Presurgical Imaging with Indium-labeled Anti-Carcinoembryonic Antigen for Colon Cancer Staging

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Abstract

Over a 4-year period, 108 patients with known or suspected colorectal cancer were studied by radioimmunoconjugate scintigraphy prior to operative procedures. Study subjects received 0.2 to 40 mg i.v. of murine anti-carcinoembryonic antigen monoclonal antibody labeled with 2-5 mCi of \(^{111}\text{In} \) (Indacea). Resected tissues were analyzed for \(^{111}\text{In} \) and carcinoembryonic antigen content. Tumor, liver, and draining lymph nodes had over 10% injected dose/kg compared to <2.5% injected dose/kg for other normal tissues. Primary tumors that were successfully imaged were significantly larger and had higher \(^{111}\text{In} \) and carcinoembryonic antigen content. In 54 patients, primary tumors were visualized with a sensitivity of 78%. Hepatic metastases (58 patients) were visualized as negative filling defects (sensitivity, 45%). Extrahepatic (intra-abdominal) metastases (25 patients) were visualized (sensitivity, 48%) as areas of increased uptake. Extrahepatic metastases were uncommon (10 patients; sensitivity, 80%). Of 56 patients with known or suspected hepatic metastases who presented with no evidence of extrahepatic disease by conventional tests (X-ray, computerized tomographic scan), 20 (36%) were documented. The effectiveness of primary tumor and metastatic tumor imaging has been analyzed and a role for Indacea scintigraphy in the management of colorectal cancer patients has been identified.

Introduction

CEA\(^3\) is a well characterized tumor-associated antigen found in over 95% of colorectal adenocarcinomas (1). Antibodies to CEA have been produced and radiolabeled for targeting to tumor tissue. Early studies used polyclonal antibodies and \(^{111}\text{In} \) labeling (2-6), but more recently murine MABs and \(^{111}\text{In} \) have been utilized (7-12). We previously reported our experience using a high affinity \(^{111}\text{In} \)-labeled MAB (Indacea) directed against CEA (12, 13). Observations in 40 patients were validated by surgical exploration.

We have now expanded the study to 108 patients with colorectal cancer who have been imaged and gone on to a surgical procedure for diagnosis and/or treatment. In this report, the association of tumor imaging with various tissue parameters is documented. The effectiveness of primary tumor and metastatic tumor imaging has been analyzed and a role for Indacea scintiscanning in the management of colorectal cancer patients has been identified.

Materials and Methods

Indacea. As previously described by Paxton et al. (14), the N-hydroxy-yuccinimide active ester of DTPA was synthesized and conjugated to T84.66, an IgGl monoclonal antibody specific for CEA. \(^{111}\text{In} \) was incubated with the antibody conjugate at a ratio of 10 \(\mu\text{Ci}/\mu\text{g} \) to form Indacea. A second anti-CEA IgGl MAB was produced by Hybritech (San Diego, CA) and designated ZCE025. This MAB was purified from murine ascitic fluid by an 18% sodium sulfate fractionation, followed by DEAE-Sepharose ion exchange chromatography. The ZCE025 was conjugated and labeled with a proprietary DTPA bifunctional chelate technique at a ratio of 5 \(\mu\text{Ci}/\mu\text{g} \). In the case of each MAB, excess EDTA or DTPA was added to the reaction mixture (Indacea) prior to patient administration to complex free \(^{111}\text{In} \) and facilitate renal excretion of the unbound radioisotope. All MAB utilized was sterile, non-pyrogenic, and without evidence of murine viral contamination. Each antibody was prepared and administered under Food and Drug Administration approval (T84.66, BB-IND-2014; ZCE025, BB-IND-2041).

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" 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 for the City of Hope National Medical Center.

Scintiscanning. Radiolabeled T84.66 (200 \(\mu\text{g} \), 2 mCi) was administered by direct i.v. infusion. \(^{111}\text{In-ZCE025} \) (1.0 mg, 5 mCi) was mixed with 19 or 39 mg of unmodified MAB (ZCE025), and infused i.v. over 30 min in 100 ml of saline. Multiple anterior and posterior scintiscan images of the trunk were obtained at least twice (24, 48, 72, 120, or 168 h) using a Technicare Omega 500 camera and a medium-energy collimator. Scintillation data were stored in a Technicare 560 computer.

Surgery. Patients underwent surgery 5 to 20 days after the MAB injection. If suspicious areas were noted on scintiscans, additional noninvasive diagnostic testing and/or transcutaneous biopsy was attempted to confirm the presence or absence of disease, at the discretion of the patient’s physician. In cases in which the presence of extra-abdominal metastasis was suggested, additional appropriate operative procedures were similarly utilized to confirm suspected disease.

