Semiquantitative SPECT Tumor Uptake of Technetium-99m-Labeled Anti-CEA Monoclonal Antibody in Colorectal Tumor

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Technetium-99m-BW431/26 images were compared to images of resected tumor specimens to evaluate the efficacy and accuracy of SPECT imaging of colorectal carcinoma. **Methods:** Immunoscintigraphy with ^{99m}Tc-BW431/26 was performed on seven patients with colorectal carcinoma and one patient with a benign colorectal tumor. Accumulation of ^{99m}Tc-BW431/26 in the tumor was expressed as the tumor-to-normal tissue count ratio (SPECT T/N ratio) calculated by a region of interest method on the SPECT images obtained 24 hr after administration of ^{99m}Tc-BW431/26. All patients underwent resection of the tumor immediately after 24-hr imaging, and the radioactivity in tumor specimen and normal tissue was measured to calculate the tissue T/N ratio. **Results:** SPECT demonstrated definite increased tracer uptake by the tumor in all colorectal cancer patients. The benign lesion showed tracer uptake to a lesser extent. SPECT, however, failed to visualize a 10-mm lesion in a patient with familial adenomatous polyposis despite a tissue T/N ratio of 4.8, while autoradiography showed radioactivity uptake in the polyps. **Conclusion:** Although SPECT has limitations in detecting small lesions because of its limited spatial resolution, T/N ratios could be measured exactly by SPECT if the lesion is of a certain volume. SPECT imaging with ^{99m}Tc-BW431/26 can precisely evaluate tracer uptake in tumors and predict the efficacy of radioimmunotherapy in patients with colorectal cancer.

Key Words: technetium-99m; monoclonal antibody BW431/26; colorectal carcinoma; semiquantitative analysis

Monoclonal antibodies (MAbs) are expected to carry radionuclides tagged to MAbs to desired sites. Radiolabeled anti-tumor MAbs have been successfully used in therapeutic regimens of some cancers, especially malignant lymphoma (1). Tumor-to-normal organ ratios (T/N

ratios) of radionuclides seemed to be the most important factors for radioimmunotherapy and radioimmunodetection of cancers. Low radiation doses to lungs and hematopoietic system were demanded before patients could be treated. Patient without sufficient distribution of radiation should be received other therapy.

Immunoscintigraphy with radiolabeled antibodies against the carcinoembryonic antigen (CEA) to detect colorectal carcinoma has been performed for more than a decade (2-5). BW431/26, described by Bosslet et al. (6), is one of many MAbs against various epitopes of CEA. Moreover, the clinical usefulness of immunoscintigraphy using ^{99m}Tc-BW431/26 has been reported (7-11).

This study examines the clinical usefulness and limitations of radioimmunodetection using ^{99m}Tc-BW431/26 with SPECT in patients with colorectal carcinoma and evaluates the accuracy of tumor-to-normal ratios.

MATERIALS AND METHODS

Patients

Eight patients, five males (age range 38 to 80 yr) and three females (age range 30 to 72 yr), with untreated primary colorectal tumors were studied (Table 1). Each patient was diagnosed on the basis of barium enema, endoscopy and histological findings of biopsy specimens. One patient (Patient 8) was diagnosed as having a benign tumor composed of chronic inflammatory tissues without malignant cells. Serum CEA values were determined in all patients using a commercially available CEA kit (12). The upper limit of normal CEA value was 2.5 ng/ml. The study protocol was approved by the hospital's ethics committee. All patients were informed of the aim and methodology of the study and gave informed consent.

Immunoscintigraphy

BW431/26 was labeled with ^{99m}Tc by a direct labeling method after reduction (8). After labeling, 1 mg of intact IgG1 with 1110 MBq of ^{99m}Tc was mixed with 100 ml physiological saline; this solution was injected intravenously for 10 min. Temperature, blood pressure, heart rate, respiratory rate and subjective symptoms were monitored during and after the infusion. Blood sampling was performed 10, 30, 60 min and 6, 24 and 48 hr postinjection to determine the blood clearance of ^{99m}Tc-BW431/26.

