Tomoscintigraphy for detecting gastrointestinal and medullary thyroid cancers: first clinical results using radiolabelled monoclonal antibodies against carcinoembryonic antigen

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Abstract

Transaxial tomoscintigraphy (or single-photon emission computerised tomography) was used to detect secondary deposits of carcinoma in 17 patients who had been injected with iodine-131-labelled monoclonal antibodies against carcinoembryonic antigen. Of 17 tumour sites studied by tomoscintigraphy 16 were detected (sensitivity 94%); five sites had a volume smaller than 10 cm³. Tomoscintigraphy also detected three unknown tumour deposits later confirmed by surgery or radiology. In contrast, when 21 tumour sites in the same patients were studied by rectilinear scintigraphy, only nine tumour sites were detected (sensitivity 43%), of which eight had a volume larger than 50 cm³.

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As compared with conventional scintigraphy, tomoscintigraphy represents a fundamental improvement which can increase both the sensitivity and specificity of immunoscintigraphy.

Introduction

Immunoscintigraphy using radiolabelled antibodies against carcinoembryonic antigen (CEA) was performed on animals in 1974¹⁻³ and on man soon after.⁴⁻⁶ Until recently polyclonal anti-CEA antibodies were used in clinical studies.⁴⁻⁸ Immunological specificity can be improved with monoclonal anti-CEA antibodies.^{9,10}

When immunoscintigraphy is performed with conventional scintigraphy the tumour contrast can be enhanced by subtracting scintigrams obtained with non-specific radiotracers¹¹ from the radioactivity due to the antibody. This subtraction technique presents limitations due, firstly, to differences in the distribution of the antibody and of the non-specific tracers in normal tissues and, secondly, to the difference in energy of the most commonly used radionuclides (¹³¹I and ^{99m}Tc).

Axial transverse tomoscintigraphy¹² allows reconstruction of the three-dimensional distribution of the radiolabelled tracers. The contrast is related to the ratio between the activity per unit mass of the tumour and that of the normal tissues. For the study of relatively small tumours immunotomoscintigraphy should therefore represent a fundamental improvement over conventional immunoscintigraphy, in which the contrast is volume dependent.

We compared the results obtained with immunotomoscintigraphy and immunoscintigraphy in 19 patients injected with anti-CEA monoclonal antibodies labelled with ¹³¹I.

Patients and methods

Monoclonal anti-CEA antibodies were produced in ascitic form by two mouse hybridoma lines.⁹ With their informed consent, we included in the study patients without personal or family histories of allergy and with negative responses to tests of hypersensitivity to normal mouse IgG. They were injected intravenously (under cover with anti-allergic drugs) with 0.3 mg of ¹³¹I-labelled monoclonal antibodies representing 37-55 MBq (1-1.5 mCi). The thyroid was blocked with Lugol's iodine. None of the 19 patients injected with mouse anti-CEA antibodies showed any adverse effect or any sign of discomfort during or after the injection.

The radioactivity of the normal thyroid was measured on surgical specimens of one patient (case 11) two days after the injection of 55.5 MBq (1.5 mCi) of anti-CEA antibodies and was equal to 20 kBq/g (0.54 µCi/g) after correction of physical decay. This was approximately equal to activities found after conventional thyroid scintigraphy with 1 MBq (27 µCi) of ¹³¹I.

Rectilinear scintigrams were produced with a scintigraph Ohio-Nuclear 84-FD. Over 40 minutes the anterior view and the sum of

Fifty-one examinations using tomoscintigraphy in all 19 patients were performed: 29 were immunotomoscintigrams using radiolabelled monoclonal anti-CEA antibodies (17 performed the first day, nine the second day, and three the third day after the injection) and 22 were tomoscintigrams with other radiolabelled compounds (nine with ^{99m}Tc-methylen diphosphonate, six with ^{99m}Tc-sulfur-colloid, four with ^{99m}Tc-pertechnetate, and three with ¹²³I). These other radiolabelled compounds were used to obtain radioactive anatomical landmarks in the same position as with immunotomoscintigraphy. Only 17 of the 22 tumour sites were studied by immunotomoscintigraphy.

