A Case of Multiple Endocrine Neoplasia (MEN) Type 1; The Immunohistochemical and Ultrastructural Studies of Its Tumors and the Analysis of Hormones in Tumor Extracts

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Abstract

We reported a case of sporadic multiple endocrine neoplasia type 1, with multiple insulinoma, parathyroid adenoma, and pituitary tumor.

Measurement of hormone contents and immunohistochemical studies of the pancreatic tumors showed that the tumors contained insulin, glucagon, somatostatin, and pancreatic polypeptide. Furthermore, the concentrations of these hormones were different in each tumor.

Insulin extracted from the pancreatic tumors analyzed by reversed-phase high performance liquid chromatography revealed no structural abnormalities. On the other hand, in gel filtration evaluation of the extract of the parathyroid adenoma, it was found that the tumor extract contained a macromolecular parathyroid hormone (molecular weight 20,000 to 25,000).

Multiple endocrine neoplasia (MEN) type 1, also known as Wermer syndrome, is a hereditary disorder characterized by hyperplasia or neoplasms of the pituitary, pancreas, and parathyroid glands. The parathyroid glands are involved in 76–97% of cases, the pancreas in 71–82%, and the pituitary gland in 54–64%. The tumors, variously combined, involve all three glands with relatively low frequency (21–33%) (Eberle and Grün, 1981; Kin et al., 1986).

Multiple endocrine neoplasia, whether they belong to MEN type 1 or not, produce multihormones such as gastrin, insulin, and glucagon, etc. Furthermore, in MEN type 1, the pancreatic lesions are considered to be multicentric (Yamaguchi et al., 1980a). In addition, recent studies reported structural abnormalities of hormones in MEN type 1 (Kothary et al., 1983; Seino et al., 1985).

This report describes a case of MEN type 1 with immunohistochemical and ultrastructural examinations of the pancreatic tumors and parathyroid adenoma and with structural analysis of insulin by reversed-phase high performance liquid chromato-
graphy (RP-HPLC) and parathyroid hormone (PTH) by gel filtration.

Case Report

In 1983 a 25-year-old woman was admitted because of unconsciousness after prolonged fasting. She had fasting-induced double vision for two years prior to the admission. She had no family history of endocrine disease. Physical examination results were normal. Fasting blood glucose was 46 mg/dl and fasting serum insulin (IRI) was 19.9 μU/ml, inappropriate to the prevailing blood glucose. After a 20-hour fasting test, she became drowsy, with blood glucose of 32 mg/dl and IRI of 14 μU/ml. An intravenous tolbutamide (1 g) tolerance test induced hypersecretion of IRI (max Δ IRI = 303.3 μU/ml) and an arginine tolerance test showed hyperresponse of plasma glucagon (IRG) (max Δ IRG = 397 pg/ml). Selective angiography demonstrated a tumor stain, 1.3 cm in diameter, in the region of the body of the pancreas. Venous sampling by means of percutaneous transhepatic portal and splenic vein catheterization (PTPC) revealed the step-up of IRI and IRG at the same point as the tumor stain and that of somatostatin (SLI) and pancreatic polypeptide (PP) at the other points (Fig. 1A). Thus a multihormone-secreting insulinoma was suggested. Laparotomy revealed six tumors at the body and the tail of the pancreas and distal pancreatectomy was performed. After the operation her symptoms were improved.

She was readmitted in 1985 because her serum calcium level was gradually elevated to 11.7 mg/dl. Serum phosphorus was normal (2.8 mg/dl). Plasma C- and N-terminal

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**Fig. 1.** Sampling points through percutaneous transhepatic portal and splenic vein catheterization (PTPC) and localization of multiple insulinoma. Table A shows the hormonal activity in each sample and table B shows the hormone contents in the acid-extracts of each tumor measured by radioimmunoassay. IRI: insulin, IRG: glucagon, SLI: somatostatin, PP: pancreatic polypeptide, n.d.: not detected.

**B. Hormone contents in the tumor**

<table>
<thead>
<tr>
<th>Tumor (size: cm)</th>
<th>IRI (U/g)</th>
<th>IRG (pg/g)</th>
<th>SLI (μg/g)</th>
<th>PP (pg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (1.4×0.7)</td>
<td>1.9</td>
<td>4.1</td>
<td>0.6</td>
<td>0.4</td>
</tr>
<tr>
<td>2 (0.5×0.3)</td>
<td>0.4</td>
<td>n.d.</td>
<td>0.1</td>
<td>1.9</td>
</tr>
<tr>
<td>3 (0.3×0.3)</td>
<td>7.3</td>
<td>3.1</td>
<td>0.7</td>
<td>0.03</td>
</tr>
<tr>
<td>4 (0.8×0.5)</td>
<td>5.8</td>
<td>1.3</td>
<td>0.09</td>
<td>0.01</td>
</tr>
<tr>
<td>5 (1.0×1.0)</td>
<td>0.4</td>
<td>1.1</td>
<td>0.03</td>
<td>0.6</td>
</tr>
<tr>
<td>6 (1.5×1.5)</td>
<td>10.1</td>
<td>1.9</td>
<td>0.1</td>
<td>0.01</td>
</tr>
</tbody>
</table>