Scintigraphic imaging was performed using a gamma camera

TABLE 1
Scintigraphic Results

Patient no.	Age/Sex	Tumor Location/Histology	Size (mm)	Serum CEA† (ng/ml)	Immunoscintigraphy* 6 hr (planar)	24 hr (planar)	24 hr (SPECT)	Tumor-to-normal tissue ratio SPECT	Resected specimen
1	54/F	Caecum/Mod	35 × 35	1.9	+	++	++	12.8	14.7
2	38/M	Transverse/Mod‡	40 × 55	<1.1	+	++	++§	8.2	10.5
3	80/M	Transverse/Mod	40 × 35	1.1	−	+	++	8.1	7.4
4	72/F	Ascending/Mod	90 × 80	1.4	++	++	++	7.4	4.1
5	57/M	Sigmoid/Mod	35 × 35	1.4	+	++	++	5.0	6.3
6	73/M	Rectum/High	56 × 42	2.4	−	−	++	4.7	3.9
7	30/F	Sigmoid/Poor	70 × 40	34.9	−	+	++	2.7	2.0
8	57/M	Caecum/Benign	70 × 90	2.6	−	+	+	2.4	2.0

*++ = markedly positive; + = positive; − = no visualization.
†Normal range of serum CEA; less than 2.5 ng/ml.
‡Familial adenomatous polyposis.
§Lesions smaller than 10 mm could not be detected.
Mod = moderately differentiated adenocarcinoma; high = highly differentiated adenocarcinoma; poor = poorly differentiated adenocarcinoma; benign = nonspecific chronic inflammatory tumor.

fitted with a low-energy, all-purpose collimator. An online data processing computer was used for image data processing. Anterior and posterior planar images of the chest, abdomen and pelvis were obtained at 6 and 24 hr postinjection followed by SPECT imaging of the abdominal region. All patients received an enema before 24-hr SPECT imaging. For planar imaging, images were acquired for 100 sec and 900 sec in a 256 × 256 matrix at 6 and 24 hr, respectively. The SPECT images were acquired in a 64 × 64 matrix over 360°, 64 steps, 30 sec/step. Butterworth and Shepp-Logan filters were used for image reconstruction. The slice thickness was 6.5 mm.

SPECT Tumor-to-Normal Ratios

Semiquantitative analysis of MAb uptake in the tumor was made by visually placing a 4 × 4 pixel region of interest (ROI) on the tumor site of the most representative SPECT image. The ROI was also placed in the contralateral normal area on the transverse sections. The SPECT T/N ratio was calculated as the average counts/pixel in the tumor over the average counts/pixel in the normal region.

Autoradiographic Tumor-to-Normal Tissue Ratios

After 24-hr imaging, all patients underwent surgical removal of the tumor. The resected specimen was then placed on the gamma camera which was fitted with a low-energy, high-resolution collimator, and an autoradiogram was obtained. Approximately 1 g of the tumor specimen and 1 g of normal tissue were removed from the resected tissue and the radioactivity was counted in a well-type gamma counter. The tissue T/N ratio was calculated as the radioactivity (cpm)/g of the tumor sample over the radioactivity (cpm)/g of the normal tissue.

RESULTS

Seven of eight tumors were confirmed as adenocarcinoma (Table 1). Tumor sizes ranged from 35 × 35 mm to 90 × 80 mm in diameter. Tracer clearance through the blood occurred within 48 hr after tracer injection and was fitted for an exponential function with a mean half-life of 27.4 ±

8.4 hr (mean ± s.d., n = 8). For the seven patients with colorectal cancer, planar images obtained 6 hr after administration demonstrated definite positive uptake in one, slightly positive uptake in three and negative uptake in three. At 24 hr, four patients showed definite positive uptake, two showed slightly positive uptake and one showed negative result. The 24-hr SPECT images demonstrated definite positive uptake in all colorectal cancer patients. The smallest tumor detected on SPECT images was 35 mm in diameter (Patients 1 and 5).