Results

RECTILINEAR SCINTIGRAPHY

The rectilinear scintigrams allowed us to determine the temporo-spatial distribution of the injected radiolabelled heterologous antibodies in the normal organs. The vascular radioactivity decreased progressively, with a half life of about 1.5 days: only the heart was

Scintigraphic and tomoscintigraphic results in 19 patients (17 with tumours and two without). Patients are classified according to their primary carcinomas

Case No	Tumour sites	Type of cancer	Serum CEA (µg/l)	Estimated tumour volume (cm ³)	Rectilinear scintigraphy*	Tomo-scintigraphy*
<i>Thyroid (medullary carcinoma)</i>						
1	Thyroid	Recurrence	78.5	3.7†	-	+
13	Thyroid	Recurrence	250.0	2 + 4 + 14†	-	+
17	Thyroid	Recurrence	244.0	20	-	+
7	Lungs	Metastasis	2105.0	50	+	NS
9	Liver	Metastasis		100	+	+
11	Liver	Metastasis	5380.0	500	+	+
	In remission		36.0	0	-	-
<i>Stomach</i>						
8	Stomach	Primary	5.8	5.4†	-	+
18	Stomach	Primary	2.9	50	+	+
<i>Pancreas</i>						
12	Pancreas	Primary	1.0	110	-	-
<i>Caecum</i>						
16	Right pelvis	Metastasis	6.8	10	-	+
<i>Sigmoid</i>						
4	Sigmoid	Primary	27.8	8.0†	-	+
	Lungs	Metastasis		50	-	NS
	Liver	Metastasis		50	-	NS
3	Sigmoid	Recurrence	47.8	10	+	+
	Lungs	Metastasis		50	+	NS
	Liver	Metastasis		50	-	NS
<i>Rectum</i>						
2	Rectum	Primary	20.0	100	+	+
5	Rectum	Primary	1.6	60-100	+	+
6	Rectum	Primary	27.3	500	NS	+
10	Rectum	Recurrence	1.0	20	+	+
14	Rectum	Recurrence	270.0	120	+	+
19	Liver	Metastasis	1990.0	21 + 210	+	+
15	In remission		7.8	0	-	-

*+ = Detected site; - = site not detected; NS = site not studied.

†Exact value found after surgery.

anterior and posterior views of the total body (except the legs) were obtained. Owing to the efficiency of this detector for ¹³¹I, it was possible to perform such examinations up to eighteen days after the antibody injection.

For tomoscintigraphy we used our tomographic prototype. It comprises a scintillation camera with a large field of view (Acti-camera CGR) which rotates around the patient as he lies on a specially designed bed. The rotation time can vary from 1 to 20 minutes for an entire turn. The collected data are stored on a minicomputer Simis 5 Informatek and are processed with original algorithms^{13,14} which enable the reconstruction of transverse sections or any other plane sections (frontal, sagittal, or oblique). Immunotomoscintigraphy could be performed up to three days after the injection of monoclonal antibodies.

Nineteen patients with a history of primary cancer were studied: six had medullary thyroid carcinomas and 13 gastrointestinal carcinomas (see table). Two patients (cases 11 and 15) who had had their primary carcinomas removed (1 thyroid and 1 rectum carcinoma) were in complete clinical remission at the time of examination. The other 17 patients presented 22 major tumour sites detected by other techniques: surgery, computed tomography, echography, radiography, and conventional scintigraphy.

