 ng/ml</td>
</tr>
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<td>Glu 35 mg/dl</td>
<td>IGF-I 54.1 ng/ml</td>
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<td>CRP 0.1 mg/dl</td>
<td>IGF-II 1,030 ng/ml</td>
</tr>
<tr>
<td>CEA 1.3 ng/ml</td>
<td>IGF-I/IGF-II 19.1 (1.7–7.1)</td>
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<td>CA19-9 &lt;10 U/ml</td>
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3. OGTT

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<th>Time (min)</th>
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<th>60</th>
<th>90</th>
<th>120</th>
<th>180</th>
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<tr>
<td>Glucose (mg/dl)</td>
<td>34</td>
<td>100</td>
<td>97</td>
<td>91</td>
<td>80</td>
<td>63</td>
</tr>
<tr>
<td>IRI (μU/ml)</td>
<td>&lt;2</td>
<td>25.4</td>
<td>15</td>
<td>9.7</td>
<td>&lt;2</td>
<td>&lt;2</td>
</tr>
</tbody>
</table>

( ) normal range.
135–185 mg/dl thereafter; postoperative serum level of IGF-II decreased to 513 ng/ml (Fig. 3). Postoperatively, the patient had no hypoglycemic episodes without continuous glucose infusion.

**Biochemical analysis of tumor tissues**

Immunohistochemical analysis revealed that IGF-II-like immunoreactivity was exclusively localized within the cytoplasm of many, but not all tumor cells (Fig. 2C). Real-time quantitative RT-PCR revealed the abundant expression of IGF-II gene transcripts, a band corresponding to 309 bp, in the patient’s tumor tissue specimens (Fig. 4). In contrast, the tissue specimens obtained from the patient’s adjacent normal tissue, colonic mucosa, and two carcinomas of the colon did not express significant IGF-II mRNA levels; the number of IGF-II gene copies in the tumor was about 30-fold greater than that of normal mucosa by quantitation of IGF-II mRNA.

Western immunoblotting analysis of IGF-II revealed the
presence of a greater fraction of a HMW (19.8 kDa) band in the patient’s serum comparable to that in another case with NICTH; control serum sample from a healthy subject displayed only a 7.5 kDa band corresponding to the size of IGF-II (Fig. 5).

Discussion

Currently, most cases with NICTH are attributed to excessive production of IGF-II by the tumors based on the recent accumulating evidence that these tumors produce and secrete IGF-II (1–8). The diagnosis of the present patient with NICTH was consistent with that of HMW form IGF-II-producing tumor causing hypoglycemia for the following reasons: 1) preoperative high IGF-II levels; 2) hypoglycemia improved and serum IGF-II levels decreased to normal after tumor resection; 3) the presence of circulating HMW form IGF-II in patient’s serum by Western blotting; 4) the expression of IGF-II mRNA and protein by the tumor as demonstrated by real-time quantitative RT-PCR and immunohistochemistry, respectively.

IGF-II consists of 67 amino acid residues with a molecular weight of 7.5 kDa (12). IGF-II gene, although ubiquitously expressed throughout the body, is most prevalent in tissues originating from the mesoderm (13, 14). IGF-II promotes fetal development and has insulin-like effects on many tissues (12, 13, 15). The levels of circulating IGF-II, although highest during the fetal development, range from 378 to 804 ng/ml in normal adults (10). The present patient had elevated serum levels of IGF-II consisting predominantly of HMW (approximately 19.8 kDa) form. The HMW form IGF-II has been shown to mimic insulin’s effects more effectively than a mature (7.5 kDa) form (1, 2, 4–8). In normal subjects, about two-thirds of the circulating mature form IGF-II is present as a ternary 150 kDa complex with IGF binding protein-3 (IGFBP-3) and acid-labile subunit (ALS). IGF-II can only mediate its insulin-like effects when it is present as a free form unbound with this complex. The HMW form IGF-II produced by the tumors as in our case apsin to form smaller binary complexes consisting of IGF-II bound to IGFBPs (mainly 1, 2 and 4) rather than ternary complexes (4–6, 16). While ternary complexes for the most part do not cross capillary walls, binary complexes do (17). Thus, binary IGF-II can more readily exert its insulin-like effects on target tissues via insulin receptors. It is possible to speculate that the tumor-derived HMW form IGF-II may circulate as a major free form in binary rather than ternary complexes to induce insulin-like activity.

During hypoglycemic episodes, several circulating counter-regulatory hormones, such as GH, cortisol, catecholamines and glucagon, could increase to prevent hypoglycemia. It is noteworthy that the present patient had a low level of GH and a normal level of glucagon in despite of severe hypoglycemia. Such paradoxical response may be accounted for by the excessive secretion of tumor-derived IGF-II via its feedback inhibition of GH secretion (18) as well as its suppressive effect on glucagon secretion (19, 20).

Taken together, it is suggested that the HMW form IGF-II produced by the mesenteric sarcoma in the present case, could be responsible for the profound hypoglycemia. To our knowledge, this is the first documented case of NICHT caused by a primary sarcoma of the mesentery in which abundant expression of IGF-II mRNA and protein by the tumor as well as the presence of circulating HMW form IGF-II was demonstrated.

References