There was definite positive uptake on the autoradiographs of resected tumor specimens. The SPECT T/N ratios in seven patients with colorectal cancer (range 2.7 to 12.8) were almost equal to the tissue T/N ratios (range 2.0 to 14.4) (Table 1). High correlation was observed between SPECT T/N ratios and tissue T/N ratios (r = 0.92, p < 0.001, n = 8) as shown in Figure 1. A small 10-mm tumor in Patient 2, who had no histological evidence of carcinoma, was clearly visible by autoradiography, whereas it was undetectable even on SPECT images obtained before surgery (Fig. 2). One patient with a nonspecific inflammatory tumor (Patient 8), however, showed slightly increased tracer uptake by the lesion, with SPECT and autoradiographic T/N ratios of 2.4 and 2.0, respectively.

Illustrative Cases

Patient 2. This 38-yr-old male was referred to our hospital because of left abdominal pain and diarrhea. Barium enema and colonoscopy revealed a large tumor of 35 mm in diameter and a small tumor of 10 mm at the transverse colon associated with various sized polyposis smaller than 10 mm throughout the colon. A diagnosis of familial adenomatous polyposis (FAP) was made based on family history. Immunoscintigraphs obtained 24 hr after tracer
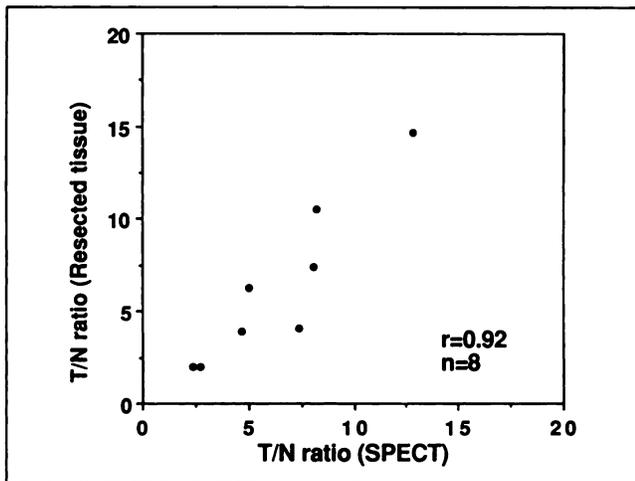
FIGURE 1. Correlation of the tumor-to-normal tissue uptake ratio determined by SPECT images and surgically resected specimens. Significant correlation was observed (r = 0.92, p < 0.001, n = 8).

administration demonstrated a markedly increased radio-activity at the region anteriorly to the left kidney (Figs. 2A, B). Autoradiography of the resected specimen showed an area of remarkably increased radioactivity at the main tumor (35 × 35 mm) and the small tumor (10 × 10 mm). In addition, many small spotty areas of radioactivity were seen mainly at the ascending and transverse colon, corre-

sponding to polyposis which showed no histology of malignancy (Fig. 2C). SPECT and autoradiographic T/N ratios of the larger tumor were 8.2 and 10.5, respectively. The smaller tumor showed a tissue T/N ratio of 4.8; SPECT could not visualize the tumor (Figs. 2B, C).

Patient 3. This 80-yr-old male had experienced mucous stool and melena for 1 mo. A barium enema indicated stenosis at the transverse colon and tumor biopsy revealed adenocarcinoma. Immunoscintigraphy with ^{99m}Tc-BW431/26 showed intense radioactivity at the site of the right kidney (Fig. 3A). Transaxial SPECT images clearly showed two areas of radioactivity at the area anteriorly to the right kidney (Fig. 3B). Autoradiography of the resected specimen showed two areas of radioactivity corresponding to the SPECT images (Fig. 3C). SPECT and tissue T/N ratios were 8.1 and 7.4, respectively.

