Preoperative Imaging of Colorectal Carcinoma with $^{111}$In-labeled Anticarcinoembryonic Antigen Monoclonal Antibody


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ABSTRACT

Patients with primary, recurrent, or metastatic colorectal adenocarcinoma were given injections of 200 $\mu$g of anticarcinoembryonic antigen (CEA) monoclonal antibody labeled with 2 mCi of $^{111}$In (Indacea). Patients were imaged at 24 and 48 h. Cellotomoy was performed on 40 patients between 3 and 17 days post-Indacea injection. Of 16 primary tumors, 11 (69%) were imaged. Of six extrahepatic recurrences, none was imaged. Intrahepatic metastases were visualized as negative images in 10 of 24 (42%) patients. On the basis of the activity in tissue expressed as a percentage of the total radioactive dose per kg injected into the patient (% ID/kg), extrahepatic tumors that were imaged using Indacea had a significant uptake of radiolabel in the tumor (5.99 ± 0.91% ID/kg (SE)) and in the associated normal mesenteric lymph nodes (12.0 ± 2.4% ID/kg). The CEA content of these tumors was high (13.3 ± 4.7 µg/g), and, histologically, the CEA was located primarily apically or intraluminally. Intrahepatic tumor imaging correlated only with tumor size. The greatest Indacea uptake was seen in normal liver (22.1 ± 3.2% ID/kg). Low Indacea uptake was seen in fat (0.21 ± 0.05% ID/kg) and bowel wall (1.11 ± 0.17% ID/kg). In conclusion, Indacea imaging of colorectal carcinoma is specific for high concentrations of accessible CEA in CEA-bearing tumors or in lymph nodes draining these tumors. The successful clinical use of monoclonal antibodies for tumor imaging and therapy will require careful selection of patients for a number of antigen-related parameters including antigen content and distribution in tumors. This information will only come from careful correlation between image results and tissue analysis. High uptake by normal liver tissue is the major unresolved problem labeled with antibody imaging.

INTRODUCTION

CEA is the best characterized tumor-associated antigen for human solid tumors. The cellular, tissue, and organism distributions of this antigen in normal and disease states have been documented (1, 2). In 1974, Reif et al. (3) attempted to detect metastatic cancer in a patient with advanced colon carcinoma using radiolabeled antibody to CEA. The total body scan showed no evidence of localization of the radiolabel in cancer tissue. Using a human CEA-producing tumor xenograft in hamsters, it was subsequently demonstrated that affinity purification of CEA antibody provided tumor localization (4). In 1978, Goldenberg et al. (5) administered $^{131}$I-labeled affinity-purified antibody to 18 patients with advanced neoplastic disease of diverse origins, and total body scans were performed. Ordinary scans proved difficult to interpret. By using radiolabeled albumin for computer subtraction of blood-pool activity, tumor localization was demonstrated at 48 h in almost all cases studied. Scans were negative in patients without demonstrable tumors or with tumors apparently devoid of CEA. High plasma CEA levels did not prevent successful tumor imaging in this study.

The advent of monoclonal antibody technology has resulted in significant progress in the development of high affinity antibodies directed against tumors. More recently, the labeling of these monoclonal antibodies with $^{111}$In has been documented by several groups (6-9). $^{111}$In has several advantages over $^{131}$I as an imaging agent: short half-life (2.83 days); two emissions per disintegration; lower energy of radioactivity (171 and 241 keV); favorable dosimetry (no high energy $\beta$ emissions); and stability of binding.

In this paper, we have used a monoclonal anti-CEA antibody (T84.66) that has shown high antigen binding affinity ($K = 2.6 \times 10^{10}$ liters/m) and an absence of cross-reactivity with CEA-like antigens (10, 11). The antibody has been labeled with $^{111}$In by an improved method of binding DTPA to the monoclonal antibody developed in our laboratory (12). The indium-labeled monoclonal anti-CEA antibody (Indacea) maintained antibody activity and remained stable under physiological conditions. The method of preparation of Indacea, including the use of EDTA to facilitate rapid clearance of free $^{111}$In, has made possible the development of a “kit” for Indacea preparation. Indacea for imaging CEA-bearing human tumors xenografted in nude mice under conditions that were comparable to those that could be used in humans (3 ng of antibody per g of body weight, labeled with $^{111}$In at a ratio of 10 $\mu$Ci per $\mu$g of antibody) was previously reported (13, 14). The effects of time, antibody dose, tumor CEA content, and tumor size upon the biodistribution of Indacea and upon tumor imaging were examined. Imaging directly paralleled the biodistribution of Indacea, with the best uptake occurring at 48 h in small tumors of high CEA content.