Forty-five examinations using rectilinear scintigraphy (in the two patients without tumours and in 16 of those with tumours) were carried out five hours to 18 days after injection. One patient with one tumour site was too tired to undergo this examination.

slightly visible at day 8, and the kidneys and bladder disappeared at day 4. Despite the treatment with iodine, the thyroid was visible on day 1 and thyroid uptake at day 2 was maximal and higher than any other uptake. The activity in the reticuloendothelial system (liver, spleen, bone marrow) was greatest on day 1; thereafter it decreased and disappeared at day 4.

The sensitivity (percentage of tumour sites detected) of rectilinear scintigraphy for detecting tumours was assessed by double-blind readings performed by four physicians. The average of their results showed that rectilinear scintigraphy detected nine of the 21 tumour sites studied in 16 patients (43% sensitivity). Out of 13 tumour sites with a volume larger than 50 cm³, eight were detected (3 in livers, 2 in lungs, 1 in the stomach, 2 in rectums; table). Of these eight, four (2 in rectums, 1 in the lung, 1 in the stomach) seemed clearer on the later scintigrams (on days 4 to 8).

Four areas of abnormal non-specific uptake were noted in four patients with tumours: three of the areas were of unknown origin (1 in the liver, 1 in the stomach, 1 in the right pelvis) and one secondary to an operation on the stomach (case 12).

TOMOSCINTIGRAPHY

Fig 1 shows the results obtained in two patients with local recurrences of thyroid medullary epitheliomas injected with ¹³¹I-anti-CEA antibodies. One patient (case 1) was suspected of having a recurrence

15 years after a left hemithyroidectomy and postoperative irradiation because of an increase in serum CEA concentration to 78.5 $\mu\text{g/l}$ (normal <5 $\mu\text{g/l}$). Fig 1a and Fig 1b show, respectively, transverse and frontal sections on day 1 passing through a tumour recurrence in the right lobe, which was shown to weigh 3.7 g at subsequent surgery. Histological examination showed that the thyroid was almost exclusively cancerous, with rare foci of sclerotic thyroid tissue. Thus, the right thyroid uptake observed on the sections was due to tumour and not to uptake of free ^{131}I in normal thyroid.

In case 17 carcinoma of the right thyroid lobe was treated by local irradiation (70 Grays (7000 rads)) one year before examination. Recurrence was suspected because of a recent rise in serum CEA concentration to 244 $\mu\text{g/l}$. Fig 1c and Fig 1d show transverse sections at the same level on the middle part of the thyroid and were obtained with monoclonal anti-CEA antibodies (c) and ^{123}I (d) on day 2. The radioactive distributions were clearly different: the maximum uptake of ^{131}I corresponded to the tumour recurrence (c) and was located on

FIG 1—Tomoscintigrams performed with ^{131}I -anti-CEA monoclonal antibody in two patients with local recurrence of medullary thyroid carcinoma (A denotes front and right). Case 1. a and b show, respectively, transverse and frontal sections (zoom 1.8) at day 1 of patient with 3.7-g tumour of right lobe of thyroid, 15 years after left hemithyroidectomy of primary tumour and local irradiation. Case 17. c and d show transverse sections at same level on middle part of thyroid (zoom 2) on day 2 in patient with tumour recurrence in right lobe of thyroid. c was obtained with ^{131}I -anti-CEA antibodies and d with ^{123}I . Tumour is seen on the right lobe of thyroid, where ^{123}I uptake is almost absent. t=tumour; r=cervical rachis; sa=salivary glands; th=normal residual thyroid tissue.

FIG 2—Case 8. Tomoscintigrams of primary carcinoma of stomach (frontal sections at same level through stomach). a was obtained with ^{131}I -anti-CEA monoclonal antibody on day 3 and b with $^{99\text{m}}\text{Tc}$ -pertechnetate. The 5.4-g tumour (t) seen in a could be localised on the lesser curvature of the stomach (s) in b. h=heart; d=duodenum with benign ulcer, visible owing to bleeding.