Patient 8. This 57-yr-old male was hospitalized for cecal tumor examination. Barium enema (Fig. 4A), computed tomography and angiography revealed a mass lesion at the cecum, but it was hard to differentiate it from malignant tumor. Serum CEA value was 2.6 ng/ml (upper limit of normal values are less than 2.5 ng/ml). Planar and SPECT images showed only slightly increased tracer uptake in comparison to other portions of the colon. The SPECT T/N ratio was 2.4 (Figs. 4B, C). Pathology confirmed a chronic inflammatory lesion without malignancy.
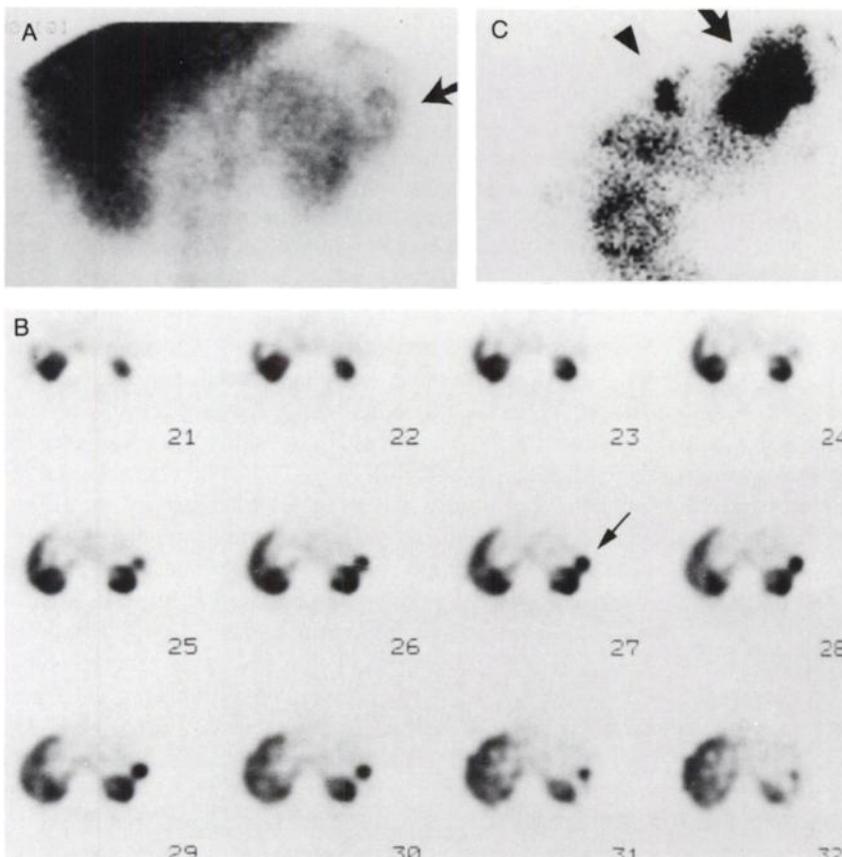
FIGURE 2. A 38-yr-old male with familial adenomatous polyposis (Patient 2). (A) Planar (A) and SPECT (B) images demonstrate intense radioactivity at the region anteriorly to the upper pole of left kidney (arrow). Tumor-to-normal colon ratio is 8.2. (C) Autoradiogram of resected colon specimen demonstrates a large area of radioactivity corresponding to scintigraphic visualization of the main tumor (arrow). Tumor-to-normal colon tissue ratio is 10.5. A smaller area of radioactivity is also seen in transverse colon (arrow head) which could not be visualized on planar or SPECT images. Tumor-to-normal colon ratio is 4.8. Multiple spotty radioactivity was observed along ascending and transverse colon, suggesting more increased CEA expression by the polyps without malignancy.
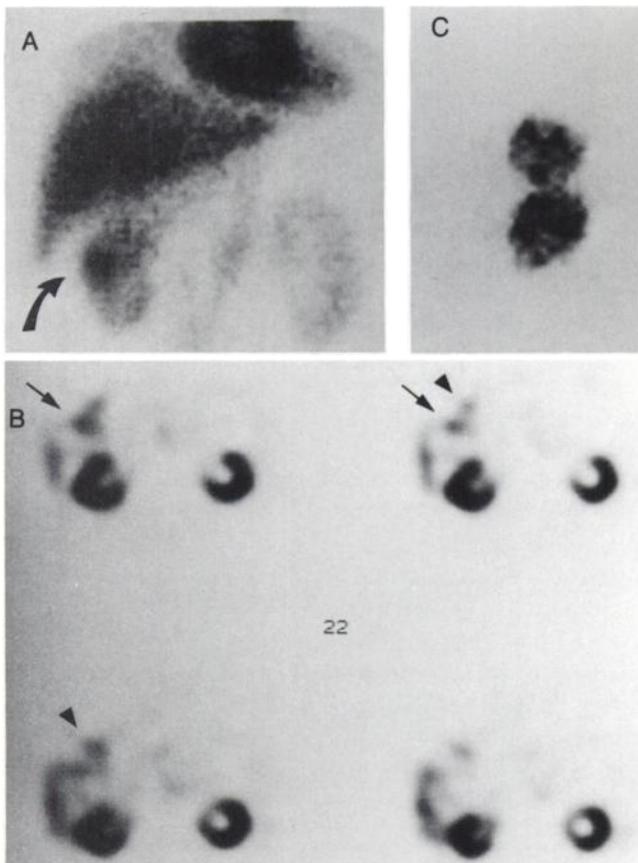
FIGURE 3. An 80-yr-old male patient with transverse colon cancer (Patient 3). (A) Planar image obtained 24 hr after administration of 99mTc-BW431/26 demonstrates intense radioactivity in the region of the right kidney (arrow). (B) Transaxial SPECT images demonstrate two areas of radioactivity (arrow and arrow head) at the right prerenal area. Tumor-to-normal colon ratio is 8.1. (C) Autoradiogram of the resected colon specimen demonstrates two distinct tumor. Tumor-to-normal colon tissue ratio is 7.4.