**A. Plasma hormone concentrations during PTPC**

<table>
<thead>
<tr>
<th>Hormone</th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>d</th>
<th>e</th>
<th>f</th>
<th>g</th>
<th>h</th>
<th>i</th>
<th>j</th>
</tr>
</thead>
<tbody>
<tr>
<td>IRI (μU/ml)</td>
<td>24</td>
<td>53</td>
<td>72</td>
<td>25</td>
<td>64</td>
<td>366</td>
<td>890</td>
<td>888</td>
<td>581</td>
<td>30</td>
</tr>
<tr>
<td>IRG (pg/ml)</td>
<td>103</td>
<td>162</td>
<td>203</td>
<td>79</td>
<td>208</td>
<td>309</td>
<td>517</td>
<td>287</td>
<td>298</td>
<td>187</td>
</tr>
<tr>
<td>SLI (pg/ml)</td>
<td>40</td>
<td>79</td>
<td>55</td>
<td>71</td>
<td>49</td>
<td>67</td>
<td>71</td>
<td>86</td>
<td>83</td>
<td>57</td>
</tr>
<tr>
<td>PP (pg/ml)</td>
<td>37</td>
<td>332</td>
<td>460</td>
<td>83</td>
<td>248</td>
<td>359</td>
<td>322</td>
<td>237</td>
<td>120</td>
<td>103</td>
</tr>
</tbody>
</table>
of PTH (C- and N-PTH) were 0.6 ng/ml and 84 pg/ml respectively (normal: C-PTH < 1.3, N-PTH < 120). Tubular resorption of phosphate slightly decreased to 79% (normal 80–92) and nephrogenous cyclic AMP (NcAMP) increased to 7.40 nmol/dlGFR (normal 0.80–2.78). Serum albumin and alkaline phosphatase, intravenous urogram, and skeletal survey results were all normal. A cervical echogram showed a hypoechoic mass behind the lower pole of the left lobe of the thyroid gland and a 201Tl-Chloride scintigram showed abnormal uptake in the same region. Digital subtraction angiography demonstrated a tumor stain (30×5 mm in size) supplied from the left superior thyroid artery. These results led to a diagnosis of parathyroid tumor. The tumor was resected and the other three glands, apparently unchanged, were left untouched. Postoperatively her serum calcium and NcAMP decreased to the normal range.

In 1987 magnetic resonance computed tomography revealed an intrasellar mass (1.5 cm in diameter) extending into the suprasellar cistern (Fig. 2). The basal pituitary hormone levels were within normal limits (PRL 19.3 ng/ml, GH 1.5 ng/ml, TSH 1.05 µU/ml, ACTH 29 pg/ml, LH 44.1 mU/ml, FSH 10.1 mU/ml, and somatomedin C 0.66 U/ml) and responded normally to pituitary function tests such as TRH, LHRH, and corticotropin releasing factor tests. However a GH releasing factor (GRF) test showed hyperresponse of GH (max J GH = 61.9 ng/ml).

### Pathological findings and analysis of hormones

#### (I) Pancreatic tumors

The cut surface of the resected specimen revealed six well-circumscribed tumors, ranging in size from 0.3 to 1.5 cm in diameter, located at the body and the tail of the pancreas (Fig. 1). Sections of the tumors showed that they consisted of islet cell-like atypical epithelial cells proliferating in trabeculae, in a solid nest, and partly in a glandular pattern with the scanty stroma focally hyalinized (Fig. 3).

The tumor sections were stained by the peroxidase-antiperoxidase (PAP) technique for IRI, IRG, SLI, and PP, using DAKO PAP kits (KyowaMedics Co. Ltd., Tokyo, Japan) (Fig. 4). Most cells in tumor 1 (see Fig. 1) were positive for IRG and most cells in tumor 6 were positive for IRI, whereas some cells in tumor 3 and 2 had immunoreactivity for SLI and PP, respectively.

The hormone contents in the acid-extracts of the tumors were determined by radioimmunoassay.
These tumors contained IRI, IRG, SLI, and PP and the concentrations of these hormones were quite different in each tumor (Fig. 1B).

Insulin in the acid-extract of tumor 6 was analyzed by RP-HPLC according to the method of Miyazaki et al. (1986). The insulin peak corresponded to that of standard human insulin (Fig. 5) and identical results were obtained with RP-HPLC of the extracts of the other tumors.

(2) Parathyroid tumor
The resected specimen revealed a 35 × 5 × 5 mm soft, well-circumscribed mass. Microscopic findings showed that the tumor cells, with granular and vacuolated cytoplasm, proliferated in cord, nests, and follicular patterns. These findings were compatible with chief cell adenoma (Fig. 6).

