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^{99m}Tc radioimmunoscintigraphy of colorectal cancer

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Summary The monoclonal antibody PR1A3 against a normal colonic columnar cell surface antigen has been labelled with ^{99m}Tc and used for imaging colorectal cancer. High uptake in undifferentiated cancer is seen. The tumour to mucosa ratio was up to 63:1 and the percentage of the injected activity in the tumour up to $1.7 \times 10^{-2}\%\ g^{-1}$. As ^{99m}Tc is continuously available in a Nuclear Medicine Department, on receipt of a request the study may be completed within 24 h enabling radioimmunoscintigraphy to be used routinely in the management of patients with colorectal cancer.

Cancer detection and management using radioimmunoscintigraphy requires an avid monoclonal antibody against a selective cancer-associated antigen, labelled in a stable way so as to preserve its immunoreactivity, with a radionuclide able to give the best possible signal, which is optimal for the gamma camera imaging system. Three types of antigen have been used to produce monoclonal antibodies for the radioimmunoscintigraphy of colorectal cancer. These are the oncofetal antigens such as carcinoembryonic antigen (CEA), cancer-related antigens where malignant tissue has been used as the immunogen such as TAG72 giving B72.3, and normal tissue antigens such as that giving the antibody PR1A3.

Antibody PR1A3 was developed against a surface antigen present in the cell membrane of the apical cytoplasmic region and microvillous brush border of the surface and upper crypt columnar absorptive cells of normal colon during a search for genetic markers for colorectal cancer (Richman & Bodmer, 1987). PR1A3 does not react with mucus cells or cytoplasmic constituents. There is a slight reaction with some cells of the stomach, ileum, oesophagus, trachea and breast, but not with any other tissue. It binds strongly to both well, moderately well and poorly differentiated colorectal carcinoma and to all stages of colorectal carcinoma. It has advantages over anti-CEA monoclonal antibody since its antigen is fixed and does not appear in lymphatics or normal lymph nodes draining a tumour as does CEA (Granowska *et al.*, 1989*a*). The colonic basement membrane prevents access of PR1A3 to normal mucosa in contrast to CEA which is released. Richman & Bodmer (1987) reported that 59/60 colorectal tumours reacted with PR1A3 whereas only 75% of colorectal tumours react with B72.3 (Salvatore *et al.*, 1989).

The signal from the radiolabel is the basis of the detection of the sites of specific uptake of the monoclonal antibody. Although ^{131}I was used initially because of its convenience (Mach *et al.*, 1980), the low count rate obtained meant that several days had to elapse for tissue background activity to fall to enable the poor signal to be detected. When the same antibody is labelled with ^{123}I to the same activity which gives 20 times the count rate of ^{131}I, the same tumour was detectable within 4 h (Britton *et al.*, 1989). Using the bifunctional chelate method of Hnatowich *et al.* (1983) to label antibody, ^{111}In has been successfully used for imaging colorectal cancer, but imaging may still take 72 h to complete. In-111 has two gamma ray energies; the higher requires that the gamma camera is used with a heavier collimator which reduces sensitivity and resolution. It has to be ordered regularly, is expensive and gives a high radiation absorbed dose to the patient. When ^{111}In-labelled antibodies are metabolized in the reticuloendothelial system, the ^{111}In is deposited in the tissue giving a high liver and bone marrow background and there is often a high large bowel activity.

The radiolabel that is continuously available in any Nuclear Medicine Department is ^{99m}Tc. It is cheap, gives a low radiation absorbed dose and is ideally suited to the modern gamma camera with a low energy collimator. The problem of labelling a gamma globulin with ^{99m}Tc has been solved by the Schwarz technique (Schwarz and Steinstraesser, 1987). The S-S bonds linking the heavy chains near the hinge region of the antibody are opened using 2-mercaptoethanol in a molar ratio of 1000:1. The antibody retains its immunoreactivity and may be frozen or stored. When required the antibody is thawed and a bone scanning methylene diphosphonate kit is added to provide the tin reducing agent and 700 MBq ^{99m}Tc generator eluate is added (Table I). The ^{99m}Tc monoclonal antibodies labelled in this way are stable *in vivo* for 24 h. There is no detectable uptake in the thyroid when imaging patients who have received no thyroid blocking agent either 30 min or 24 h after the intravenous injection of the ^{99m}Tc-labelled antibody.

This indicates that there is no release of free ^{99m}Tc as pertechnetate. The high renal uptake is likely to be due to breakdown with time of the antibody to a ^{99m}Tc peptide that is filterable. This would be followed by its reabsorption in the proximal tubules and metabolism with the deposition of the ^{99m}Tc there. This is thought to be the mechanism for deposition of radiometal-labelled Fab fragments in the kidney, and would help to account for the relatively lowered liver and reticuloendothelial uptake of the ^{99m}Tc-labelled antibody.

