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Radioimmunoscintigraphy of advanced gastrointestinal carcinomas employing I-131 labeled CEA-79 monoclonal antibody

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CEA-79 is a murine IgG2a type monoclonal antibody (MoAb) generated using purified CEA from culture supernatants of a human colon cancer cell line, LS174T. The association constant and immunoreactivity of the I-131 labeled CEA-79 ranged from 2.0 to 3.2×10^9 *l*/mole, and from 54 to 74%, respectively. The purpose of this study was to evaluate the feasibility of radioimmunoscintigraphy employing MoAb CEA-79 in patients with advanced gastrointestinal carcinomas. Two mgs of MoAb CEA-79 was labeled with 111 MBq (3 mCi) of I-131, and infused intravenously in 6 stomach cancer and 16 colon cancer patients. Out of 6 patients with stomach cancer, immunoscintigraphy was able to detect the tumors in 4 cases. However, immunoscintigraphy found out tumors in all patients with colon cancer. Moreover, 1 patient with stomach cancer and 2 patients with colon cancer showed increased uptake of MoAb in the tumor lesions despite normal serum levels of CEA. We could conclude that this antibody has a potential as a new imaging agent for the diagnosis of gastrointestinal carcinoma.

Key words: radioimmunoscintigraphy, carcinoembryonic antigen, monoclonal antibody, gastrointestinal carcinoma

INTRODUCTION

GASTROINTESTINAL CARCINOMA is one of the most frequent cancers in many Asian countries, including Korea and Japan. Until now there was no acceptable therapy except early diagnosis and surgery.[1,2] Recently radioimmunoscintigraphy has been studied as a new method to detect malignant tumors. From the beginning of radioimmunoscintigraphy, several studies have utilized radiolabeled anti-carcinoembryonic antigen (CEA) antibodies.[3–7] However, studies with these anti-CEA antibodies have had different results, probably due to the diverse characteristics of the antibodies.[8]

Recently we made an in house anti-CEA monoclonal antibody[9] which was shown to bind highly to tissues of gastrointestinal carcinoma *in vitro*.[10] In this investigation, we developed a radioimmunoscintigraphic system employing this anti-CEA antibody and evaluated the feasibility of this method in the detection of advanced gastrointestinal carcinoma.

MATERIALS AND METHODS

Patients

Twenty-two patients between thirty-five and sixty-nine years of age were subjected to our study from November, 1990 to March, 1992. All the patients had advanced cancers confirmed by pathologic examination. Twelve patients had colon carcinoma, and 6 had stomach carcinoma.

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Monoclonal antibody

We used monoclonal antibody CEA-79. It is an IgG_{2a} antibody to CEA. This antibody was generated by using CEA purified by immunoaffinity chromatography from supernatants of LS174T colon cancer cells. It was made by Dr. Chung of Seoul National University in 1988.[9]

Labeling of this antibody with I-131 was performed by the chloramine T method. Two mgs of antibody was reacted with 111 MBq (3 mCi) of I-131 (New England Nuclear, Boston, MA) by adding 12.5 μg of chloramine T and stopped by adding 43.8 μg of sodium thiosulfate. The reaction time was 2 minutes. A PD-10 column (Pharmacia, Piscataway, NJ) was used for the separation of radiolabeled protein and free I-131. More than 90% of the purified antibody was precipitable with 10% trichloroacetic acid.

Immunoreactivity of the radiolabeled antibody was determined by a serial cell binding assay. In 200 μl, 0.25 to 10 million SNU-C4 human colon cancer cells which expressed CEA were reacted with 5 ng of radiolabeled antibody. Nonspecific binding was measured by adding 25 μg of unlabeled antibody. After 2 hours' incubation, all tubes were centrifuged. Cell pellets were counted and expressed as a percentage of the total count (corrected with nonspecific binding). The immunoreactivity was calculated by a double inverse plot, as described by Lindmo et al.[11] The antibody was tested for pyrogenicity (LAL test), sterility and pathologic murine viruses (MAP test) before being injected into the patients.

Scintigraphic technique

Immunoscintigraphy was done after obtaining informed consent. Thyroid uptake of I-131 was blocked by oral administration of potassium perchlorate (500 mg/day) and potassium iodide (360 mg/day) for 7 days beginning from 1 day prior to injection of the radiolabeled antibody. Three, five and seven days after injection, static planar scintigrams were obtained with a large field-of-view gamma camera linked to GAMMA-11 computer and a medium energy collimator.

Seventy-four to 111 MBq (2–3 mCi) of the antibody was injected into each patient. The radiolabeled antibody was mixed in 20 m*l* of normal saline and given intravenously over a period of 5 minutes. Anterior images of the chest, abdomen and pelvis were recorded, each accumulating 200,000 counts.