In electron microscopic studies, the tumor cells were seen to contain large aggregations of glycogen granules and lamellar arrays of granular endoplasmic reticulum, but no secretory granules (Fig. 7).

The acid-extract of the tumor contained a large amount of PTH measured by radioimmunoassay with C-terminal antisera (6.5 µg PTH/g wet tissue). On Sephadex G-50 gel filtration, the peak of PTH was eluted as a single peak with molecular weight of 20,000 to 25,000 (Fig. 8).

Fig. 4. Pancreatic tumors stained with hormonal antiserum (peroxidase-antiperoxidase technique). GLU: Staining with glucagon antiserum (tumor 1). IRI: Staining with insulin antiserum (tumor 6). SLI: Staining with somatostatin antiserum (tumor 3). Some tumor cells react (arrow). PP: Staining with pancreatic polypeptide antiserum (tumor 2). Some tumor cells react (arrow). (X400)
Fig. 5. Reversed-phase HPLC (RP-HPLC) profiles of the acid-extract of pancreatic tumor (tumor 6). A: The profiles of standard human and porcine insulin. B: The profiles of the tumor extract by the same retention time as that in A. The continuous lines represent the absorbance units detected at 210 nm. C: The elution pattern of insulin immunoreactivity obtained from the fractions through RP-HPLC of the tumor extract.

Discussion

This patient was a sporadic case of MEN type 1 presented with multiple insulinoma, parathyroid adenoma, and pituitary tumor. Endocrinological examinations for pituitary hormones were all normal except GRF test, suggesting that this pituitary tumor may secrete GH. Further examinations should be required.

Multiple lesions in the pancreas are considered to be one of the characteristic features of MEN type 1 (Yamaguchi et al., 1980a). In contrast, the pancreatic tumors are solitary in up to 80% of patients who do not belong to MEN type 1 (Stefanini et al., 1974). Furthermore, the tumors produce multiple hormones irrespective of whether the patients have MEN type 1 or not, and in MEN type 1, the hormone predominantly produced in each of the multiple tumors is quite different (Yamaguchi et al., 1980a). Our patient had six adenomas of islet cells. The immunohistochemical staining and measurement of hormone content proved that the tumors produced multiple hormones such as insulin, glucagon, somatostatin, and pancreatic polypeptide, and that the concentrations of these...
hormones were quite different in each tumor. The largest tumor (tumor 6) contained a high concentration of insulin which had a normal amino-acid sequence of human insulin proved by RP-HPLC, and the PTPC showed a marked step-up of insulin near the tumor. These results indicate that insulin predominantly secreted from this tumor caused the clinical picture of fasting hypoglycemia. The multihormone-producing tumors can be divided into two types; monocellular-multihormonal type (Heitz et al., 1981) and multicellular-multihormonal type (Capella et al., 1978). The immunohistochemical staining of consecutive serial sections should be required to clarify which type our case belongs to.
The chief cells of parathyroid adenoma have been reported to contain large aggregations of glycogen granules, lamellar arrays of granular endoplasmic reticulum, and various numbers of secretory granules (Capen and Roth, 1973). In our case, we detected the former two, indicating increased protein synthesis, but no secretory granules. This may be due to the increased rate of PTH secretion, consequently exceeding the ability of tumor cells to form mature storage granules.

It is well recognized that the polypeptide hormones in serum or tumor extracts from patients with endocrine tumors sometimes have size heterogeneity or structural abnormalities. Yamaguchi et al., (1980b) proved the macromolecular vasoactive intestinal polypeptide (VIP) in the VIP producing tumors. In MEN type 1, abnormal structure of insulin (Seino et al., 1985) and a large molecule of gastrin (Kothary et al., 1983) were reported. In our case, insulin extracted from the pancreatic tumors and analyzed by RP-HPLC revealed no structural abnormalities. On the other hand, on gel filtration of the extract of the parathyroid adenoma, we found a substance immunoreactive to C-terminal antisera with a molecular weight of 20,000 to 25,000, which was much greater than previously reported PTH components such as intact PTH (MW 9,500), pro PTH (MW 10,200), and pre-pro PTH (MW 14,000). But the biological significance of this macromolecular PTH remains unclear. It was eluted as a single peak and normal PTH components were not eluted, suggesting that the macromolecular PTH might be an aggregation of PTH or non-specific binding of PTH to the other molecule. However, it is also possible to speculate that there may be an abnormality in the processing of hormone at the DNA, transcriptional, or translational level, since the pre-pro PTH is the initial product synthesized from the specific mRNA (Habener et al., 1977). Habener et al. (1976) analyzed PTH in tissue extracts of parathyroid adenoma in 3 patients who did not belong to MEN type 1, but did not detect the macromolecular PTH. Whether or not the present finding is universal in MEN type 1 requires further clarification.

References


Seino, S., A. Funakoshi, Z. Z. Fu and A.