FIG 3—Case 5. Tomoscintigrams of primary carcinoma of rectum (sagittal sections at same level, A denotes the front). a was obtained with ^{131}I -anti-CEA antibodies on day 1. The 6-cm tumour (t) was seen behind the bladder (b). b shows a bone tomoscintigram with $^{99\text{m}}\text{Tc}$ -methylen diphosphonate in the same position as shown by immunotomoscintigram. This section showed no $^{99\text{m}}\text{Tc}$ uptake in the tumour area behind the bladder. Rachis (r) was detected on both scintigrams, and non-specific uptakes on the penis (pe) and testes (te) are seen in a.

FIG 4—Case 4. Tomoscintigram with ^{131}I -anti-CEA antibodies on day 1 of primary carcinoma of sigmoid (A denotes front and right). a and b show, respectively, transverse and sagittal sections passing through 8-g primary tumour (t) located between bladder (b) and sacrum (s) or pelvic bones (p). c shows transverse section at lower part of bladder passing through lymph-node metastasis (m) on left side of rectum. It also shows non-specific bone marrow uptakes (bo). d shows sagittal section passing through same lymph node metastasis, which is located behind bladder and below pelvic bones.

the right lobe of the thyroid, whereas there was almost no ^{123}I uptake in this area (d). The remaining normal left lobe of the thyroid showed a definite uptake of ^{123}I .

Fig 2 shows frontal sections at the same level passing through the middle of the stomach in a patient with a primary carcinoma of the lesser curvature of the stomach (case 8). Fig 2a was obtained with ^{131}I -anti-CEA antibodies on day 3 and fig 2b with $^{99\text{m}}\text{Tc}$ -pertechnetate to visualise the stomach. Tumour was seen on a and could be localised to the lesser curvature of the stomach delineated by $^{99\text{m}}\text{Tc}$ (b). At subsequent operation the tumour weighed 5.4 g.

The patient shown in fig 3 (case 5) had a primary rectal carcinoma of 6-cm spread. Fig 3 shows sagittal sections at the same level obtained with ^{131}I -anti-CEA antibodies and $^{99\text{m}}\text{Tc}$ -methylen diphosphonate. The tumour was seen on a behind the bladder. The tumour uptake did not correspond to any $^{99\text{m}}\text{Tc}$ uptake as shown in b. On the rachis the antibody and methylen diphosphonate bone uptakes did not have exactly the same distribution because the antibodies were fixed on bone marrow.

Fig 4 shows the results of immunotomosintigraphy on day 1 in case 4—a patient with an untreated primary sigmoid carcinoma. Fig 4a and fig 4b show, respectively, transverse and sagittal sections passing through the primary tumour located between the bladder and the sacrum or the pelvic bones. At subsequent surgery the primary tumour was resected and found to weigh 8 g (2.5-cm diameter). Fig 4c and fig 4d show, respectively, transverse and sagittal sections passing through a left rectal lymph-node metastasis detected by immunotomosintigraphy and confirmed by subsequent surgery.

SENSITIVITY

For evaluating the sensitivity of tomoscintigraphy double-blind readings were performed by three observers. Of the 17 tumour sites studied, 16 were detected (see table). The only undetected site was a primary carcinoma of the pancreas with a volume of about 110 cm³. In this patient (case 12) the serum CEA concentration was 1 µg/l.

Of the 16 tumour sites in 16 patients studied by both tomoscintigraphy and rectilinear scintigraphy, 15 sites were detected by tomoscintigraphy (94% sensitivity) compared with seven detected by rectilinear scintigraphy (44% sensitivity).

Moreover, for three patients with tumours immunotomosintigraphy revealed areas of uptake which did not correspond to a previously known tumour site. At subsequent surgery two of these uptakes were found to be lymph-node metastases (see fig 4). The third corresponded to a bone metastasis which was confirmed by x-ray examination. These previously unknown tumour sites were not taken into account for assessing the diagnostic efficiency.