To evaluate the pharmacokinetics, serial blood samples were obtained before injection and 5, 30 minutes and 1, 2, 4, 8 hours after infusion as well as at 24, 48, 72 hours in 3 patients, and the activity was measured. Serial urine collections were obtained at 0 to 2 hours, 2 to 24 hours, and 24 to 48 hours. We also measured the serum levels of CEA with a radioimmunometric assay kit (Abbott, North Chicago, IL).

RESULTS

The labeling efficiency was above eighty-five percent. The range of immunoreactivity was between fifty-four and seventy-four percent. Pyrogen test and several sterility tests gave negative results. Mouse antibody production (MAP) tests were negative for 16 pathologic viruses of mouse (Table 1). No adverse reaction or change in vital signs was seen except in one case of transient skin rash.

Faint activities in the liver, spleen and bone marrow interfered with tumor detection. Almost all tumor lesions showed dense uptake of the antibody. Table 2 shows the results for the patients. Serum levels of CEA were within the normal range in 3 patients. Despite this fact, immunoscintigraphy showed positive uptake in all of these patients. Immunoscintigraphy detected the tumors in all patients with colon cancer. In 2 out of 6 patients with stomach cancer, immunoscintigraphy was negative. Most patients with stomach cancer had low levels of serum CEA.

Figure 1 shows the scan of a patient with cancer of the descending colon. Dense uptake of I-131 labeled CEA-79 was seen in the tumor mass. There was also some uptake into the bone marrow and liver. Figure 2 shows another case with colon cancer in whom palliative operation was done. Immunoscintigraphy showed multiple uptakes into the whole abdomen. Abdominal computerized tomography (CT) showed liver metastasis, pancreatic invasion, and multiple seedings in the omentum and peritoneum. The case in Figure 3 also had colon cancer. Abdominal CT showed a huge mass in the right abdomen with central necrosis. The scintigraphic image revealed antibody uptake in the tumor but no uptake into the necrotic area.

It is noteworthy that in one patient with stomach cancer, and two with colon cancer there was increased uptake of radiolabeled antibody into tumor

Table 1 *In vitro* tests of radiolabeled antibody

Labeling efficiency	85.5%–97.3%
Immunoreactivity	54.7%–74.4%
Pyrogen test	Neg
Sterility tests	
Bacteria culture	Neg
Mycoplasma culture	Neg
Virus culture	
viro cell	Neg
MRC-5 cell	Neg
Mouse antibody production test	Neg

Table 2 Result of radioimmunoscintigraphy of advanced gastrointestinal carcinoma

Patient No.	Age/Sex	Primary carcinoma	CEA (ng/ml)	Tumor site	Immuno scintigraphy
1	46/F	colon ca	60.0	transverse colon	+
2	52/M	colon ca	28.9	multiple liver metastases	+
3	51/M	colon ca	14.8	rectum	+
4	38/F	colon ca	61.0	ascending colon	+
5	56/M	stomach ca	7.2	lesser curvature	−
6	42/F	stomach ca	2.1	lesser curvature	+
7	44/M	colon ca	82.0	rectum, liver metastasis	+
8	46/F	colon ca	161.0	right iliac bone, pelvic mass	+
9	58/F	colon ca	5.4	multiple abdominal masses	+
10	62/M	colon ca	75.0	liver metastasis, abdominal mass	+
11	42/F	stomach ca	2.1	lesser curvature	+
12	49/M	colon ca	5.4	splenic flexure, liver metastasis	+
13	45/M	stomach ca	—	paraaortic node	−
14	63/M	stomach ca	—	lesser curvature, paraaortic node	+
15	53/M	colon ca	2.9	sigmoid colon	+
16	51/M	colon ca	16.0	pelvic mass	+
17	41/F	colon ca	121.0	sigmoid colon, liver metastasis	+
18	63/F	colon ca	76.0	hepatic flexure	+
19	65/F	colon ca	1,053.0	liver metastasis	+
20	35/M	colon ca	262.0	pelvic mass	+
21	69/M	stomach ca	11.4	antrum	+
22	64/F	colon ca	262.0	abdominal lymph node, liver metastasis	+

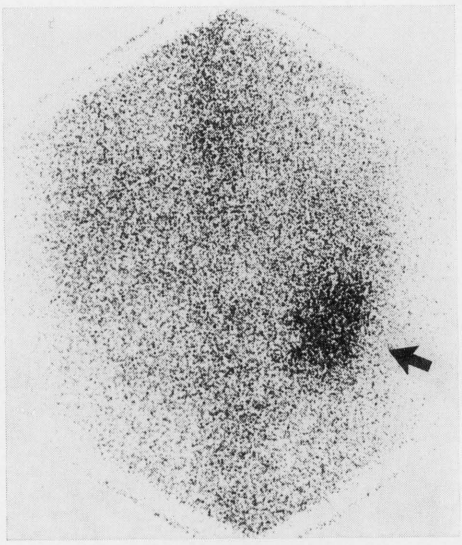


Fig. 1 Anterior abdominal image of a patient with carcinoma in the descending colon. I-131 labeled anti-CEA antibody is shown to be accumulated in the tumor.