In order to determine the clinical value of Indacea for localization of occult cancer, a study using Indacea was undertaken in colorectal cancer patients prior to the surgical resection of their tumors. Imaging of colorectal carcinoma at an early stage of the disease by surgical exploration has allowed evaluation of the true specificity and sensitivity of this technique. A systematic comparison of imaging results with gross and histological operative findings has not previously been reported. This comparison is important for the development of a better understanding of the tumor, host, and antigen characteristics affecting tumor imaging. Scintiscanning patients prior to surgical resection has also allowed evaluation of resected tissues for Indacea biodistribution, CEA content, and histological distribution of CEA within the tumor.
MATERIALS AND METHODS

Conjugation of Monoclonal Antibody with DPTA. The method was a modification of the technique of Krejcarek and Tucker (15) as described in detail by Paxton et al. (12). DTPA (12 mg, 0.0305 mmol) and triethylamine (0.017 ml, 0.122 mmol) were dissolved in 0.2 ml of dry acetonitrile with gentle heating. After cooling the solution to room temperature, N-hydroxysuccinimide (1.76 mg, 0.0153 mmol) and disopropylcarbodiimide (0.0024 ml, 0.0153 mmol) were added, and the reaction was stirred for 2 h. The reaction mixture (DTPA-OSU) was used directly to prepare the DTPA-conjugated monoclonal antibody.

A solution of anti-CEA monoclonal antibody (T84.66; 1 mg in 0.2 ml of 0.1 M NaHCO3, pH 7.0) was stirred for 1 h at room temperature with 8 μl of a freshly prepared DTPA-OSU reaction mixture and was then kept at 4°C for 16 h. The resulting DTPA-labeled antibody was separated from free DTPA using a 12-ml Sephadex G-50 superfine column equilibrated with metal-free 0.1 M sodium acetate, pH 5.0. The resultant solution of DTPA-labeled anti-CEA monoclonal antibody was sterilized by passage through a 0.2-μm filter.

Labeling of the DTPA-labeled Antibody with 111In. 111In (a gift from Medi-Physics, Emeryville, CA) was prepared for labeling by adding an equal volume of 1 M sodium acetate. Sterile 111In (10 μCi/μg of protein) was added to the DTPA-labeled antibody, and after 4 h, sterile EDTA (1 mM) was added to complex any free 111In. The final preparation was referred to as "Indacea." This material was approved for investigational use by the Food and Drug Administration (BB-IND-2014). The extent of labeling of the Indacea was tested by gel filtration chromatography on a Sephadex G-50 superfine column equilibrated with 0.1 M sodium acetate, pH 5.0.

Patients. Patients eligible for the study were those: (a) with previously untreated colorectal carcinoma in whom laparotomy was planned for bowel resection; (b) with hepatic metastases of colorectal carcinoma in whom laparotomy was planned for hepatectomy and/or for continuous infusion pump placement; and (c) previously having had "curative" bowel resection for colorectal carcinoma in whom an elevated serum CEA developed and in whom a "second look" laparotomy was planned.

Patients signed informed consent prior to participation in the study. The study and consent procedures were approved by the Institutional Review Board of the City of Hope National Medical Center.

Scanning. All patients were initially shown to be negative to a 48-h skin test using 20 μg of monoclonal antibody T84.66. After 48 h, radiolabeled immunoglobulin (Indacea) was administered i.v. over 5 min in a dose of 200 μg of antibody and 2 mCi of 111In. Anterior and posterior scintiscan images of the trunk were obtained 24 and 48 h following infusion of Indacea on a Technicare Omega 500 camera using a medium energy collimator. The scans were stored on a Technicare 560 computer.

Surgery. Patients had preoperative serum CEA levels measured by radioimmunoassay (Hoffman-La Roche, Inc., Nutley, NJ) prior to injection of Indacea. Surgical exploration was performed in the standard fashion for the circumstances of the individual patient. Examination of areas of increased uptake on the Indacea scan was undertaken, but always in accordance with the best interests of the patient. Surgical procedures were planned for performance between 5 and 17 days following Indacea injection and usually occurred between Days 5 and 9. At the time of exploration, the total body dose of 111In had dropped to under 5% due to body clearance and radionuclide decay.

Tissue Analysis. Tumor and other tissue removed at exploration were examined in routine fashion by the surgical pathologists. The tissue sections were fixed in buffered formalin and B5 solution, embedded in paraffin, and stained with hematoxylin and eosin for routine histological examination. Preparation of the paraffin-embedded, fixed tissue for immunohistochemical studies has been described in detail (16). The sections were cut at 6 μm, and 2 sections were placed on one glass slide. After deparaffinization and rehydration, sections were studied with immunohistochemical procedures. A modification of the highly sensitive avidin-biotin-complex technique was used (17, 18). The procedure is briefly summarized as follows. (a) The primary antibody (T84.66) at a dilution of 1:50 is placed on 1 of 2 sections on each slide and incubated for 30 min. (b) Excess antibody is removed by washing with bovine serum albumin:phosphate-buffered saline, and the sections are overlaid with biotinylated, anti-mouse antibody at a dilution of 1:100 for 20 min. (c) A preformed avidin-biotinylated horseradish peroxidase complex at a dilution of 1:80 is applied for 15 min. (d) Excess reagent is removed with bovine serum albumin:phosphate-buffered saline. (e) The substrate color is developed with 3-amino-9-ethylcarbazol (Polysciences, Inc., Warrington, PA) or diamobenzidine (Sigma Co., St. Louis, MO). Primary antibody was added to only 1 of the 2 sections on each slide. The second section served as a control for endogenous peroxidase activity. An additional control in each case was substitution of primary antibody by mouse ascitic fluid or nonimmune mouse serum. A section was considered to be positive when we could identify at least several distinct positively stained neoplastic cells, easily distinguished from negative stromal cells on low-power examination. The intensity and pattern of immunostaining were recorded.

