A Study of Cyst Fluid and Plasma Carcinoembryonic Antigen in Patients with Cystic Ovarian Neoplasms

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SUMMARY

Cyst fluid and plasma carcinoembryonic antigen (CEA) levels were measured in 11 patients with ovarian cystadenocarcinoma and in 16 patients with benign ovarian neoplasms. In patients with ovarian cancer, plasma CEA levels were not elevated above 2.5 ng/ml unless cyst fluid CEA levels were 4 to 16 µg/ml. In this series, cystic and plasma CEA levels were elevated most consistently in patients with mucinous ovarian tumors. Furthermore, on the basis of molecular size and immunoreactivity by immunodiffusion, ovarian cancer cyst fluid CEA and colonic cancer CEA had similar immunochromical properties. Consistent with the findings in other neoplasms, follow-up studies showed that plasma CEA levels returned to the normal range between 2 and 12 weeks after surgical excision of the ovarian tumor. It is concluded that plasma CEA is of value in the management of patients with ovarian mucinous cystadenocarcinoma.

INTRODUCTION

Since the discovery of CEA1 in patients with adenocarcinoma of the colon, much effort has been directed toward its application as an immunological test in patients with cancer of other organ systems. Thus, although CEA was initially demonstrated only in the plasma of patients with entodermally derived gastrointestinal tumors (22), subsequent investigators have identified elevated plasma CEA levels in patients with urological, pulmonary, mammary, and other tumors (1, 2, 7, 10–12, 16–19, 24). Recently, elevated levels of plasma CEA have been reported in patients with ovarian cancer (10, 24). Furthermore, ovarian carcinoma CEA has been described as immunologically similar to the CEA present in colonic carcinoma (17). The present study was undertaken to determine the CEA content of cystic ovarian neoplasms and to correlate intracystic and plasma CEA levels to tumor cell type.

MATERIALS AND METHODS

Subjects in this investigation were admitted to the Gynecology Services of the University of Kentucky Medical Center from January 1973 to July 1974. Eleven patients with ovarian cystadenocarcinoma and 16 patients with benign cystic ovarian neoplasms were studied prior to therapy. The tumors were excised at surgery, and the cyst fluids were removed. Cyst fluid and plasma samples were analyzed by radioimmunoassay for CEA by the Hansen Z-gel procedure (6, 12) in 0.01 M ammonium acetate, pH 6.5, using Roche G-23 anti-CEA antisera, which recognizes an ion-sensitive site on the CEA molecule (6). CEA reagents, including G-176 anti-CEA antisera for immunodiffusion, were kindly provided by Dr. H. J. Hansen, Department of Immunology, Hoffmann-La Roche, Inc., Nutley, N. J. A plasma CEA value of 2.5 ng/ml was taken as the upper limit of normal.

Cyst fluid from patients with high intracystic concentrations of CEA was applied to glass columns (2.5 x 100 cm) packed with Bio-Gel A 1.5-m resin equilibrated with 0.05 M KH2PO4, pH 4.7, containing 0.154 M NaCl and 0.02% sodium azide. Buffer was pumped upward at a flow rate of 15 to 20 ml/hr. Six-ml fractions were collected, and the CEA activity of each fraction was measured by radioimmunoassay. The methods of gel filtration and radioimmunoassay have been described elsewhere (16).

Immunodiffusion was performed in 1% agarose dissolved in 0.025 M barbital buffer, pH 8.6. Sample wells were 2 mm in diameter and located 8 mm from the center well. Precipitin bands were developed at room temperature for 24 to 48 hr.

All ovarian specimens were examined histologically and were classified according to cell type and degree of cellular differentiation. The patients with malignant ovarian lesions were staged according to the International Federation of Gynecology and Obstetrics Staging System. Each patient’s red blood cell type was also recorded. Follow-up plasma CEA values were obtained 2, 4, and 6 weeks following surgery as well as monthly thereafter in all patients with ovarian cancer whose initial plasma CEA values were elevated.

RESULTS

Plasma and cyst fluid CEA values in patients with ovarian cancer are depicted in Table 1. Cyst fluid CEA levels ranged from 5.4 to 16,500 ng/ml and were directly related to the cell type of the tumor. CEA levels were above 4175 ng/ml in 4 of the 5 mucinous cystadenocarcinomas, whereas serous tumors all contained less than 308 ng/ml in their cystic fluids. The available data suggested that cyst

1 This investigation was aided by Contract N01-CB-33869 from the National Cancer Institute, NIH.
2 To whom reprint requests should be directed.
3 The abbreviations used are: CEA, carcinoembryonic antigen; PCA, perchloric acid.

