Perfusion of Tumor-Bearing Kidneys as a Model for Scintigraphic Screening of Monoclonal Antibodies

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Tumor-bearing human kidneys were used in an ex vivo perfusion model to screen monoclonal antibodies, recognizing renal cell carcinoma-associated antigens for diagnostic potential in vivo. Perfusion of tumor-bearing kidneys with ^{99m}Tc-labeled G250 and RC38 antibody resulted in visualization of the tumor, whereas perfusion with two other monoclonal antibodies, RC2 and RC4, did not lead to tumor visualization. Uptake of radiolabel in normal kidney tissue was low for G250 and RC38 antibody. Tumor-to-kidney tissue ratios after perfusion with G250 and RC38 antibody were 2.7 and 2.2, respectively. After rinsing for 3 hr with unlabeled perfusion fluid the tumor-to-kidney tissue ratios increased to 8.6 for G250 antibody and to 2.7 for RC38 antibody. We conclude that perfusion of tumor-bearing human kidneys with radiolabeled monoclonal antibodies is a relatively simple way to evaluate renal cell carcinoma associated monoclonal antibodies as diagnostic agents in vivo.

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In the last few years, many monoclonal antibodies (Mabs) directed against tumor-associated antigens have been tested for their ability to localize tumors in animal or man (*1,2*). These Mabs were tested in various animal tumor models of which the nude mouse xenograft model is the most widely used (*3,4*). The usefulness of this animal model lies in the ability to perform experiments under controlled conditions. A wide variety of different variables such as the kind of labeling method, choice of isotope or the best time of imaging can be studied. This model can also be used to select Mabs for human tumor imaging. This model, however, does not reliably predict the usefulness of antibodies for diagnostic purposes. The difference in size of various compartments between mouse and man and also differences in biodistribution of injected Mabs are limitations to the use of animal models (*5,6*). Furthermore, normal human tissue cannot be used in control experiments as it cannot be grafted in the nude mouse. For this reason, Sears et al. developed an ex vivo perfusion model in which segments of human colon containing colorectal carcinoma were perfused with anticolorectal Mabs (*7*).

In previous studies (*8,9*) we described a number of antibodies to renal cell carcinoma (RCC) that reacted with most RCC in immunohistochemistry. However, direct testing of these Mabs in patients cannot always be performed. Before starting clinical studies with these Mabs, we decided to test these Mabs in a new model in which antigenic makeup, vascularization, and size ratios more closely resemble the actual situation in man.

In this paper we present data on an ex vivo model in which resected human tumor-bearing kidneys were perfused with radiolabeled Mabs. We show that with this model Mabs that recognize RCC-associated antigens in tissue sections can be screened for detection of primary RCC by applying radioimmunoscintigraphy (RIS).

MATERIALS AND METHODS

Monoclonal Antibodies

Four Mabs—G250 (IgG1), RC38 (IgG1), RC2 (IgG1) and RC4 (IgM-isolated previously in our laboratory (*8,9*) were labeled with technetium-99m (^{99m}Tc).

One milligram of G250, RC38, or RC2 antibody purified to homogeneity from mouse ascites by protein-A Sepharose-CL4B (Pharmacia, Uppsala, Sweden), and 1 mg of RC4

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antibody purified by chromatography on S-500 (Pharmacia, Uppsala, Sweden) was dialyzed against 0.9% NaCl and labeled with 10–14 mCi ^{99m}Tc using a newly developed method (*10*). Briefly, to a mixture of N,N dimethylformamide and hydrochloric acid was added a [^{99m}Tc]pertechnetate solution in 0.9% NaCl. The glass tube with this solution was heated for 4 hr. After cooling to 40°C, 200 μl of solution of the Mab (10 mg/ml) was added to the dry intermediate in the tube and incubation was continued for 1 hr. The labeled antibodies were separated from free ^{99m}Tc by gel filtration over a 10 × 100 mm G25 column (Pharmacia, Uppsala, Sweden), saturated with 0.5% gelatin in 0.9% NaCl.