Aliquots of tissue in excess of that required for histopathological analysis were weighed and analyzed quantitatively for CEA and 111In content. The Roche enzyme immunoassay kit used for CEA content measurement (μg of CEA per g of tumor) uses the T84.66 antibody. The kit was a gift of Hoffman-La Roche, Inc. (Nutley, NJ). The tissue content of 111In (% ID/kg of tissue) was measured on a Searle 1185 series automatic gamma counter (Searle Analytic, Inc., Des Plaines, IL). Corrections for counting efficiency and decay time were made.

Statistical analysis was performed using the Student's t test.

RESULTS

Scanning. Fifty scintiscan studies were undertaken in 49 patients. In 40 patients, correlation between Indacea scintiscan and operative findings was possible. Exclusions were due to: unevaluable scintiscans (first 2 patients studied); failure to undertake exploration (6 patients, 7 scintiscan studies); and diagnosis other than colorectal carcinoma (1 patient found to have lymphoma at operation).

Primary colorectal carcinoma was identified in 16 patients at exploration, hepatic metastasis in 24 patients, and local recurrence and/or extrahepatic metastasis in 6 patients. Five of the patients with primary bowel disease also had hepatic metastasis, and one patient with an anastomotic recurrence had hepatic metastasis.

The 4 scintiscan studies (anterior and posterior, upper and lower trunk) done on each patient at 24 and 48 h were performed by setting the γ camera for 2.5 × 106 counts. Extrahepatic tumor images were visible at 24 h and were more intense at 48 h. Several patients were imaged at longer intervals (72 and 120 h), but no improvement in tumor imaging was noted.

Normal tissues visualized included blood pool, liver, spleen, kidneys, bone, colon, bladder, and testes. Normal tissues were most intense at 24 h and, except for liver, thereafter faded relative to tumor. The blood pool visualized was greatest in the first few hours after i.v. injection of the Indacea (Fig. 1). It was sometimes seen at 24 h, but rarely at 48 h. Liver uptake was high within 10 min of i.v. injection and remained dominant in all subsequent scintiscans. Thus, by counting a fixed number of γ rays, our scintigraphy was documenting changes in normal and tumor tissue uptake relative to that of liver. Spleen was clearly visualized in most patients studied. Bladder and kidney uptake was usually seen at 24 h, but diminished markedly by 48 h. The high early uptake in the urinary tract correlated with the initial excretion of free 111In chelated by EDTA. Uptake in osseous tissue resulted in visualization of vertebrae, pelvis, femurs, and other bones when scanning was extended outside the trunk. Once again, bone uptake diminished from 24 to 48 h. Concentration of isotope in colon was often seen in the early studies and could be prevented by mild laxatives in those patients not on a formal preoperative bowel preparation. Testicular uptake was seen in 20 of 27 male patients. Like bladder...
Fig. 1. Anterior trunk Indacea scintiscans of patient during first h and at 24 h after injection of Indacea (200 µg antibody, 2 mCi 111In). Initially, radioactivity is primarily in the heart and great vessels. By 24 h, these structures are no longer visualized. Uptake in liver and spleen is seen as early as 10 min postinjection, and these organs continue to be visualized at 24 h.

Imaging, it was seen best anteriorly and was less at 48 than at 24 h. Testicular uptake is discussed further in the companion paper (19).

Primary tumors were correctly imaged in 11 of 16 cases (Figs. 2 and 3). In one case, the primary tumor in the pelvis was not imaged, but intense uptake was noted in the left abdomen adjacent to the upper lumbar spine. No tumor was identified at laparotomy in this region despite careful examination.

Extrahepatic metastatic disease was not correctly identified on any of the Indacea scintiscans. One patient previously had a colectomy followed by a partial hepatectomy for a liver metastasis. This patient’s serum CEA again became abnormal (20 ng/ml). Conventional radiological and radionuclide studies revealed no tumor, and a second look laparotomy was planned. The Indacea scan showed uptake in the upper mediastinum compatible with tumor (Fig. 4). No tumor was identified at laparotomy or mediastinoscopy. A CT scan of the chest showed a small 1-cm lesion in the left lower lobe of the lung. At thoracotomy, an adenocarcinoma consistent with a colorectal carcinoma metastasis was resected.