The observers noted seven areas of abnormal radioactive uptake (six in five patients with tumours and one in one without) which did not correspond to known tumour deposits. Three of these areas were related to a major gastrointestinal operation (out of eight such operations) and one other to a bleeding duodenal ulcer (fig 2). The remaining three areas (stomach, left sacrum, liver) were not explained by any known lesion.

Discussion

SENSITIVITY OR TRUE-POSITIVE RATE

Ninety-four per cent of the tumour sites studied by the two methods were detected by tomoscintigraphy compared with 44% by rectilinear scintigraphy. The percentage of tumours detected by tomoscintigraphy was also much higher than the 50% (14 out of 28 gastrointestinal tumours) obtained by Mach *et al.*,¹⁰ who used the same labelled monoclonal anti-CEA antibodies with conventional scintigrams and a subtraction technique.¹¹ Moreover, the four observers of the rectilinear scintigrams presented divergent results because of the poor tumour contrast in the scintigrams, and we had to consider a rectilinear scintigram as showing a positive finding when at least two observers considered it so. This probably enhanced the results for rectilinear scintigraphy compared with those for tomoscintigraphy, for which the responses of the three observers were concordant.

Furthermore, rectilinear scintigraphy mainly detected tumours larger than 50 cm³ whereas tomoscintigraphy showed tumours as small as a few cm³. Detection by tomoscintigraphy seems to be relatively independent of tumour size (except perhaps for very small tumours) but dependent on the concentration of labelled monoclonal antibodies in the tumour as compared with adjacent normal tissues. The only tumour site not visualised by tomoscintigraphy (case 12) was large (110 cm³), and this negative result was probably due to a low concentration of antibody in the tumour. This result and the low serum CEA concentration of this patient suggested that this tumour was synthesising very little CEA.

SPECIFICITY AND FALSE-POSITIVE RESULTS

In four patients with tumours a surgical procedure was carried out after antibody injection, and the radioactivity of resected tumour tissues was found to be 1.4 to 22 times higher than that

of normal adjacent tissues and 1 to 5 times lower than that of sera. These findings confirmed a specific uptake of anti-CEA monoclonal antibodies in the tumour.¹⁰

Non-specific uptakes of anti-CEA antibodies do exist, however, and remain a problem. When located near the site of a tumour these non-specific uptakes can be interpreted as true-positive results—for example, the most evident false-positive finding on rectilinear scintigraphy corresponded to non-specific uptake in the stomach (probably due to a previous operation) front of the pancreatic tumour (case 12). It was detected by three physicians (out of four) and was scored as positive. In fact tomoscintigraphy showed that the uptake was not in the tumour but in the stomach. In addition, at least three other tumour sites (three rectums) detected by rectilinear scintigraphy corresponded to superimpositions of tumour uptakes with non-specific uptakes (see fig 3). Several so-called tumour uptakes on conventional scintigraphy might be due to non-specific uptakes. Thus we think that the actual sensitivity of conventional immunoscintigraphy might be lower than that reported^{4, 6, 7}: some so-called true-positive results might in fact be false-positive. Such artefacts might also partially explain the surprisingly low number of false-positive results reported by some authors⁷ with standard scintigraphy.

The number of abnormal non-specific uptakes (seven in five patients with tumours and one without) detected by tomoscintigraphy was higher than for rectilinear scintigraphy (four in four patients with tumours). Nevertheless, only one (left sacrum) of the seven false-positive uptakes detected by tomoscintigraphy was higher than the true-positive uptakes detected by tomoscintigraphy and four of these uptakes were explained by using landmark tomoscintigraphy with conventional radionuclides (fig 2). Hence, with complementary tomoscintigraphy, we could exclude four false-positive results.

Specificity is related to the false-positive rate (specificity = 1 – (number of false-positive results/number of negative zones)), which depends on the number of negative patients or zones⁷ studied. Obviously, the number of patients without tumours in this study (two) was too low for a conventional evaluation. The patients with tumours may, however, be considered negative when we exclude their tumour areas. With this method the specificity of rectilinear scintigraphy was 78% (1 – (4/18)) and the specificity of tomoscintigraphy varied from 63% (1 – (7/19)) to 84% (1 – (3/19)) according to whether or not we took account of the results of the complementary landmark tomoscintigraphy.