For each labeled antibody preparation the final immunologic activity was determined. The immunologic activity was measured by adding an aliquot of the labeled Mab preparation (1,000,000 cpm) to duplicate tubes each containing 100,000 cells of an RCC-cell line (SK-RC-1) reactive with the antibodies (*9*). The tubes were incubated at room temperature for 1 hr. After this time the cells were pelleted and counted for radioactivity bound to the cells. The bound radioactivity was expressed as the percentage of total radiolabeled Mab added. The specific activity of G250, RC38, RC2 and RC4 antibody ranged between 5 and 9 mCi/mg. The immunologic activity for all four Mabs ranged between 60% and 70%. For experiments, ^{99m}Tc-labeled antibodies were administered corresponding to ~0.5 mg antibody and ranging in activity between 2.6 and 4.5 mCi.

Kidney Perfusion Model

Tumor-bearing kidneys from four patients, all clinically diagnosed as RCC, were nephrectomized. Directly after the arteries and veins of the kidney were separated from the systemic circulation, the renal artery was connected by means of an arterial catheter (Talas, Ommen, The Netherlands) to an extracorporeal circuit (Gambro, USA) (Fig. 1A,B). This circuit consisted of a roller pump with an adjustable flow rate which was fixed in our experiment at 100 ml/min and a reservoir in which the kidney was held. This flow rate in our experiments correspond with a systolic and diastolic pressure of 120 and 80 mmHg, respectively (Hewlett Packard, USA). Polyvinyl tubing was used, except for the tubing in the roller pump, which was silicone rubber. The reservoir was externally cooled, keeping the temperature of the kidney between 4° and 7°C during the experiments to minimize formation of edema. The arterial pressure was measured continuously. The kidney was perfused with 300 ml Collins preservation fluid (*11*), supplemented with 100 ml albumin (20%), 100 mg methylprednisolone, and 5 mg sodium bicarbonate 8.4%. Oxygen was directly supplied by blowing a flow of oxygen with a flow rate of 200 ml/min over the perfusion fluid in the reservoir.