Liver metastases were the most difficult to visualize because of the high radiodensity of normal liver. For this reason, the metastases were visualized as negative defects, much the same as in conventional liver/spleen imaging with colloidal substances (Fig. 5). Of 24 patients with liver metastases, only 10 patients had tumor identified in the liver. No patient had a liver defect without a metastasis being identified. One patient with a liver metastasis only had uptake in the left lower quadrant of the abdomen. This image could not be confirmed as tumor at exploration.

The sensitivity of Indacea imaging was 69% (11 of 16) for primary colorectal carcinoma, 42% (10 of 24) for hepatic metastasis, and 0% (0 of 6) for extrahepatic recurrence or metastasis (Table 1). The specificity was 83% (33 of 40) for extrahepatic metastasis.
PREOPERATIVE COLON CANCER IMAGING WITH INDACEA

Fig. 3. Lower trunk Indacea scintiscans at 48 h of patient with primary rectal carcinoma (T) and solitary metastatic lesion (MC) in the right lobe of the liver (L). The anterior scintiscan (A) shows pelvic uptake in the urinary bladder (B). The posterior scintiscan (P) shows splenic and rectal tumor (T) uptake. Anterior pelvic intensity is normally much greater than posterior pelvic intensity due to the bladder. The presence of posterior pelvic intensity comparable to anterior intensity is diagnostic of tumor in the posterior pelvis.

Fig. 4. Anterior upper trunk Indacea scintiscan of patient with elevated serum CEA (20 ng/ml). Patient had previously had colectomy for colonic carcinoma and partial hepatectomy for liver metastasis of colon carcinoma. Patient received 200 μg of antibody, labeled with 2 mCi of 111In. Indacea scintiscan showed 2 nodules in the upper mediastinum (T). Chest CT scan identified a 1-cm lesion in the left lower lobe of the lung and, at thoracotomy, adenocarcinoma containing 7.9 μg of CEA per g of tumor was confirmed. Liver uptake (L) of the Indacea is marked.

CEA Content. Serum CEA was evaluated preoperatively in all 40 patients. Sufficient resected tumor was available in 24 patients to allow assay for tumor CEA content. Patients with primary colorectal cancer that were imaged by Indacea had lower serum CEA values (1.9 ± 0.5 ng/ml; Table 2). Patients with primary tumors that did not image with Indacea tended to have higher serum CEA values (16.9 ± 8.4 ng/ml). In contrast, the CEA content of the tumor was higher for primary tumors that were imaged (13.3 ± 4.7 μg/g) and lower for primary tumors that were not imaged (2.9 ± 2.0 μg/g). No correlation was seen between the histological Dukes' stage of primary colorectal carcinoma and the imaging of these tumors.

As expected, patients with metastatic and/or recurrent colorectal cancer, had an elevated serum CEA (>5.0 ng/ml) in a greater proportion of cases (17/29, 59%) than did patients with only primary cancer (1/11, 9%). The serum CEA in patients with hepatic metastases that were visualized on the Indacea scan was 205 ± 104 ng/ml and in those patients whose hepatic tumors were not visualized was 23.4 ± 9.2 ng/ml. The serum CEA in the 5 patients with recurrent extrahepatic disease and no hepatic metastases was 30.5 ± 15.0 ng/ml. None of these recurrent tumors was imaged. The CEA content of the metastatic colorectal cancer resected from the liver was 14.8 ± 4.6 μg/g and there was no significant difference between the CEA content of visualized and non visualized hepatic tumors. The CEA content of recurrent extrahepatic disease was uniformly low (3.48 ± 1.52 μg/g).

By examining all extrahepatic tumors (primary and recurrent), we noted that the CEA content of extrahepatic tumors (primary) that were imaged by Indacea (13.3 ± 4.7 μg/g) was significantly higher (P < 0.05) than the CEA content of extrahepatic tumors (primary or metastatic) that were not imaged (3.29 ± 1.22 μg/g). All tumors with CEA greater than 8 μg/g were positively imaged by Indacea, whereas only one-third of tumors with CEA less than 8.0 μg/g was imaged. The serum CEA level in patients with extrahepatic tumors that were imaged by Indacea (1.9 ± 0.5 ng/ml) was significantly lower (P < 0.025) than the serum CEA of patients with extrahepatic tumors that were not imaged (26.6 ± 8.9 ng/ml).

Size of Liver Metastases. An operative scoring system for semiquantitatively estimating the extent of hepatic metastases was devised (Table 3). Using this system with a maximum score of 6 and a minimum score of 1, we documented that the larger, more extensive tumors were identified by the photopenic hepatic image (score, 5.1 ± 0.3) and that the smaller and less extensive hepatic metastases were not identified (score, 2.6 ± 0.4) (P < 0.001). Hepatic metastases with scores of 1 to 3 (9 patients) were not imaged on Indacea scans, while hepatic metastases with scores of 4 to 6 were visualized in 10 of 15 patients.