DISCUSSION

Semiquantitative analysis showed that the SPECT T/N ratios were almost identical to the resected tissue T/N ratios. High correlation was noted between SPECT T/N ratios and tissue T/N ratios ($r = 0.92$, $n = 8$, $p < 0.001$). The count rate of the 24-hr SPECT images (106 to 516 counts/pixel at the tumor and from 21 to 76 counts/pixel at the normal region) was sufficient for quantitative evaluation. The highest counts/pixel of 76 at the normal background was obtained in a patient with FAP. Accurate quantification of BW431/26 uptake by the tumor and normal organs is important for successful radioimmunotherapy and evaluation of viable neoplastic tissue (1).

Baum et al. reported the first clinical results using 99mTc-BW431/26 (8), noting the efficacy and convenience of the 99mTc label as well as the high diagnostic accuracy in patients with CEA-producing recurrent tumors. The clinical usefulness of 99mTc-BW431/26 in the early detection of recurrent colorectal carcinoma has also been reported (9,10). Muxi et al. compared autoradiographic and planar

imaging results (11). Muxi et al., however, did not perform quantitative analysis on the SPECT images.

Of radionuclides used to label MAb, 131I and 111In have been most widely employed (1–5). Early studies on the clearance of iodinated IgG1 from the circulation demonstrated that the half-life ranged from 15.4 to approximately 100 hr (13–17). Therefore, 99mTc is regarded to have too short a half-life for labeling intact IgG, although 99mTc is a most desirable radioisotope because of its availability, low cost and photon energy. Our results show that with a mean blood clearance of 27.4 hr, high quality SPECT images may be obtained even at 24 hr after infusion, although nonspecific uptake by the liver and kidneys as well as blood pool are demonstrated. Antibody fragments labeled with 99mTc may lead to higher T/N ratios by reducing background radioactivity. Immunogenicity of whole antibody derived from mouse has also disadvantage for clinical use.

Twenty-four hour SPECT imaging demonstrated all seven primary colorectal tumors as definitely positive and is superior to planar imaging because of previously reported high tumor-to-background ratios (18). For the patient with the lesion that was indistinguishable from nonspecific renal uptake of 99mTc-BW431/26 on planar images, SPECT easily demonstrated the lesion as two isolated tumors anteriorly to the kidney (Fig. 3B). Because of limitations in spatial resolution, SPECT is not able to identify small lesions (<10 mm), despite a tissue T/N ratio of 4.8. These results indicate that visualization of early-stage colorectal cancer with immunoscintigraphy is still difficult.

Although one patient (Patient 8) with inflammatory colon tumor showed equivocal lesion uptake of 99mTc-BW431/26, the degree of uptake was low and quantitative analysis revealed a T/N ratio lower than that of the other seven patients (Fig. 4).

