Roles of Circulating Carcinoembryonic Antigen and Calcitonin in Diagnosis of Medullary Thyroid Carcinoma: A Comparative Study

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Synopsis

Carcinoembryonic antigen (CEA) and calcitonin (CT) were simultaneously determined in sera and tumor tissues from 15 patients with medullary carcinoma of the thyroid (MCT). Serum CEA was increased in all but one patient, and CT did in all of them. Both levels were significantly related to the weight of excised tumor, but not to the presence of metastasis. Furthermore, a significant correlation was noted between the basal levels of CT and CEA. Both levels fell to normal after a radical operation had been performed. Tissue concentrations of CEA and CT in the MCT were more than 100 times those in hyperthyroidism, and the ratios of tissue over serum levels averaged 770 in CEA and 1000 in CT. In the calcium infusion test, CEA levels were not significantly changed in contrast with a distinct increase in CT levels. The results indicate that CEA and CT represent separate activities of the tumor cells, and that circulating CEA together with CT is a useful indicator in the diagnosis and follow-up of the disease.

Medullary carcinoma of the thyroid (MCT), originated from the parafollicular cells (Williams, 1966), is shown to secrete calcitonin (CT) (Melvin and Tashjian, 1968), histaminase (Baylin et al., 1970), DOPA-decarboxylase (Atkins et al., 1972), prostaglandin (Williams et al., 1968), serotonin (Moertel et al., 1965), and ACTH (Donahower et al., 1968). Of these bioactive substances the CT, a hypocalcemic hormone, is most consistently elevated in plasma, and further, seemingly normal levels, if any, are remarkably increased to diagnostic levels after calcium infusion (Clark et al., 1969; Tashjian et al., 1970; Melvin et al., 1972; Jackson et al., 1973; Deftos, 1974). Consequently, the radioimmunoassay for CT is thought to be the most sensitive, specific method for the diagnosis of disease.

Quite recently we have demonstrated that carcinoembryonic antigen (CEA), a tumor-associated fetal antigen described by Gold and Freedman (1965), is markedly increased in sera and tissues of the MCT in contrast with other types of thyroid carcinomas (Ishikawa and Hamada, 1976). The results obtained prompted us to investigate the relationship between CEA levels and cellular activity of the tumor, making comparison with the CT secretion.

The study reported here has shown that the production of CEA as well as CT is closely correlated with cellular activity of the tumor, although the mechanism involved is different from each other.
Materials and Methods

Fifteen patients with histologically proven MCT were studied. Resting levels of both CEA and CT were measured prior to operation. In 14 patients the calcium infusion test was performed at a rate of 15 mg/kg of body weight for four hr. In some patients postoperative determinations were performed after 5-14 months. Tissue specimens were obtained surgically from 4 patients with the MCT and a patient with Graves' disease. Extraction of CEA was performed according to the procedure described by Martin and Martin (1972). Tissue was homogenized in 9 volumes of saline using a Virtis "60" homogenizer, an equal volume of 1.2 M perchloric acid was added to the homogenate, and the mixture was stirred for 1 hr at room temperature. After centrifugation for 30 min at 4°C and 30,000 x g, the supernatant was dialyzed thoroughly, followed by lyophilization.

Radioimmunoassay for CEA was performed by the double antibody technique of non-equilibrium system (Ishikawa and Hamada, 1976), modified from that of Laurence et al. (1972). In this assay system, a 100 pl serum sample was used without extraction, and the upper limit of normal was 10 ng/ml since only 2 out of 84 normal subjects (2.4%) showed CEA levels exceeding this limit. Purified CEA for standard and labeling with $^{125}$I was obtained from human colonic adenocarcinoma tissue employing preparative disc gel electrophoresis in combination with sequential gel filtrations on Sepharose 4B and Sephadex G-200. The preparation obtained was immunologically indistinguishable from Gold’s CEA (kindly supplied by Dr. P. Gold, Montreal General Hospital). CEA was labeled with $^{125}$I by a minor modification of Hunter and Greenwood method. Specific antiserum to CEA was prepared after the method of Gold and Freedman (1965).

Radioimmunoassay for CT was performed after the method of Deftos (1971). The procedure was described in detail elsewhere (Morita et al., 1975). A synthetic human calcitonin M and antiserum to human CT were kindly supplied by Ciba-Geigy Pharmaceuticals, Basel. In this assay procedure, basal levels in plasma were distributed less than 0.3 ng/ml in all 21 normal subjects.

In addition, no cross-reactivity was demonstrated between CEA and CT in the respective assays.