Biodistribution of Indium in Resected Tissues. As would be anticipated, tumors with the highest CEA content had the greatest uptake of Indacea. For example, primary colorectal carcinoma that was imaged using Indacea had 13.3 ± 4.7 μg of CEA per g and 5.99 ± 0.91% ID/kg compared to 2.9 ± 2.0 μg/g of normal colorectal cancer and 3.67 ± 1.63% ID/kg in primary tumors that were not identified by scintiscan (Table 2). Similarly, tumors metastatic to the liver had 14.8 ± 4.6 μg of CEA per g and 8.38 ± 1.96% ID/kg, whereas extrahepatic metastases or local recurrences had 3.5 ± 1.5 μg of CEA per g and 1.03 ± 0.32% ID/kg. Interestingly, tumor in mesenteric lymph nodes had an
Fig. 5. Large hepatic metastasis of colorectal carcinoma seen on standard liver/spleen scintiscan (**Tc-sulfur colloid) (left) and Indacea scintiscan at 48 h (200 µg of antibody, 2 mCi of **In) (right). On both scans, the metastasis was seen as a negative defect (decreased uptake). Computer subtraction of the **Tc from the **In resulted on 2 occasions in a “hot rim” suggestive of increased tumor uptake in the peripheral areas of the tumor.

Table 1 Correlation of Indacea imaging of colorectal carcinoma with operative findings (40 patients)

<table>
<thead>
<tr>
<th>Tumor location</th>
<th>Tumors imaged (true positive)</th>
<th>False positive tumor image</th>
<th>Sensitivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary (16)†</td>
<td>11</td>
<td>1</td>
<td>69</td>
</tr>
<tr>
<td>Metastatic (29)</td>
<td>10</td>
<td>2</td>
<td>34</td>
</tr>
<tr>
<td>Intrahepatic (24)</td>
<td>10</td>
<td>0</td>
<td>42</td>
</tr>
<tr>
<td>Extrahepatic (6)</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, number of tumors.
† Numbers in parentheses, number of patients.

Intermediate **In uptake (3.47 ± 0.54% ID/kg). No lymph node specimens in this series were assayed for CEA content. Overall, the uptake of **In by extrhepatic tumors (primary) that were imaged by Indacea (5.99 ± 0.91% ID/kg) was significantly higher (P < 0.025) than the **In uptake of extrhepatic (primary or metastatic) tumors that were not imaged (2.45 ± 1.07% ID/kg).

The uptake of Indacea within individual tumors was not uniform. This was most easily seen with hepatic metastases. The outer rim of these tumors had a high level of radioactivity (8.38 ± 1.96% ID/kg), and the central area of the tumor had a much lower level (2.16 ± 0.82% ID/kg).

In all specimens, the tumor uptake of Indacea was higher than the normal bowel wall uptake (1.11 ± 0.17% ID/kg), the gallbladder wall uptake (0.21 ± 0.08% ID/kg), and the uptake in fat (0.22 ± 0.05% ID/kg). However, the level of radioactivity in the normal liver (22.1 ± 3.2% ID/kg) was substantially higher than the hepatic metastases of colorectal carcinoma in all but one case. The average liver:tumor ratio of radioactivity for hepatic metastases was 5.6 ± 1.2 (n = 14).

Lymph nodes that were replaced histologically by tumor had an increased amount of **In (3.47 ± 0.54% ID/kg) relative to the adjacent fat. As expected, the positive mesenteric lymph nodes from specimens that had been imaged had more Indacea uptake than those that had not been imaged (Table 4; 4.59 ± 1.01 and 2.91 ± 0.62% ID/kg, respectively). However, normal mesenteric nodes had an even higher Indacea content than the tumor-containing nodes (10.8 ± 2.2 and 3.47 ± 0.54% ID/kg, respectively; P < 0.005). In addition, a dramatic difference in the normal node content of radioactivity was seen between the imaged and nonimaged tumors (12.0 ± 2.4 and 6.61 ± 2.15% ID/kg, respectively; P < 0.05).

Tumor Immunohistochemistry Using T84.66. Almost all resected colorectal adenocarcinomas (51 of 52) contained CEA by immunohistochemistry using T84.66. Generally, 50% or more of the cells were CEA positive, and the staining intensity was moderately (++) to strongly (+++) positive. No correlation was observed between tumor imaging and degree of CEA staining assessed by these conventional parameters.