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fluid content of CEA was directly related to the stage of disease in mucinous cystadenocarcinomas but not in serous tumors. Assuming that red blood cell type would reflect blood group antigens in the cystic fluids of secretors, the data in Table 1 do not support a correlation between CEA level and any particular blood group type.

In order to partially characterize the physicochemical nature of the substance that reacted in the CEA radioimmunoassay, aliquots of cyst fluid were extracted in 0.6 M PCA and cochromatographed on Bio-Gel A 1.5-m columns with 125I-CEA, which has a molecular size of approximately 200,000 daltons (16). Chart 1 shows the profile of CEA activity of a PCA extract of cyst fluid from an ovarian mucinous cystadenocarcinoma (Patient M. A.). Approximately 60% of the recovered CEA activity cochromatographed with the 125I-CEA marker, while the remainder eluted in the void volume. Profiles of 2 additional samples (Patients L. P. and M. T.) were similar. The profile of CEA in the PCA extract of cyst fluid from a patient with an ovarian mucinous cystadenoma (E. W.) was also similar. Column profiles of CEA from patients J. C. and K. R. with cystic teratomas differed only in the relative distribution of CEA in the 2 peaks, i.e., approximately 80% of the CEA eluted in the void volume and only a small percentage of the anti-CEA neutralizing activity cochromatographed with the marker (Chart 2). PCA extracts of cyst fluids from ovarian mucinous cystadenocarcinomas were reacted in immunodiffusion against anti-CEA antibody, and lines of identity were seen with colonic cancer CEA (Chart 3). With some specimens (Chart 3, Wells 2 and 5), a 2nd precipitin line to the anti-CEA antibody was observed. Thus, on the basis of molecular size and immunoreactivity by immunodiffusion, ovarian cancer CEA appeared to be identical to colonic cancer CEA. Chemical identity, however, remains to be established.

Plasma CEA levels were generally directly related to intracystic CEA levels in the patients with malignant ovarian neoplasms (Table 1); 4 of the 5 patients with mucinous tumors had elevated plasma CEA levels. In contrast, none of the patients with serous cystadenocarcinomas had elevated plasma CEA values, and no patient whose cyst fluid CEA concentration was less than 4175 ng/ml had an elevated plasma CEA. Plasma CEA values following surgery in 4 patients with malignant ovarian tumors whose initial plasma CEA was elevated are illustrated in Chart 4, which shows that plasma CEA values returned to normal within 2 to 12 weeks of surgery in all patients. All but 1 of the patients are clinically free of recurrent ovarian cancer at this time, 6 to 12 months following operative removal of their tumors. Two of these patients have been treated with monthly 5-day courses of L-phenylalanine mustard (1 mg/kg/course) throughout the follow-up period. In the 1 patient with recurrent ovarian cancer, plasma CEA became abnormally elevated 2 weeks prior to clinically detectable recurrence.

Plasma and cyst fluid CEA levels in patients with benign ovarian neoplasms are presented in Table 2. Serous cystade-
nomas and cystic teratomas were the most common lesions studied. Two of 3 cystic teratomas and the 1 mucinous cystadenoma contained high (>1070 ng/ml) concentrations of CEA. Plasma CEA levels were elevated in 3 patients with benign lesions. Of the 16 cases with benign ovarian tumors studied, the 1 with a significantly elevated plasma CEA titer of 5.9 (Patient E. W.) was a mucinous cystadenoma with an increased intracystic CEA concentration. Two patients with benign cystic teratomas had markedly elevated intracystic CEA levels but normal plasma CEA values. This may be a reflection of the relatively small proportion of intracystic CEA that is in the 200,000-dalton size range. As in the patients listed in Table 1, cystic CEA levels in the benign ovarian tumors did not show any relationship to the patients' blood types (Table 2).