For an overall diagnostic evaluation we used an index called gain,¹⁴ which is the percentage of the true-positive results (sensitivity) minus the percentage of the false-positive results. The gain can be interpreted as the value of sensitivity when there is no false-positive result. Thus, the gain with rectilinear scintigraphy was weak (22%) compared with the gain with tomoscintigraphy, which varied from 57% to 78% according to whether complementary tomoscintigraphy was taken into account.

The gain with tomoscintigraphy is essentially dependent on the false-positive rate, which is related to the ratio of uptake in the tumour to non-specific uptake. Fragments of polyclonal antibodies or of monoclonal antibodies (such as F(ab')₂ fragments¹⁰) might be better tracers than the antibodies in current use. Other radionuclides with shorter physical half lives than ¹³¹I and emitting photons with lower energy, such as ¹²³I or ¹¹¹In, may further increase the spatial resolution of immunotomosintigraphy.

In conclusion, the clinical use of rectilinear scintigraphy for immunoscintigraphy with anti-CEA antibodies seems limited to the detection of large tumours, which are often best appreciated on late scintigrams (days 4 to 8). Immunotomosintigraphy, in contrast, allows the detection of smaller tumours, and it can be used even where non-specific uptakes are important, especially on days 1 and 2. Thus, immunotomosintigraphy with anti-CEA monoclonal antibodies may represent an efficient diagnostic technique for detecting occult CEA-producing tumours pre-

operatively, and, in particular, for localising recurrences or metastases of medullary thyroid carcinomas.

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Association of specific immune response to pork and beef insulin with certain HLA-DR antigens in type 1 diabetes

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Abstract

To test the association of HLA-DR antigens with high-responder and low-responder status to either beef or pork insulin, insulin antibodies in diabetic sera were separated into those with average low and those with average high affinity and their insulin-binding capacities for each insulin determined. Significantly less binding of pork insulin by the high affinity antibodies occurred in the group of patients with DR3 antigens compared with those with DR4 antigens ($p < 0.01$) and DR3/4 antigens ($p < 0.01$). The difference in the binding capacity of beef insulin by the high affinity antibodies between the groups with DR3 and DR4 antigens was less pronounced but still significant.

The high-responder status of DR3/4 antigens to pork insulin suggests that the gene or genes associated with HLA-DR4, and responsible for a high response to pork

insulin, are dominant to genes associated with HLA-DR3 and a low response. If extended to human insulin and different HLA-DR and HLA-B antigen patterns, these findings should help in the therapeutic selection of the appropriate insulin and thus reduce the induction of an anti-insulin response in patients with diabetes.

Introduction

The immune response to insulin is under the control of genes, the so-called immune response genes, which are located within the major histocompatibility complex. Immune response genes to insulin have been most convincingly demonstrated in mice and guinea pigs, whereas their investigation in humans is far less advanced. Scherthaner *et al*¹ and Bertrams *et al*² correlated the high responder status of antibody production to insulin with the HLA-DR4 and B15 antigens, while DR3 and B8 antigens were more closely associated with a low responder status. Their particular technical approach did not allow discrimination between a high responder and low responder status with respect to the most commonly used insulins, beef and pork.

The insulin molecule is small and carries on its surface only a limited number of antigenic determinants. Furthermore, beef insulin differs from human insulin by three amino-acid exchanges: Ala for Thr at A8 (A-chain loop region), Val → Ile at A10, and Ala → Thr at B30 (B-chain). Pork insulin differs only by one exchange: Ala → Thr at B30. To detect an antibody with binding specificity for one of these sites it is important that the antigen in the assay retains its native structure as far as possible. If the antibody-bound and free insulin is, however,

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