Table 1. Circulating CEA and CT levels in 15 patients with medullary carcinoma of the thyroid

<table>
<thead>
<tr>
<th>Case</th>
<th>Tumor Weight (g)</th>
<th>Metastasis</th>
<th>Preoperative levels</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>CEA (ng/ml)</td>
</tr>
<tr>
<td>Y. H.</td>
<td>20</td>
<td>(+)</td>
<td>6</td>
</tr>
<tr>
<td>T. T.</td>
<td>50</td>
<td>(+)</td>
<td>14</td>
</tr>
<tr>
<td>S. A.</td>
<td>60</td>
<td>(+)</td>
<td>47</td>
</tr>
<tr>
<td>I. K.</td>
<td>20</td>
<td>(-)</td>
<td>48</td>
</tr>
<tr>
<td>A. E.</td>
<td>10</td>
<td>(-)</td>
<td>51</td>
</tr>
<tr>
<td>K. N.</td>
<td></td>
<td>(+)</td>
<td>55</td>
</tr>
<tr>
<td>A. Y.</td>
<td>12</td>
<td>(-)</td>
<td>59</td>
</tr>
<tr>
<td>S. T.</td>
<td>70</td>
<td>(+)</td>
<td>74</td>
</tr>
<tr>
<td>I. Y.</td>
<td>10</td>
<td>(+)</td>
<td>78</td>
</tr>
<tr>
<td>T. K.</td>
<td>16</td>
<td>(-)</td>
<td>80</td>
</tr>
<tr>
<td>M. E.</td>
<td>115</td>
<td>(+)</td>
<td>118</td>
</tr>
<tr>
<td>T. H.</td>
<td>15</td>
<td>(-)</td>
<td>125</td>
</tr>
<tr>
<td>K. K.</td>
<td>150</td>
<td>(+)</td>
<td>130</td>
</tr>
<tr>
<td>A. I.</td>
<td>210</td>
<td>(+)</td>
<td>145</td>
</tr>
<tr>
<td>T. H.</td>
<td>200</td>
<td>(+)</td>
<td>170</td>
</tr>
</tbody>
</table>
Results

Values for serum CEA and CT in 15 patients with medullary carcinoma of the thyroid (MCT) are shown in Table 1. Serum CEA ranged from 6 to 170 ng/ml, and positive CEA results were obtained in 14 cases (93.3%), including all five patients without metastasis. The CEA level was significantly related to the size of tumors ($r=+0.74$, $P<0.01$), but not to the metastasis (Fig. 1, left). High values above 40 ng/ml were noted even in localized tumors weighing about 10 g. A simultaneous elevation of plasma CT ranging from 1.2 to 230.0 ng/ml was seen in all the patients (Table 1). Plasma CT level was also significantly related to the size of tumor ($r=+0.73$, $P<0.01$), but not to the presence of metastasis (Fig. 1, right).

The relation between CEA ($X$) and CT levels ($Y$) is shown in Fig. 2. Despite considerable scattering a significant correlation ($r=+0.62$, $P<0.05$) existed between the two, and the regression equation obtained was $Y=0.0052X^2-0.087X-2.25$.

Shown in Fig. 3 are the changes in levels of CEA and CT after surgical treatment of the MCT. Both levels fell to or near to normal when a radical operation was performed. However, they remained elevated when the resection was incomplete.

Fig. 2. Relation between circulating CEA ($X$) and CT levels ($Y$). A significant positive correlation was noted between the two ($r=+0.62$, $P<0.05$). The regression curve shown is expressed by the equation: $Y=0.0052X^2-0.087X-2.25$.

Fig. 3. Effects of surgical treatment of medullary carcinoma with localized (open circles) or metastatic lesion (closed circle) on the circulating levels of CEA and CT. The shaded area shows the normal range.

Fig. 4. Effects of four-hour calcium infusion on circulating CEA and CT in fourteen patients with medullary thyroid carcinoma.
Table 2. Concentrations of CEA and CT in sera and tumor tissue from patients with medullary carcinoma

<table>
<thead>
<tr>
<th>Case</th>
<th>Carcinoembryonic Antigen Serum (µg/ml)</th>
<th>Carcinoembryonic Antigen Tissue (µg/g)</th>
<th>T/S</th>
<th>Calcitonin Serum (ng/ml)</th>
<th>Calcitonin Tissue (µg/g)</th>
<th>T/S</th>
</tr>
</thead>
<tbody>
<tr>
<td>H. T.</td>
<td>170</td>
<td>57</td>
<td>335</td>
<td>98.0</td>
<td>80.1</td>
<td>817</td>
</tr>
<tr>
<td>I. A.</td>
<td>145</td>
<td>105</td>
<td>724</td>
<td>96.8</td>
<td>70.5</td>
<td>728</td>
</tr>
<tr>
<td>A. S.</td>
<td>47</td>
<td>69</td>
<td>1468</td>
<td>10.0</td>
<td>55.2</td>
<td>552</td>
</tr>
<tr>
<td>H. I.</td>
<td>48</td>
<td>26</td>
<td>541</td>
<td>14.6</td>
<td>27.6</td>
<td>1890</td>
</tr>
<tr>
<td>Mean ± S.D.</td>
<td>103±64</td>
<td>64±33</td>
<td>767±494</td>
<td>54.9±49.2</td>
<td>58.4±22.9</td>
<td>997±606</td>
</tr>
<tr>
<td>Graves' disease</td>
<td>5</td>
<td>0.25</td>
<td></td>
<td>&lt;0.1</td>
<td>&lt;1</td>
<td></td>
</tr>
</tbody>
</table>

The effect of calcium infusion on blood levels of CEA and CT was examined. As displayed in Fig. 4, no significant change was demonstrated in the CEA levels, while a distinct increase in CT levels was seen in all the patients.