**DISCUSSION**

The presence of high amounts of CEA activity in mucinous ovarian lesions raises the question of whether the assay may be measuring mucinous substances other than CEA that cross-react with anti-CEA antiserum. Ovarian cyst fluid is known to contain large amounts of blood group substances (14), and since it has been suggested that the CEA molecule has a blood group A-like determinant (4, 5, 8, 23), we must of course consider the possible contamination of our specimens with blood group substances. In our assay for CEA, blood group A plasma was used to dilute the antibody and the radiolabeled antigen standard, thus eliminating any cross-reactive blood group A immunoreactivity. Further, no correlation between red blood cell antigen status and CEA activity of the ovarian cyst fluids seemed evident in our series of patients. It should also be pointed out that chemical differences between CEA and blood group A substances from tumor ovarian cyst fluid have been found (21).

Chromatographic analysis of ovarian cyst fluid indicated that at least one-half of the CEA activity of benign and malignant mucinous ovarian tumors resides in the 200,000-dalton molecular size fraction, which was identical in size to the marker 125I-colonic cancer CEA. The nature of the substances reactive with anti-CEA antibody that eluted in the void volume of our gel filtration profile has not been elucidated. Treatment of the sample with sulfhydryl reagents and detergents, such as sodium dodecyl sulfate, did not affect the chromatographic profile (Q. Pletsch and D. M. Goldenberg, unpublished results). This fraction was highly viscous, preventing any further characterization. Thus, it is not clear whether the CEA-like activity in the...
void volume is due to aggregation of CEA molecules or to nonspecific inhibition in the radioimmunoassay by mucinous substances unrelated to CEA.

The findings reported here thus confirm numerous other observations that CEA is not restricted to digestive tract cancer (1, 2, 6, 10–12, 16–19, 24), since small quantities of CEA or CEA-like substances were found in nearly all ovarian cystic neoplasms examined. Although CEA was also present in benign ovarian tumors, it appeared highest in malignant cystadenocarcinomas and specifically in those of the mucinous cell type. Elevated intracytic CEA was more directly related to cell type than to cancer, since both benign and malignant mucin-producing ovarian lesions were observed to have high cystic contents of CEA. On the other hand, none of the patients with malignant serous tumors had markedly elevated (5.4 to 308 ng/ml) intracytic CEA levels. In addition, none of the 5 patients with a serous ovarian cystadenocarcinoma had an elevated (> 2.5 ng/ml) plasma CEA value. This lack of correlation with cancer for intracytic CEA levels does not explain why 3 of 16 cases with benign tumors had elevated plasma CEA levels, which correlated with intracytic CEA elevation in only 1 case, a mucinous cystadenoma. Interestingly, 2 of 3 cases with cystic teratoma had very high intracytic CEA values, yet plasma CEA levels in these cases were within normal limits. The 1 case of mucinous cystadenoma in which intracytic and plasma elevations correlated well had a gel filtration profile for CEA similar to those obtained in the malignant counterparts. The gel filtration profile of CEA in both cystic teratomas with elevated intracytic CEA but normal plasma CEA values may help explain such differences, since only a small portion of material with CEA-like activity co-chromatographed with the 200,000-dalton CEA marker. Thus, in the series of ovarian cancer patients included in this study, it appears that increased plasma CEA levels reflected increased amounts of 200,000-dalton CEA present in the cystic fluids, thus suggesting that it is this particular intratumoral CEA size that might be entering the circulation and producing elevated plasma CEA values. The finding that ovarian cancer cyst fluid CEA is similar in molecular size by gel filtration and immunoprecipitation to colonic cancer CEA supports our previous observations that ovarian cancer CEA and colonic cancer CEA may be identical (16, 17).

This study perhaps also explains the earlier observations that antibody to intestinal mucosa cross-reacts with mucinous cystadenoma (15) and antibody to ovarian mucinous cyst fluid has colon cancer specificity (13). It would appear that CEA represented at least 1 common antigen in these ovarian and colonic tissues. However, the intestinal antigenicity could not be demonstrated in mucinous cystadenocarcinomas (15), which seems to conflict with our interpretation because of the high levels of CEA that we have found in this tumor type.

The pattern of decline in plasma CEA following surgical removal of ovarian cancer is similar to that reported by Khoo and Mackay (10) in gynecological cancer and by others in digestive tract cancer (3, 7, 9, 11, 20). CEA may prove, therefore, to be useful in predicting recurrent ovarian cancer prior to clinical recognition, as was indeed the experience of 1 of the cases included in this study. Since plasma CEA is apparently more consistently increased in patients with mucinous cystadenocarcinoma, the CEA blood assay would seem to be of value in the management of patients with this type of ovarian cancer.

REFERENCES


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