The pattern of CEA staining was also examined. It was divided into 3 categories: apical (Fig. 6); cytoplasmic (Fig. 7); and intraluminal (Fig. 8). Cytoplasmic staining was the predom-
Table 3 Operative scoring of hepatic metastases of colorectal carcinoma

<table>
<thead>
<tr>
<th>Score</th>
<th>Characteristics</th>
<th>No.</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Single metastasis</td>
<td></td>
<td>&lt;2-cm diameter</td>
</tr>
<tr>
<td>2</td>
<td>Multiple metastases</td>
<td></td>
<td>All &lt;2-cm diameter</td>
</tr>
<tr>
<td>3</td>
<td>1-3 Metastases</td>
<td></td>
<td>Largest 2- to 4-cm diameter</td>
</tr>
<tr>
<td>4</td>
<td>Single metastasis</td>
<td></td>
<td>&gt;4-cm diameter</td>
</tr>
<tr>
<td>5</td>
<td>Multiple metastases</td>
<td></td>
<td>Largest &gt;4-cm diameter</td>
</tr>
<tr>
<td>6</td>
<td>Extensive metastases</td>
<td></td>
<td>&gt;50% liver replacement</td>
</tr>
</tbody>
</table>

Table 4 Correlation of Indacea imaging with indium content (% ID/kg) of mesenteric lymph nodes in patients with primary colorectal carcinoma

<table>
<thead>
<tr>
<th>Node status histologically</th>
<th>Tumor imaged</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes (11)*</td>
<td>4.59 ± 1.01*</td>
<td>12.0 ± 2.4</td>
<td>(49)</td>
</tr>
<tr>
<td>No (5)</td>
<td>2.91 ± 0.62</td>
<td>6.61 ± 2.15</td>
<td>(14)</td>
</tr>
<tr>
<td>Total</td>
<td>3.47 ± 0.54</td>
<td>10.8 ± 2.2</td>
<td>(63)</td>
</tr>
</tbody>
</table>

* Numbers in parentheses, number. * Mean ± SE.

DISCUSSION

Successful tumor imaging with labeled monoclonal antibodies will depend upon a fuller understanding of tumor cell biology and optimization of all parameters that contribute to successful scans. A recent review by Bradwell et al. (20) examined theoretical and practical considerations for tumor imaging. They stressed the need for tumor-specific antigens and commented that CEA is as "specific" as many of the more recently described antigens. They pointed out that, compared to the more commonly used iodine isotopes (131I, 125I), 111In is an attractive isotope. The major problems with 111In have been the long biological half-life giving it a higher absorbed dose and the marked uptake in the normal liver.

Most of the human work to date with antibodies directed against CEA or other colorectal cancer antigens has utilized iodine isotopes and subtraction techniques. The original work of Goldenberg (1, 21) and Mach (22) used affinity-purified polyclonal antibody. Since that time, improved imaging has been noted for monoclonal antibodies (23), antibody fragments (24), combinations (24), second antibodies for clearance of the blood pool (25, 26), and transaxial tomoscintigraphy (27). All
showing strong intraluminal staining with monoclonal anti-CEA antibody were evaluated for antigen and radionuclide content, and (d) correlation was obtained for most patients, (c) resected tissues and have compared tumor imaging with other imaging tests (X-rays, CT scan, nuclear scan, ultrasound).

The results reported in this article differ from those previous reports in that (a) antibody was labeled with \( ^{111} \text{In} \), (b) operative correlation was obtained for most patients, (c) resected tissues were evaluated for antigen and radionuclide content, and (d) resected tissues were examined by immunohistology using the same antibody as for imaging. Our purpose was not only to assess the efficacy of colorectal cancer imaging using another preparation, but also to identify those antigen, tumor, and host factors associated with imaging.

While a large number of monoclonal antibodies to CEA have been produced, not all have been appropriate for imaging. Recently, 5 different epitopes of CEA were outlined by Shively's laboratory (10, 11). They identified the monoclonal antibody that had the greatest specificity for CEA, the least cross-reactivity with other antigens, and the highest antigen-antibody binding affinity (T84.66). We have utilized this antibody in these studies and have avoided the problem of cross-reacting monoclonal antibodies reported by Dillman et al. (28).

Several investigators have reported more reliable tumor imaging in animals and humans for antibodies labeled with \(^{111}\text{In} \) compared to radionuclides of iodine (6, 7, 29, 30). Paxton et al. (12) described a method of conjugating DTPA to the T84.66 antibody that minimized the structural change of the antibody and, unlike the reports of Murray et al. (31) and Fawwaz et al. (32), avoided loss of immunological activity of the antibody. The conjugate was labeled with \(^{111}\text{In} \) to make “Indacea.” This preparation was stable under physiological conditions. Similar preparations have been documented to be stable by others in vitro and in vivo (6, 7, 33).

We have previously shown that Indacea was effective for imaging CEA-bearing human tumor xenografts grown in nude mice (13, 14). The dose used in this human study was comparable on a weight basis (3 ng/g) to that used in the animal studies. The dose was kept low to minimize side effects of foreign protein or subsequent development of anti-murine antibodies. The presence of antibodies against murine immunoglobulin and anti-idiotype antibodies following injection of murine monoclonal antibodies in humans has recently been reported (34, 35). This immune response may affect the serial use of murine monoclonal antibodies for imaging or therapy. Following injection of 200 \( \mu \text{g} \) of Indacea in this study, no side effects were noted in any of the patients. This dose is much lower than generally reported by others in the field (31). One patient had 2 injections of Indacea 3 mo apart. No side effects were noted, and no anti-murine antibodies could be detected following the second injection.