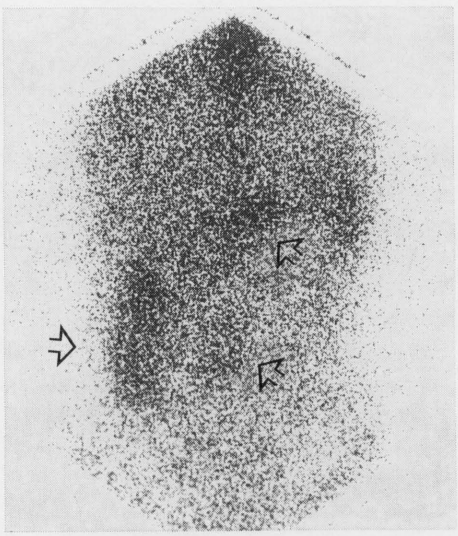
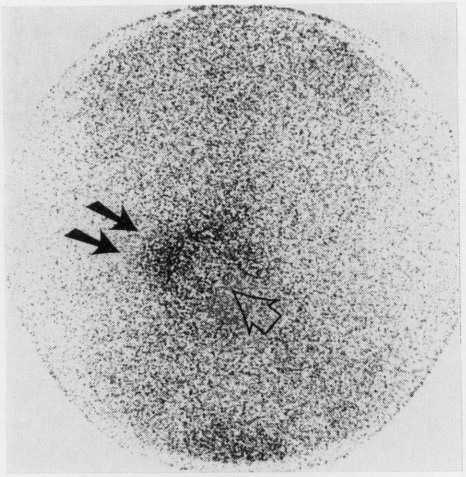


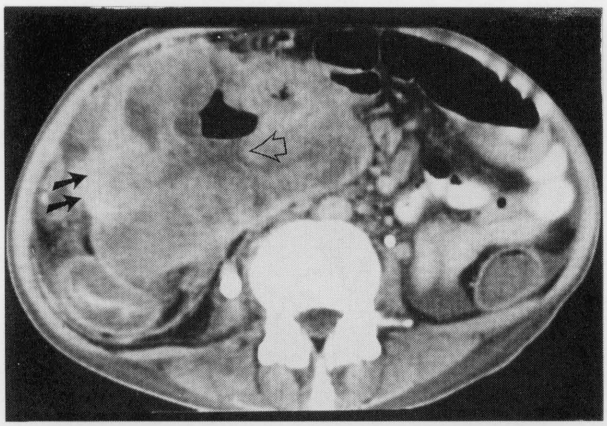
Fig. 2 Immunoscintigram of a patient with multiple abdominal metastases of colon cancer. Anterior abdominal image showed multiple activities in the abdomen.

lesions despite normal levels of serum CEA. Figure 4 shows the scan of a patient with stomach cancer and a normal level of serum CEA.

The serum clearance of the labeled antibody was analyzed (Table 3). The volume of the central compartment (Vc) was between one and three liters, which is approximately the plasma volume. The volume distributed in the steady state (Vdss) ranged from 8,218 to 12,078 m*l*, $T_{1/2}$ from 42 to 79 hours, mean retention time (MRT) from 54 to 102 hours and clearance from 117 to 148 m*l*/hour. Within 3 days after injection, 60–70% of the injected MoAb was excreted in urine.



(A)



(B)

Fig. 3 (A) Anterior abdominal image of a patient with carcinoma in the cecum. Increased activity was seen in the margin of the tumor (solid arrow) without uptake in the central area (empty arrow). (B) CT scan showed tumor mass (solid arrow) with the central necrosis (empty arrow).

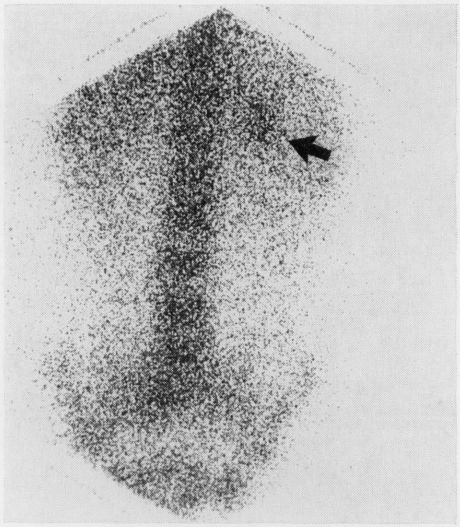


Fig. 4 Immunoscintigram of a patient with stomach cancer. Abnormal uptake was seen in the epigastrium corresponding to the tumor lesion.