When surgical procedures were performed, a careful examination of all accessible regions identified any previously unsuspected disease. In those patients with suspicious uptake on CEA scan particularly careful examination and appropriate tissue sampling were utilized to confirm or reject the diagnosis of extrahepatic disease. Tissues removed in the region of "hot spots" were subjected to careful gross and microscopic pathological analysis as well as evaluation of radioactivity content and CEA content.

Tissue Analysis. Tumor and other tissue removed at operation were examined in standard fashion by the surgical pathologist as described previously (12). Aliquots in excess of that required for histopathological analysis were weighed and analyzed quantitatively by enzyme immunoassay for CEA content and by gamma counter for \(^{111}\text{In} \) content. The Roche enzyme immunoassay kit (a gift from Hoffman La Roche Inc., Nutley, NJ), used for measurement of CEA content, employs the T84.66 antibody. For each tissue specimen the \(^{111}\text{In} \) content was corrected for radiation decay and expressed as percentage of injected dose/kg of tissue. Mean values ± SE, median values, and range have been calculated. The observed data for the tissue analysis parameters were skewed toward large values and thus statistical comparisons were performed using the Wilcoxon rank-sum test.

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2To whom requests for reprints should be addressed, at Department of General Oncologic Surgery, City of Hope National Medical Center, 1500 E. Duarte Road, Duarte, CA 91010.
3The abbreviations used are: CEA, carcinoembryonic antigen; MAB, monoclonal antibody; Indacea, \(^{111}\text{In} \)-labeled anti-CEA MAB; TP, true positive; FP, false positive; TN, true negative; FN, false negative; CT, computer-assisted tomography; DTPA, diethylenetriaminepentaacetic acid.
Results

Between June 1984 and June 1988, 108 evaluable scintiscan studies were undertaken in colorectal cancer patients and subsequently followed by an operative procedure for diagnosis and/or treatment. Four patients had two Indacea studies; each study prior to a different operative procedure. Sixty-five scintiscans followed administration of 200 μg of 111In-labeled T84.66 and 43 followed 20 to 40 mg of 111In-labeled ZCE025. The 200 μg of T84.66 were labeled with 2 mCi of 111In and scans were performed at 24 and 48 h. The 20 and 40 mg of ZCE025 were labeled with 5 mCi of 111In. These Indacea scans could be performed at 48 to 72 h and 5 to 7 days because of the higher dose of radionuclide.

The individuals responsible for scintiscan interpretation (J. D. B., D. Y.) were provided with relevant clinical data such as location of known primary or metastatic tumor at the time of scan evaluation. The operating surgeon was notified immediately regarding areas of increased Indacea uptake that did not correspond to known tumor locations. The operative procedures were undertaken so that suspected tumor sites were examined and biopsied, provided this was in the best interest of patient care. Postoperatively, the extent of disease and scan status were compared in each of four categories: primary tumor; hepatic metastasis; extrahepatic abdominal metastasis; and extraabdominal metastasis. In each category, the scan was scored as either positive or negative and, on the basis of the surgical/pathological assessment, each scan study was recorded as one of: TP, FP, TN, or FN. In each category, the Indacea scintiscan sensitivity [TP/(TP+FN)], specificity [(TN/(TN+FP)], and predictive value [positive scan, TP/(TP+FP); negative scan, TN/(TN+FN)] were calculated.

Scintiscan Accuracy. We analyzed scintigraphy findings for primary tumors by individual tumors so that tissue analysis parameters could be compared for imaged (TP) and nonimaged (FN) lesions. We analyzed metastases for their presence or absence by region (hepatic, extrahepatic abdominal, extraabdominal) as treatment decisions were generally based upon presence or absence of disease in these areas, not the exact lesion number in an area.

Thirty-six (78%) of 55 primary adenocarcinomas of colon or rectum in 54 patients were visualized as positive images by Indacea scintigraphy (Table 1). Positive Indacea scintiscans had an excellent predictive value (100%) for the presence of primary tumors (Fig. 1) but were not able to distinguish between luminal tumor (primary) and extrahepatic abdominal metastasis.

Fifty-eight patients had one or more hepatic metastases from colorectal adenocarcinoma of which only 26 (45%) were visualized by Indacea scintigraphy. The extensive uptake by histologically normal liver (Fig. 1) resulted in large or extensive metastases appearing as photopenic areas (Fig. 2). On several occasions, a hepatic metastasis had increased uptake relative to the normal liver. Differentiation of hepatic metastasis from extrahepatic metastasis or histologically normal retroperitoneal lymph nodes adjacent to the liver was often not possible using planar Indacea scintigraphy (Fig. 3).