In humans, Indacea has behaved in a similar fashion to other antibodies labeled with \(^{111}\text{In} \) by other techniques. The radioactivity was seen first in the cardiovascular pool (Fig. 1). The plasma half-life of Indacea (26 h) was very similar to the report of Rosenblum et al. (35) for an indium-labeled monoclonal antibody against malignant melanoma (27 to 39 h). In this same patient, a drop of serum CEA from 128 ng/ml preinjection to 95 ng/ml at 75 min was noted. By 48 h, the serum CEA had risen to 130 ng/ml, and a wk later, it was 158 ng/ml. This dip in antigen level followed the same pattern recently reported for CA 19.9 (27).

Kidney and bladder intensity dropped rapidly in the first 24 h as the free \(^{111}\text{In} \) chelated by the EDTA in the preparation was cleared by the urinary tract. Rosenblum et al. (36) noted that 9% of the injected dose was excreted in the first 8 h, followed by 5% in the next 40 h. As noted by others (33, 36), splenic intensity decreased with time in a fashion similar to that observed for the kidney.

Bone uptake was sometimes substantial, but decreased significantly during the first 48 h. By chromatography of human serum, Hnatowich et al. (33) examined the change in indium profile with time following injection of indium-labeled antibody. They reported that only 1 to 2% of the total \(^{111}\text{In} \) became bound to the transferrin, but that 9 to 10% of the serum \(^{111}\text{In} \) activity at 20 h postinjection was bound to transferrin. This \(^{111}\text{In} \) exchange could account for the observed bone uptake.

The colon uptake of \(^{111}\text{In} \), similar to colon uptake seen on conventional \(^{68}\text{Ga} \) citrate and \(^{111}\text{In} \) chloride scans, was minimized by the use of mild laxatives after the 24-h scintiscan. Testicular uptake has not previously been documented with anti-CEA or other antibody imaging techniques and is discussed in the companion paper (19).

Hepatic uptake was seen within 10 min and increased rapidly over the first hour following injection (Fig. 1). Thereafter, liver image intensity was relatively stable except for the decrease due to isotope decay. Hnatowich et al. (33) estimated the liver uptake as 20 ± 8% of the injected dose. They documented that the injected activity in the liver remained at this level for over 60 h from the time of injection. Clearly, liver uptake is the major problem with a \(^{111}\text{In} \)-labeled antibody and is a consistent finding in all the animal and human studies reported to date. Speculation on the mechanism of hepatic uptake includes free \(^{111}\text{In} \) uptake by liver, transfer of \(^{111}\text{In} \) from DTPA to transferrin, and receptor-mediated uptake of glycoproteins. Recent nude
mouse studies from our laboratory (14) and the San Diego group (37) have documented that increased tumor size, increased tumor CEA content, and increased rate of secretion of CEA by tumor all increase the hepatic uptake of $^{111}$In-labeled anti-CEA monoclonal antibody. These results suggest that the uptake of antigen-antibody complexes by the liver is another important reason for the hepatic accumulation.

In contrast to normal tissues (except liver), positive tumor images (Fig. 2) were more prominent at 48 h than at 24 h. This observation was most important in the pelvic region, where, due to bladder uptake, anterior scans were normally more intense than posterior scans. The presence of an intense region in the posterior pelvic scintiscan, equal or greater than that seen on the anterior scan and with increasing intensity from 24 to 48 h, proved diagnostic for rectal or posterior pelvic tumor (Fig. 3).

The patients selected for Indacea imaging in the current study were those without advanced disease. The sensitivity of conventional imaging tests for evaluating intraabdominal tumor (e.g., CT scan, ultrasound) in comparison with operative findings (38) has been reported as low as 30% with specificity of 80%. Thus, the use of operative findings for Indacea scan evaluation would have been expected to result in a very low sensitivity rate. However, the overall incidence of tumor imaging with Indacea, considering that imaging without subtraction was utilized, was similar to previous results with other antibodies and other radiolabels referred to above. The breakdown into clinically significant categories of disease is a departure of the current study from other reports. The imaging of 69% of primary colorectal adenocarcinomas is very encouraging. It is slightly better than the 57% (13 of 23) preoperative localization of primary colorectal carcinoma reported by Armitage et al. (39) with an iodinated monoclonal antibody (791T/36). The failure of Indacea in the imaging of any operable recurrent extrahepatic metastases and in the photopenic imaging of hepatic metastases is disappointing.

The major contribution of the current report to the monoclonal antibody imaging field is the correlation of scintiscan findings with the assay of tumor CEA content, tumor $^{111}$In content, and immunohistology that was facilitated by the routine operative exploration. Basically, we have observed that (a) primary tumors with a high CEA content have the best Indacea uptake and are most easily imaged; (b) hepatic metastases have a high CEA content and high Indacea uptake, but do not image well because of the large uptake by normal liver; (c) extrahepatic metastases have a low CEA content, low Indacea uptake, and are not imaged well; (d) apical and intraluminal staining of tumors by anti-CEA monoclonal antibody immunohistology correlates with Indacea uptake; and (d') uptake of Indacea by normal lymph nodes draining colon tumors is greater than the primary tumor uptake of Indacea.