Table I Standard protocol for labelling antibody with technetium-99m

1. By ultrafiltration, concentrate antibody to approximately 10 mg ml^{-1}
2. To stirred solution of antibody add sufficient 2-mercaptoethanol (2-ME) to provide a molar ratio of 1000:1 2-ME: antibody
3. Incubate at room temperature for 30 min with continuous rotation
4. Purify reduced antibody by gel filtration of Sephadex-G50 using phosphate buffered saline as mobile phase
5. Collate antibody fractions and divide into 0.5 mg aliquots. Freeze immediately at −20°C

Label antibody as follows:

6. Thaw frozen antibody aliquot
7. Reconstitute Amerscan MDP kit with 5 ml of 0.9% saline injection
8. Add 50 µl MDP solution to antibody aliquot and mix well
9. Add required amount of ^{99m}Tc pertechnetate (700 MBq approx.) to antibody/MDP mixture. Wait 10 min
10. Assess labelling efficiency by chromatography using ITLC developed in 0.9% saline (should be >95%)
11. If necessary, the labelled antibody can be further purified by gel filtration on Sephadex G-50 prior to injection

N.B. Labelled antibody is stable for some hours after preparation.

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Stability of the labelled antibody *in vitro* with a 100-fold molar excess of diethylene triamine penta acetate (DTPA) as a competitive chelating agent showed a loss of ^{99m}Tc bound to the antibody of only 8% in 24 h, with no loss at 5 h.

Patients and methods

Patients were selected by the surgeons of St Mark's Hospital. Three types of patient were studied: those thought to have a primary colorectal cancer; those thought to have a recurrence of cancer on follow-up; and those who were symptom-free 1 year after resection of a Dukes' C colorectal cancer, at which time there is a 50% likelihood of recurrence. The study was accepted by the City & Hackney District Ethical Committee and informed signed consent was obtained from each patient. A history of allergy to foreign protein or other atopy was sought from each patient but was obtained in none. No skin test was made with the antibody to avoid sensitization. The monoclonal antibody PR1A3 provided by the ICRF was labelled with ^{99m}Tc using the technique described above. The labelling efficiency is over 95% and the immunoreactivity unaffected. The patient was placed supine on the imaging couch with the gamma camera set over the pelvis. The gamma camera (Siemens ZLC75 Tube Digitrac System) was set for 140 kev with a 20% window and a low energy general purpose collimator was used. Data were transferred directly on-line to a V77 Nodecrest computer for subsequent analysis.

The patients received the injection intravenously over 30 s. No untoward reactions occurred. Imaging was performed at 5 min, 2–3 h, 5–6 h and at 22–24 h. Anterior and posterior views of the lower chest and upper abdomen, and lower abdomen and pelvis were obtained together with images of six radioactive marker sources set on the bone landmarks to check repositioning of the patient and the image at each time point. Single photon emission tomography (SPET) was performed at 5 h and sometimes at 22 h.

Gamma camera images were also made of the excised surgical specimen where possible. The histological staging and grading of the tumour was undertaken. Specimens of the tumour, nearby mucosa and lymph nodes known to be involved or not involved with tumour were selected and counted, together with standards and appropriate background samples. Serial blood and urine samples were also obtained and assayed in some cases.

Results

Representative images are shown in Figures 1 and 2 and the imaging findings are set out in Table II. All 13 patients with malignant tumours were correctly identified by imaging. In four patients local recurrences were correctly identified and in two of these a single liver metastasis was demonstrated and confirmed by ultrasound. Two patients had a villous adenoma, one primary and one recurrent. Although both are technically benign tumours, both required surgery. This gives the sensitivity for colorectal cancer of 90% and for tumours requiring operation 100%. Because of the selection procedure, only two patients were imaged who had no tumour. One presented at follow-up with bowel symptoms with narrowing seen on colonoscopy suggestive of a recurrence, but was image negative. Surgical excision of the narrowed region of bowel showed no malignancy on histology. Another patient with bowel disorder at follow-up was shown to have mild inflammatory bowel disease on biopsy. Imaging