False-positive results may occur since CEA is expressed on normal tissues as well as on the cell membranes of colorectal cancer cells. Nonspecific distribution of 99mTc-BW431/26 in the ascending colon has been reported (10). In this study, all the patients received an enema before undergoing 24-hr imaging, which might result in correct quantitative evaluation of 99mTc-BW431/26 tumor uptake on SPECT images. Our semiquantitative analysis suggests that equivocal uptake of antitumor MAb by a lesion with a ratio twice as high as normal tissue should be considered to be nonspecific (Patient 8). A low T/N ratio was obtained in patients with poorly differentiated adenocarcinoma of the sigmoid colon (Patient 7). The resected specimen showed that severely necrotic tissue was contaminated within the neoplastic tissue. Poorly differentiated colorectal adenocarcinoma usually contains much less demonstrable surface CEA (19). Rhenium-186 has favorable properties for cancer therapy and has a similar chemical character to that of 99mTc (20). Although directly labeled 186Re-MAb is reported to be unstable either in serum and saline because of reoxidation, a new labeling procedure to increase its stability has been reported (21,22). The clinical usefulness of 186Re-MAb remains to be investigated.

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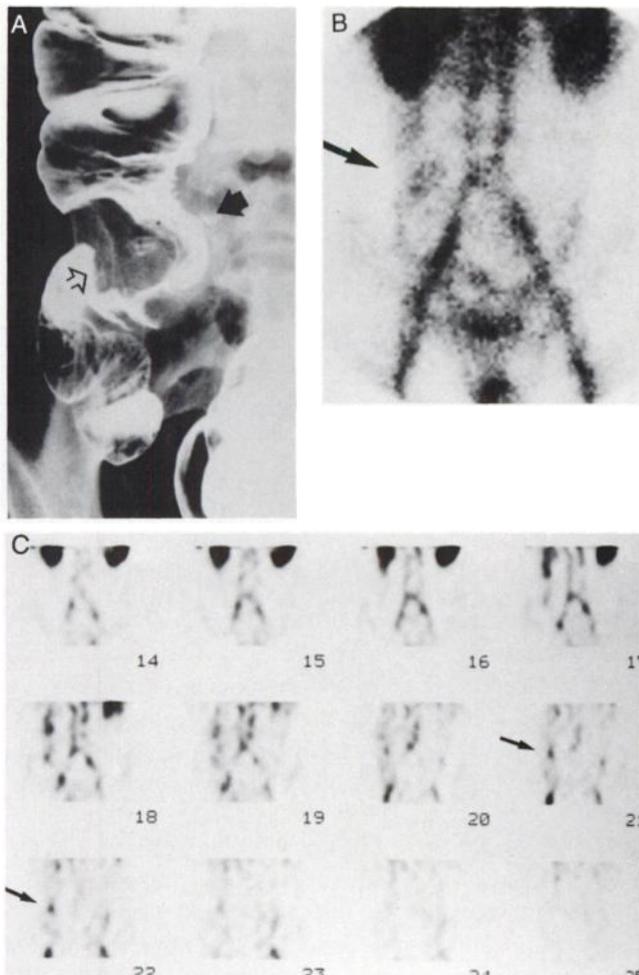
FIGURE 4. A 57-yr-old male with nonspecific benign tumor of the cecum (Patient 8). (A) Barium enema demonstrates a large mass lesion at the cecum (arrow). (B) Planar image of anterior view demonstrates slightly increased radioactivity in the ileocecal region (arrow). (C) SPECT image demonstrates slightly increased radioactivity corresponding to the cecal lesion (arrow).

This study demonstrated increased uptake of ^{99m}Tc-BW431/26 by adenomatous polyps on autoradiography prior to histological manifestations of malignancy (Fig. 2C). As O'Brien et al. noted, CEA expression in colon polyps may correlate with parameters of malignant potential (19) and CEA expression may be accelerated before histological differentiation of the carcinoma. Therefore, SPECT using ^{99m}Tc-labeled anti-CEA MAb may be useful in detecting lesions with increased CEA expression when there is no histological differentiation of carcinoma, despite spatial resolution constraints for detecting small nodules.

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