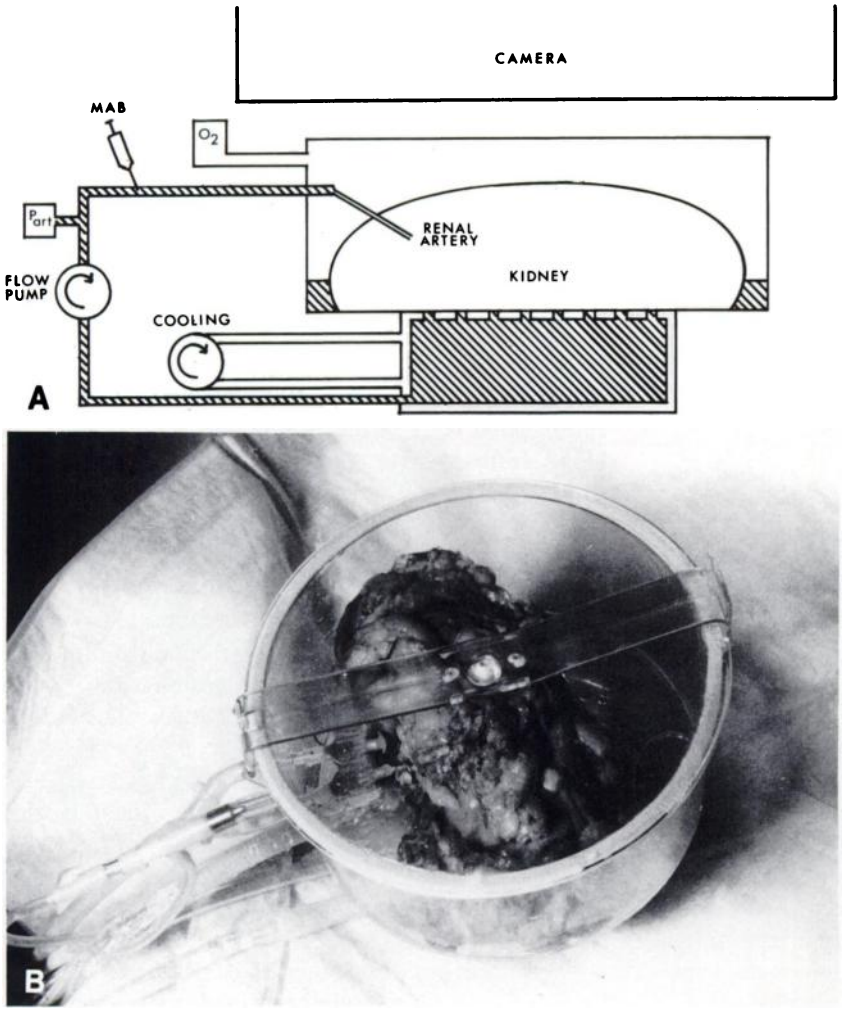

FIGURE 1
Schematic picture of the kidney perfusion model (A) and a photograph of a perfused kidney (B). P_{art}: arterial pressure gauge; O_2: Oxygen supply. The hatched area represents the perfusion fluid. The renal vein was left open.

The renal vein was left open, permitting the perfusion fluid to flow through the kidney into the reservoir below the kidney from where it was recirculated.

After 2 hr of perfusion, ^{99m}Tc-labeled Mabs were added to the perfusion fluid (Fig. 1A) and the kidneys were perfused for 16 hr. During the perfusion experiments, the oxygen flow remained at 200 ml/min, the systolic pressure varied between 80 and 120 mmHg, and the diastolic pressure was between 30 and 90 mmHg. The temperature never exceeded 7°C. A photograph of a perfused kidney is shown in Figure 1B. To rinse out unbound antibody, the perfusion fluid was then changed twice with unlabeled fluid and perfused for 1 hr. After this period the perfusion fluid was changed again and the kidney was perfused for another 2 hr. After this time a final image with the gamma camera was taken.

Scintigraphic Analysis

After the injection of radiolabeled Mabs, images were obtained over 15 min intervals with a gamma camera (Toshiba GCA 102S, Japan) linked to a computer system (Medical Device System A2). A 128-by-128 pixel matrix was used. The camera was equipped with a standard low-energy collimator. A 20% window centered on the 140 keV photopeak of ^{99m}Tc was used. The distance between the camera and the kidney was 2 cm and for each image 150,000 counts were acquired.

To determine the tumor-to-kidney tissue ratios, regions of interest (ROIs) were drawn corresponding to presumed tumor and normal kidney tissue. Tumor-to-kidney tissue ratios were determined 4 and 12 hr postinjection of radiolabeled Mabs and after 3 hr rinsing with unlabeled perfusion fluid. Integrated counts in all images and ROIs were corrected for radionuclide decay to yield comparable counts.

Immunohistochemistry

After the perfusion experiments, the tumor-bearing kidneys were dissected and tissue samples were formalin-fixed for routine pathologic investigation. Macroscopically the identity of tumor regions in the regions of the computer images was confirmed. Tissue samples of tumor and normal kidney were taken, snap-frozen and stored at −70°C. Immunohistochemistry was performed as described before (*12*). Briefly, frozen sections of tumor and normal kidney were fixed in acetone and incubated for 60 min with the Mab used in the perfusion experiment. After rinsing, rabbit anti-mouse immunoglobulin conjugated to horseradish peroxidase was added and the sections were developed with 0.05% 3-3′ diaminobenzidine and 0.03% H_2O_2, followed by counterstaining with hematoxylin. Sections were examined under a light microscope.