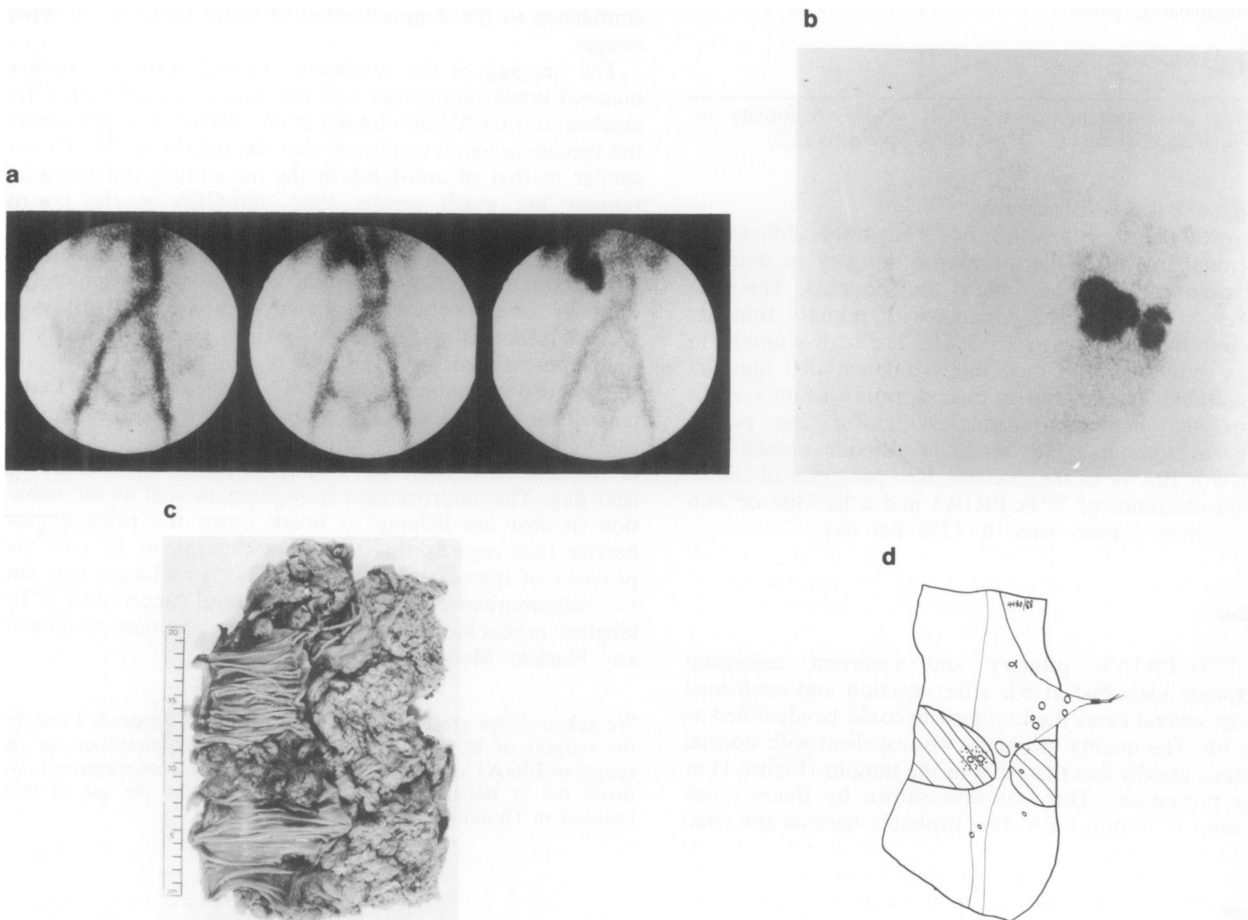

Figure 1 Poorly differentiated adenocarcinoma of the transverse colon. **a** Anterior abdominal images at 5 min, 6 and 22 h after injection of ^{99m}Tc-PR1A3. Note the increasing tumour uptake with time at the top of the image, the fading blood pool, the 'empty' pelvis and the absence of large bowel and urinary activity. The liver has less uptake than the tumour. **b** Image of the tumour specimen shortly after surgery on the same day as the 22 h image. Note no lymph node uptake. The tumour to mucosa ratio was 63:1 and 1.4×10^{-2}% g^{-1} of the injected dose was in the tumour. **c** Pathological specimen. **d** Diagram of pathological specimen Dukes' B. No lymph nodes were involved. Open circles, uninvolved lymph nodes; hatched areas, tumour site.

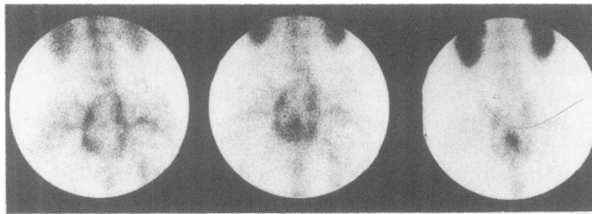

Figure 2 Moderately differentiated adenocarcinoma of the rectum. Posterior pelvic images at 5 min, 6 and 22 h show increasing focal uptake with time in the low pelvis, clearing of vascular activity, low marrow uptake and high renal concentration of activity increasing with time. Anterior and SPET views showed no bladder activity. Rectal tumour was confirmed surgically.