In 4 cases of the MCT and 1 case of Graves' disease, the tissue concentrations of CEA and CT were determined and compared with the blood levels (Table 2). The CEA contents in the tissue of the MCT were variable, but the values obtained were more than 100 times than in Graves' disease. Although the number of cases examined was small, the serum level of CEA appeared to be related with the tissue contents, and the ratios of tissue over serum levels averaged about 770. A similar relationship was observed in CT between the plasma and tissue levels.

**Discussion**

The simultaneous assay has revealed that serum levels of CEA and CT in the MCT have the following common characteristics: remarkably high levels even in small tumor without metastasis, the levels significantly correlated with tumor size, and decrease in the levels after surgical treatment. As a result, a significant positive correlation exists between the levels of CEA and CT.

In contrast to CT, however, the serum level of CEA was not changed after the calcium infusion (Fig. 4). It follows that the secretory mechanism involved is different from that of CT. CEA is shown to locate on the cell surface (von Kleist and Burtin, 1969), particularly in the glycoalyx (Gold et al., 1970), and more recently demonstrated within the cells in both cancerous and non-cancerous tissues (Rogalsky, 1975; Huitric et al., 1976). Although the biological role of this antigen is not fully understood, it may be a structural constituent produced by the cells.

Despite the possible difference in their biological roles, basal levels of CEA and CT have been shown to be significantly correlated with the weight of excised tumor (Fig. 1) and furthermore, simultaneously decreased after surgical treatment (Fig. 3). The results indicate that the levels in serum are closely related with cellular activity or cell population of the tumor. With regard to CT, however, the secretion from tumor cells is at least in part calcium-dependent (Fig. 4), and may well be affected by other regulatory factor(s). Therefore, it is likely that CEA is a more direct indicator for cell population of the MCT than CT.

In 15 patients with the MCT positive findings were obtained in 100% for CT and in 93.3% for CEA. The CT assay is known to be most sensitive for detection of the MCT, especially when used in combination with the calcium infusion test (Jackson et al., 1973; Deftos, 1974). The present results were in general agreement with previous reports. Positive CEA find-
ings were also very high, though slightly lower compared with CT, and further, the levels in excess of 40 ng/ml ("diagnostic levels") were shown in 86.7%. Because elevated levels of CEA are rarely seen in other thyroid diseases (Ishikawa and Hamada, 1976), CEA assay appears very useful for diagnosis of the MCT.

In the MCT, serum histaminase activity is reported to increase with appreciable consistency (Baylin et al., 1970; Melvin et al., 1972; Keiser et al., 1973; Deftos, 1974). The elevated activity is found only in half the patients, and especially in patients with metastatic disease. In addition, it bears no significant correlation with CT level (Melvin et al., 1972; Jackson et al., 1973). It is concluded, therefore, that circulating CEA level is a more reliable indicator for the disease than histaminase activity.

The concentration of CEA in tissue of MCT is extremely high compared with that in Graves' disease. Martin and Martin (1972) reported that CEA contents in the perchloric extracts of colonic and gastric carcinomas are 8.9 to 233 μg/mg and less than 0.1 to 3.0 μg/mg, respectively. Because the yield of perchloric acid extraction averages about 3 mg/g of tumor tissue (Krupey et al., 1972), CEA content should be 27 to 700 μg/g for colonic carcinoma and 3.0—9.0 μg/g for gastric carcinoma. Therefore, the tissue content in the MCT is nearly comparable with that of colonic carcinoma.

Despite the comparable content in the tissue, circulating CEA levels are much higher in the MCT than in colorectal carcinoma. The remarkably high level in the MCT is probably ascribed to abundant blood supply to the tissue, because the thyroid is an organ of internal secretion receiving much larger quantities of blood supply compared with the kidney (Ingbar and Woeber, 1974).

Acknowledgements

We wish to thank Dr. Phil Gold, the Montreal General Hospital, Montreal, Canada, for his kind gift of purified CEA and anti-CEA antiserum, Profs. Yoshihiro Hamashima and Osamu Midorikawa, Dept. of Pathology, Kyoto University, for their kind supply of autopsy material, and Dr. Kanji Kuma, the Kuma Hospital, Kobe, for his kind supply of patients' material.

This work was supported in part by a Grant-in-Aid for Cancer Research from the Ministry of Education, Science and Culture.

References