CEA is constantly being synthesized by colorectal adenocarcinoma cells, migrating through the cytoplasm, associating with the cell membrane, and being shed into the tissues around the tumor cells as soluble CEA. As the soluble glycoprotein (CEA) accumulates in the interstitium, it is concentrated by the lymphatics, passed to adjacent lymph nodes, and transported as chyle into the liver (2). From our results, we hypothesize that the higher the cellular content of CEA in colorectal carcinoma, the greater the amount shed by the cells and concentrated in the lymphatics, and the greater the amount of CEA that is generally accessible to Indacea. Indeed, the tumor images that we have seen may not be due so much to Indacea on the tumor cell surface, but due to Indacea bound to CEA in the interstitial fluid around the tumor and in adjacent lymph nodes. For example, one patient (Fig. 3) had an upper thoracic intensity following Indacea injection that was interpreted as mediastinal CEA-bearing tumor. At mediastinoscopy, 5 lymph nodes were removed, radioactivity was measured (Table 5), and routine histology was performed. None of the nodes contained tumor, but high levels of indium were measured in the right inferior paratracheal node (59.0% ID/kg) and, to a lesser extent, the left upper paratracheal node (5.07% ID/kg). The distribution of radioactivity suggested a CEA-secretting tumor in the left lower lobe of the lung. A CT scan of the chest was performed, and a 1-cm defect compatible with the tumor was identified in the left lower lobe. At thoracotomy, an adenocarcinoma of the left lower lobe was removed by wedge resection. The CEA content was 7.9 μg of CEA per g of tumor. Unfortunately, the CEA content of the lymph nodes was not measured in this case, since it was not routinely measured in this series of cases. Preliminary results with subsequent lymph node samples have confirmed our suspicion that soluble CEA glycoprotein is present in lymph nodes draining tumors that shed CEA.

Two conclusions can be drawn from the observation of intense uptake of $^{111}$In-labeled antibody by normal lymph nodes draining CEA-bearing colon adenocarcinoma. (a) The careful examination in humans of tumor and adjacent tissues is essential to an understanding of the mechanisms of labeled monoclonal antibody imaging and therapy. (b) Tumor images following injection of labeled monoclonal antibodies may be due to the high concentration of antibody in lymph nodes draining the tumor, a reflection of high antigen concentration in these sites. This represents an extension of the autoradiography observations of Moshakis et al. (40) and Lewis et al. (41) that labeled anti-CEA antibody interacts predominantly with CEA in the extracellular tumor space, rather than the cell membrane or the cytoplasm. Our immunohistological observation that apical and intraluminal CEA correlates best with tumor imaging by Indacea also supports this hypothesis. The strong correlation between tumor content of CEA and tumor imaging by Indacea suggests that total tumor content of CEA is predominantly a reflection of the quantity of CEA available in the extracellular spaces of the tumor. Quantitative measurement of tumor content of CEA, or other antigen, at the time of initial tumor imaging or resection may prove to be important for selection of appropriate monoclonal antibodies for subsequent imaging or therapy. Further studies correlating antibody uptake, antigen content, cellular location of antigen, and tumor imaging will be required for rational development of this technology and for validation of imaging claims.

REFERENCES


3. Reif, A. E., Curtis, L. E., Duffield, R., and Shaufler, I. A. Trial of radiolabeled antibody localization in metastases of a patient with a tumor containing

<table>
<thead>
<tr>
<th>Node location</th>
<th>Radioactivity (% ID/kg)</th>
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<tr>
<td>Left upper paratracheal (Level 2)</td>
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<tr>
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<td>1.99</td>
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<td>Right lower paratracheal (Level 4)</td>
<td>59.0</td>
</tr>
<tr>
<td>Subcarinal (Level 7)</td>
<td>1.25</td>
</tr>
</tbody>
</table>
we have identified the following key points from the document:

- The document discusses the use of monoclonal antibodies in colorectal carcinoma imaging.
- It highlights the importance of using radiolabeled monoclonal antibodies for detecting colorectal cancer.
- The authors mention the use of techniques such as radioimmunoassay, immunohistochemistry, and radiolabeled antibodies to improve detection accuracy.
- The document also covers the development and testing of new antibodies and their applications in colorectal cancer imaging.

These points are crucial for understanding the advancements in colorectal cancer imaging and the role of monoclonal antibodies in this field.
Preoperative Imaging of Colorectal Carcinoma with $^{111}$ In-labeled Anticarcinoembryonic Antigen Monoclonal Antibody

J. David Beatty, Rosemary B. Duda, Lawrence E. Williams, et al.


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