Table II Colorectal cancer: results using ^{99m}Tc-PR1A3 in 21 patients with 23 lesions

	Image positive	*Surgery/histology positive*
Malignant tumours		
Primary rectal cancer	6	6
Primary colonic cancer	7	7
Recurrent cancer	4	4
Liver metastases	2	2
Total	19	
Benign tumours		
Recurrent villous adenoma	1	1
Primary villous adenoma	1	1
Total	2	
	Image negative	*Histology negative*
No tumour		
Mild inflammatory bowel disease	1	1
Suspected recurrence	1	1
Total	2	

Sensitivity for colorectal cancer (19/21) = 90%. Sensitivity for tumour requiring operation (21/21) = 100%. Specificity (2/2).

in this patient was also negative.

Because of the short half-life of ^{99m}Tc it was difficult to time the final image on the morning of surgery so that the excised specimen could be imaged and counted. This was undertaken in seven of the patients with primary tumours and the results are shown in Table III. The higher uptake in poorly as compared with moderately differentiated tumours is demonstrated. The tumour to mucosa ratio was on average 2.5:1 for the moderately and 26.6:1 for the poorly differentiated tumours. The range of tumour uptake was $3.01-16.89 \times 10^{-3}$% of the injected dose per gram of tissue. The blood clearance of ^{99m}Tc-PR1A3 had a half-life of 24 h and the urinary activity was 10–12% per day.

Discussion

Using ^{99m}Tc-PR1A3, primary and recurrent colorectal tumours were identified at 6 h after injection and confirmed at 22 h. In several cases the tumour site could be identified as early as 3 h. The quality of images was excellent with normal liver uptake usually less than that in the tumour (Figure 1) or the liver metastases. This was also shown by Baum *et al.* (1989) using ^{99m}Tc-anti-CEA. It is probably because the renal uptake is relatively increased. Bone marrow and large bowel uptake were generally low. This is in contrast to our experience with ^{111}In-labelled PR1A3 where high liver and marrow uptake were typical (Granowska *et al.*, 1989*b*). Pelvic tumours were distinguishable from bladder activity usually on the planar images. However, SPET was helpful to confirm the separation.

Table III ^{99m}Tc-PR1A3 in excised specimens (uptake by tumour as percentage injected dose (i.d.) g^{-1}

Histology	*No.*	*Tumour (% i.d. g^{-1} × 10^{-3})*	*Mucosa (% i.d. g^{-1} × 10^{-3})*	*Tumour:mucosa ratio*
Moderately differentiated	4	3.01	2.06	1.5
		4.16	2.02	2.1
		4.42	1.47	3.0
		12.08	3.44	3.5
				(mean 2.5:1)
Poorly differentiated	3	13.92	1.78	7.8
		16.89	1.87	9.0
		13.82	0.22	63.0
				(mean 26.6:1)

Range of tumour uptake is $3.01-16.89 \times 10^{-3}$% i.d. g^{-1}. Range of tumour:mucosa ratio is 1.5–63.0:1.

The improved image quality is due to the high count rates obtained. The higher the count rate, the greater the reduction in intrinsic noise which has a square root relationship with the signal (Britton & Granowska, 1987). It is this reduction in intrinsic noise that enables a signal to be readily demonstrated inspite of a relatively high tissue background activity. Furthermore the specific uptake of a monoclonal antibody increases with time, whereas that of a non-specific uptake, after an initial distribution, decreases with time. The high intensity signal and the kinetic changes of specific uptake add confidence to the demonstration of small tumours on serial images.

The imaging of the specimens showed again a very low mucosal uptake compared with our previous work with ^{111}In-labelled anti-CEA (Granowska *et al.*, 1989*a*). The analysis of the specimens again confirmed that the uptake of PR1A3 was similar to that of anti-CEA in the moderately differentiated tumour but much greater than anti-CEA in the poorly differentiated tumour. It is of course the poorly differentiated tumour that is more likely to spread locally and to metastasize. In our series 80% of Dukes' C stage primary colorectal tumours were poorly differentiated, compared with those of Dukes' A and B stages, 92% of which were moderately or well differentiated.

The great advantage of the ^{99m}Tc-labelled PR1A3 is that a request for radioimmunoscintigraphy received in the morning can be undertaken and a provisional result given at the end of the same afternoon and confirmed on the 22 h image the next day. This improvement in logistics as well as the reduction in cost are helping to break down the psychological barrier that regards this type of technique to be only the province of specialist departments. This is no longer true and the radioimmunoscintigraphy of colorectal cancer with ^{99m}Tc-labelled monoclonal antibodies can now become routine in any Nuclear Medicine Department.

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