RESULTS

In Figure 2, a panel of images of perfused tumor-bearing kidneys are shown. Figure 2A shows an image of a tumor-bearing kidney perfused for 16 hr with 2.6 mCi G250 antibody and rinsed for 3 hr with unlabeled perfusion fluid. Accumulation of radiolabeled Mab is observed in the upper pole of the kidney. In Figure 3, the corresponding time-activity curves of accumulating ^{99m}Tc-labeled antibodies in tissue sections are shown. For ^{99m}Tc-labeled G250 antibody, initially fast accumulation of radiolabel is observed in the tumor followed by a continued slower uptake, whereas no continued accumulation of radiolabel was seen in normal kidney tissue (Fig. 3A). The tumor was later found to be indeed present in the region of high uptake of the tracer and was diagnosed as RCC. Immunohistochemical staining of tumor sections with G250 antibody showed that 60% of the tumor cells were positive for G250 antibody. The tumor-to-kidney tissue ratio as determined by computer analysis 4 hr postinjection of G250 antibody was 1.7, whereas perfusion for 12 hr increased the tumor-to-kidney tissue ratio to 2.7 (Table 1). Rinsing for 3 hr with unlabeled perfusion fluid increased the tumor to kidney tissue ratio to 8.6.

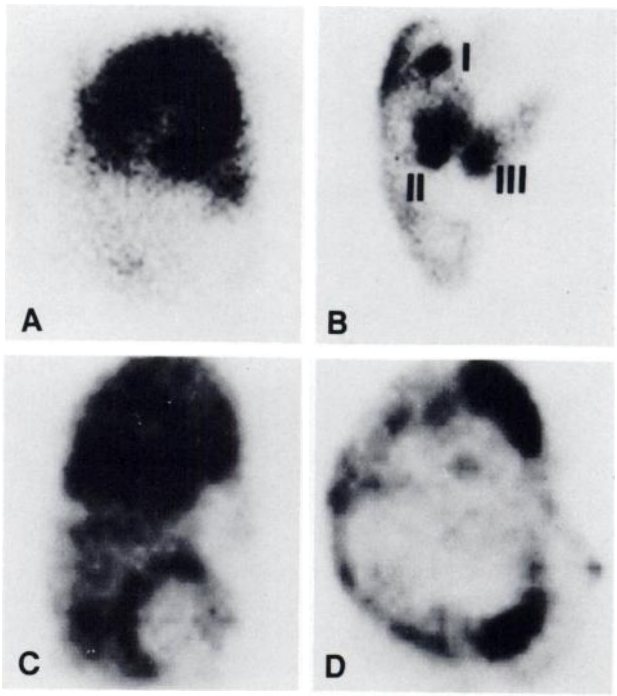

FIGURE 2
Gamma scintilation images of G250 (A), RC38 (B), RC2 (C) and RC4 antibody (D). Images were obtained 16 hr postinjection with 3 hr rinsing. Because of the heterogeneity of the tumor in Figure B, the three spots were designated as tumor I, II, and III. Bar at left shows common scale with highest value at top representing maximum pixel value.

Somewhat different results were obtained after perfusion of a tumor-bearing kidney with 3.1 mCi RC38 antibody. Although three areas of intense accumulation, later diagnosed as RCC, can be observed (Fig. 2B, 3B), tumor-to-kidney tissue ratios were low after 12 hr perfusion (Table 1). Rinsing for 3 hr with unlabeled perfusion fluid gave only a slight increase of tumor-to-kidney tissue ratio. The tumor nodules were positive in immunohistochemistry with RC38 antibody: 80% of the tumor cells were stained.

In contrast, with two other Mabs, RC2 and RC4, we were not able to accumulate label in tumor tissue. After