Table 1 Accuracy of INDACEA scintigraphy of colorectal cancer operative correlation

<table>
<thead>
<tr>
<th>Tumor location</th>
<th>True positive</th>
<th>False positive</th>
<th>True negative</th>
<th>False negative</th>
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</thead>
<tbody>
<tr>
<td>Primary tumor</td>
<td>43</td>
<td>0</td>
<td>54</td>
<td>12</td>
</tr>
<tr>
<td>Hepatic metastasis</td>
<td>26</td>
<td>1</td>
<td>49</td>
<td>32</td>
</tr>
<tr>
<td>Extrahepatic metastasis</td>
<td>12</td>
<td>7</td>
<td>76</td>
<td>13</td>
</tr>
<tr>
<td>Extraabdominal metastasis</td>
<td>8</td>
<td>2</td>
<td>96</td>
<td>2</td>
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Fig. 1. Primary cancer of rectum. Scintiscan of posterior abdomen and pelvis in 61-year-old male 7 days following administration of Indacea (ZCE025, 20 mg; 111In, 4.2 mCi). The Indacea scan showed uptake in normal liver (L), spleen (S), and a faint outline of vertebrae and pelvic bones. The primary rectal tumor (T) was clearly visualized on this posterior pelvic scintiscan but was not seen on the anterior pelvic scintiscan (not shown). This localized the tumor in the presacral region. At laparotomy, in addition to the rectal tumor, five 1-cm liver metastases not visualized on the Indacea scan were found.
logically normal mesenteric lymph nodes draining a primary tumor was low (5 to 15 μg/g). The lowest mean CEA content was found in fat.

The effectiveness of imaging primary tumors was correlated with tissue analysis parameters (Table 3). The tumors that were visualized had significantly more $^{111}$In (P < 0.005), had significantly higher CEA content (P = 0.05), and were significantly larger (P < 0.05) (all using the Wilcoxon rank-sum test).

**Clinical Relevance.** Of the 108 evaluable preoperative Indacea scans, 56 were from patients that presented with known or suspected hepatic metastasis and no evidence of unresectable extrahepatic metastatic disease by conventional investigation (X-rays, CT, magnetic resonance imaging, nuclear medicine) or from patients suspected of having recurrent colorectal cancer because of an elevated plasma CEA, but without localization of recurrence site by conventional investigation. Twenty (36%) of these 56 patients were documented to have extrahepatic metas-
ease correctly identified at both sites. In 6 of the 56 patients correctly identified was extraabdominal. One patient had dis
tastases by the subsequent surgical procedure. In 10 (50%) of these 20 patients the extrahepatic disease was visualized on the Indacea scan and correctly interpreted. In 8 patients the disease correctly identified was extraabdominal. One patient had disease correctly identified at both sites. In 6 of the 56 patients (11%), the Indacea scan proved to have a false positive inter
tation of more sites of extrahepatic metastasis significantly alter the management or treatment decisions.

Although Indacea immunoscintigraphy is sensitive (78%) for primary colorectal cancer (Fig. 1), barium enema and colonoscopy are more reliable for the diagnosis and localization of primary lesions. In the 54 patients with colorectal primaries the Indacea scan findings did not result in cancellation of the standard surgical exploration. Although a role for Indacea scanning has not been documented in primary disease patients, continued preoperative study of these patients is important at least for objective optimization of radioimmunoscintigraphy and clarification of mechanisms.

The identification of hepatic metastases by Indacea scintig
graphy is comparable to conventional liver scintigraphy using labeled colloid or albumin. The imaging is nonspecific and lesions are usually seen as filling defects. A clinical role for Indacea scanning of hepatic metastases must await methods to decrease the nonspecific uptake of Indacea by normal liver.

The low sensitivity (48%) of Indacea scintigraphy for extra
apillary or extraabdominal metastases, the relatively high false positive rate (7 of 83, 8%), and the low predictive value of a positive scan (63%) are disappointing and indicate the need for caution in interpretation of Indacea scans for extrahepatic abdominal disease.

The identification of extraabdominal metastases by Indacea scanning was more reliable than identification of abdominal (hepatic or extrahepatic) metastases (Table 1). Although the numbers were small, the Indacea scan had its most important impact in this area, often leading to further investigations and major changes in management decisions (Fig. 2).

Analysis of resected tissues has provided some reasons for these imaging successes and shortcomings. Primary tumors tend to be small (relative to hepatic metastases) but have a high content of CEA and good uptake of $^{111}$In (Table 2) accounting for their effective imaging with Indacea. Hepatic metastases also have high content of CEA and reasonable uptake of Indacea but are not well visualized because of the high normal liver uptake of Indacea. Normal liver does contain measurable CEA that may account for some of this uptake, but preliminary results in the nude mouse model suggest that the formation of CEA:Indacea complexes in the blood is responsible for much of this normal liver visualization. The CEA content of extrahepatic metastases tends to be low and as a result Indacea uptake and imaging of extrahepatic metastases are also low. Examination of primary tumors indicates that tumor imaging is associated with high uptake of Indacea, large tumor size, and high CEA content (Table 3).