Table 3 Pharmacokinetic data of I-131 labeled CEA-79 antibody

Index	Patient 1	Patient 2	Patient 3
Vc (m*l*)	3,155.3	1,591.9	1,053.0
Vdss (m*l*)	8,218.2	12,091.6	12,077.9
Vd area (m*l*)	9,004.5	13,897.4	13,388.4
MRT (hr)	54.1	86.5	101.9
$T_{1/2}$ (hr)	42.2	69.8	79.1
Clearance (ml/hr)	147.6	137.9	117.2

Vc: volume of the central compartment, Vdss: volume of distribution in the steady state, MRT: mean retention time

DISCUSSION

Despite advances in the diagnosis and treatment of gastrointestinal malignancy, a large proportion of patients have extensive disease at the time of the initial diagnosis. It is therefore apparent that there is considerable clinical need of the development of a diagnostic technique for the accurate detection and staging of gastrointestinal malignancy.[12] Carcinoembryonic antigen (CEA) is a glycoprotein described by Gold and Freedman[13] as a tumor-associated antigen in colon cancer. Nowadays CEA is considered as a "pan-carcinoma antigen" which means it is expressed in many other carcinomas as well as gastrointestinal malignancy. Although serum CEA level determination has limited value in screening colorectal cancer, preoperative and serial postoperative CEA determinations are considered to be useful procedures for early detection of recurrent colorectal cancer.[14]

In 1978, the first successful clinical immunoscintigraphy was reported by Goldenberg et al.[3] with I-131 labeled polyclonal antibody. Since the introduction of hybridoma techniques, monoclonal anti-CEA antibodies have been widely used.[5,7,15] A number of monoclonal antibodies to CEA have been produced and have been used both for experimental and clinical imaging of CEA producing tumors. This has been done following appropriate radiolabeling, particularly with I-131, I-123 and In-111.

In general, immunoscintigraphy with anti-CEA antibodies has resulted in sensitivity greater than 80%. However, the sensitivity for tumor detection has been inconsistent.[8,12] Comparison of published data is problematical because of the differences between groups of patients and the differences in antibody preparation. Immunological characteristics of the monoclonal antibodies which can target one of several epitopes of the CEA antigen and physico-

chemical properties of the antibodies are especially important factors influencing the results of clinical immunoscintigraphy.[16,17]

We made anti-CEA monoclonal antibodies utilizing CEA purified by immunoaffinity column chromatography.[9] Unlike conventional methods, this method purified CEA homogeneously with a high yield. Among several anti-CEA monoclonal antibodies, CEA-79 was found to have better immunological characteristics and could be radiolabeled by the chloramine-T method. The immunoreactivity of I-131 labeled CEA-79 and the antigen/antibody association constant were 60–70% and 1.2×10^9 *l*/m, respectively.[16] With *in vitro* quantitative autoradiography, we found this antibody to bind to 33 out of 34 stomach cancer tissues and all 20 colon cancer tissues. However, the maximal binding concentration of CEA-79 was higher in colon cancer tissues (median value: 676 pm/g) than that of stomach cancer (112 pm/g).[10] In animal experiments where SNU-C4 human colon cancer cells were implanted into nude mice, we found that I-131 CEA-79 localized in tumor tissue with a concentration of 14.0% ID/G.[17]

In this study, the tumor mass was visualized by I-131 CEA-79 in all cases with colon cancer. However, we failed to detect the tumor in two cases of stomach cancer. One reason was that the stomach cancers expressed lower concentrations of CEA. Another reason was that tumor uptake in the gastric region can be obscured by vertebral activity as this antibody binds nonspecifically to reticuloendothelial cells.

In three cases, serum levels of CEA were normal but the scintigraphy nevertheless showed increased uptake of antibody in tumor lesions. Also, the uptake of antibody did not correlate with the level of serum CEA. Similar findings were reported by Kim et al.[18] We can speculate that some tumor cells express CEA on their cell surfaces without secreting the antigen into the blood.

We found immunoscintigraphy with I-131 labeled CEA-79 can be performed safely and successfully in patients with advanced gastrointestinal carcinomas. In order to improve tumor detectability, the choice of radiolabel, fragment, and imaging technique is obviously important. Tc-99m labeling appears to result in an increase in detectability.[19] Fragmented antibodies such as $F(ab')_2$ and Fab accumulated at a relatively higher concentration than the intact whole antibody.[12] In addition, SPECT has been found to be of particular value. With the improvement of these technologies, further studies should be carried out to assess whether this antibody can be a useful tool in the staging and monitoring of gastrointestinal cancer patients.

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