Perhaps the most interesting tissue analysis findings relate to the mesenteric lymph nodes that drain primary tumors. It was originally hypothesized that antibody imaging could be used to identify nodal metastases preoperatively. Unfortunately, although histologically positive nodes contain substantial CEA and have good uptake of Indacea, these nodes are usually too small to be visualized. On the other hand, histologically negative lymph nodes contain much less CEA but have as much or more Indacea. We have seen that these nodes can lead to false positive scan interpretation (Fig. 3) and we hypothesize that they represent concentration of Indacea by the lymphatics that drain a tumor where the Indacea has accumulated.

The identification of peritoneal, retroperitoneal, and pelvic metastases by CT scan in high risk patients has been disappoint-
ing (15, 16). In 17 of 56 patients (30%) with known or suspected hepatic metastases and no evidence of other abdominal/pelvic disease by conventional studies, recurrent extrahepatic intraabdominal cancer was documented at celiotomy. Operative in-

<table>
<thead>
<tr>
<th>Table 3 Correlation of primary tumor imaging with tissue analysis parameters</th>
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<tbody>
<tr>
<td>Tumor imaged (true positive)</td>
</tr>
<tr>
<td>$^{111}$In content (% ID/kg)</td>
</tr>
<tr>
<td>Median</td>
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<tr>
<td>Range</td>
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<tr>
<td>No.</td>
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<tr>
<td>CEA content (µg/g)</td>
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<tr>
<td>Median</td>
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<tr>
<td>Range</td>
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<tr>
<td>No.</td>
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<tr>
<td>Tumor size (cm$^3$)</td>
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<td>Median</td>
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<td>Range</td>
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<td>No.</td>
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* % ID/kg, percentage of injected dose of $^{111}$In/kg of tissue.

Discussion

Radiolabeled antibody imaging of tumors has been tested in humans for over a decade with progressive improvement of sensitivity for identification of overt and occult lesions. However, a clinical role for radioimmunoscintigraphy has remained undefined. The purpose of this study has been to examine a large series of Indacea scintigraphy studies in a presurgical colorectal cancer population, to identify important tissue characteristics associated with tumor imaging, and to explore a role for Indacea scintigraphy in patient management.

Clinical, and especially surgical, decisions in colorectal cancer depend upon the presence and extent of disease in four basic anatomic areas. Primary tumors are usually treated by bowel resection for attempted cure and to prevent subsequent obstruction, bleeding, or fistulization. Liver metastases may be resected and/or treated by continuous hepatic artery infusional chemotherapy, provided the primary disease is controlled and there are no extrahepatic metastases. Extrahepatic metastases, either intraabdominal or extraabdominal, are usually palliated by radiotherapy and/or chemotherapy. Occasionally operative procedures are indicated for local control and/or palliation of extrahepatic metastatic colorectal cancer. Rarely does identification of more sites of extrahepatic metastasis significantly increase the management or treatment decisions.

Although Indacea immunoscintigraphy is sensitive (78%) for primary colorectal cancer (Fig. 1), barium enema and colonoscopy are more reliable for the diagnosis and localization of primary lesions. In the 54 patients with colorectal primaries the Indacea scan findings did not result in cancellation of the standard surgical exploration. Although a role for Indacea scanning has not been documented in primary disease patients, continued preoperative study of these patients is important at least for objective optimization of radioimmunoscintigraphy and clarification of mechanisms.

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vention would not have been undertaken in many of these patients had the extrahepatic disease been evident preoperatively. In this highly selected but high risk population of patients, Indacea scintigraphy identified 8 of 17 patients (47%) with extrahepatic abdominal disease. In a further two patients extraabdominal metastasis was identified by Indacea imaging and documented histopathologically. Thus, the preoperative use of the Indacea scan for staging of colorectal cancer patients with known or suspected hepatic metastases, and no known extrahepatic disease, identified a significant (16 of 56, 29%) subgroup that were at very high risk (10 of 16, 63%) for the presence of extrahepatic disease. The management of each of these ten patients was, or could have been, modified by the scan findings. In some patients and in several others ineligible for this series (Fig. 2) unnecessary surgery was eliminated as a result of Indacea scan findings. In short, a clinically relevant role for Indacea immunoscintigraphy in the preoperative work-up of colorectal cancer patients with known or suspected hepatic metastases has been documented. This role complements conventional imaging techniques such as CT and magnetic resonance imaging for identification of extrahepatic and extraabdominal metastases.

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References

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