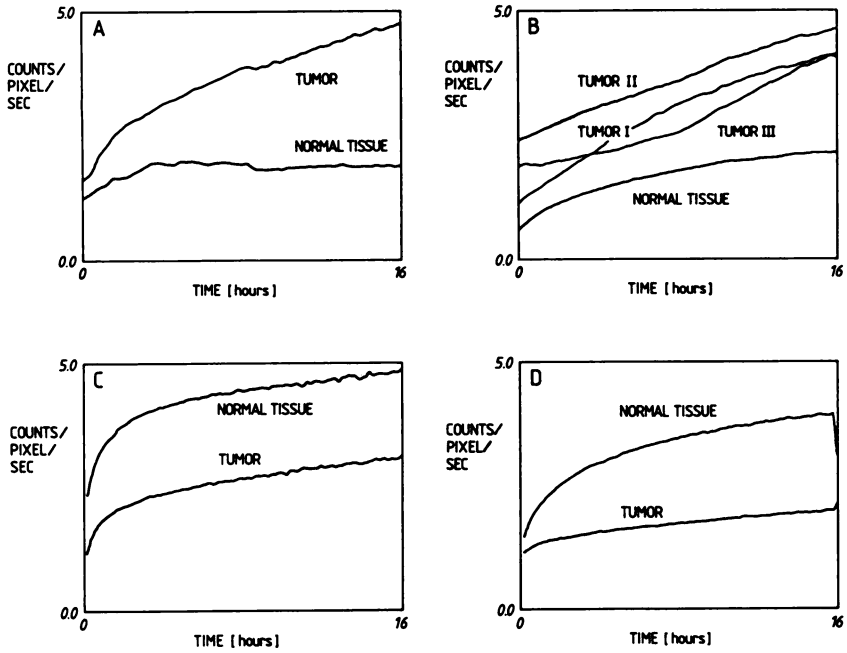

FIGURE 3
Uptake curves of G250 (A), RC38 (B), RC2 (C) and RC4 antibody (D) labeled with ^{99m}Tc of normal kidney tissue and tumor tissue during 16-hr perfusion. The uptake of radiolabeled RC38 antibody in tumor I, II and III (Fig. B) refers to the three spots in Figure 2B.

perfusion of a tumor-bearing kidney with 2.6 mCi RC2 antibody, a region with relative low radioactivity as compared to normal renal tissue can be seen in the lower pole of the kidney (Fig. 2C). Upon dissection, the tumor was found to be present in this region. Although the tumor was diagnosed as RCC, only 10% of the tumor cells were positive in immunohistochemistry with RC2 antibody.

Perfusion with 4.5 mCi RC4 antibody resulted in a picture in which the tumor was faintly outlined as a whitish mass in the middle and lower region of the kidney (Fig. 2D). After examining the kidney, the tumor was located in the middle and in the under pole of the kidney. In immunohistochemistry again only 10% of the tumor cells were stained with RC4 antibody.

For both antibodies, RC2 and RC4, tumor-to-kidney ratios remained low during the perfusion experiment, even after rinsing for 3 hr with unlabeled perfusion fluid (Table 1). In both cases, normal kidney tissue is clearly visible, due to accumulation of radiolabel throughout the entire kidney (Fig. 3C,D). Our light-microscopic studies also showed that cell morphology after perfusion was normal, indicating that the continued perfusion did not lead to cell damage.

DISCUSSION

In this paper we describe an ex vivo model in which resected human tumor-bearing kidneys were perfused with ^{99m}Tc-labeled RCC-associated Mabs. Using this model we were able to demonstrate increased Mab uptake in RCC with two Mabs, G250 and RC38, with maximal tumor-to-kidney tissue ratios of 8.6 and 2.7, respectively. G250 does not react with normal kidney tissue (*9*). RC38 reacts with glomerular visceral cells and with epithelial cells of the proximal tubules (*8*). The tumor-to-kidney tissue ratio for RC38 antibody is low as compared to G250 antibody possibly because of leaking of RC38 antibody through the basal membrane. Two other Mabs also reacting with RCC in immunohistochemistry, RC2 and RC4, did not show preferential uptake in RCC in this ex vivo model. This may be because of the fact that RC2 and RC4 reacted with only 10% of the tumor cells in immunohistochemistry. RC2 antibody mainly reacts with intracellular antigens. This may also contribute to our negative imaging results. However, from the literature it is known that Mabs detecting an intracellular and nonsecreted antigen are able to localize in melanoma (*13*). Therefore we cannot exclude the possibility that RC2 antibody may be able to visualize tumor tissue in vivo, provided a larger percentage of cells contain the antigen. The negative

TABLE 1
Tumor-to-Kidney Tissue Ratios After Perfusion with Anti-RCC Antibodies and After Rinsing

Monoclonal antibodies		4 hr perfusion	12 hr perfusion	3 hr rinsing
G 250		1.7	2.7	8.6
RC38	I*	1.6	1.9	2.1
	II	2.1	2.2	2.7
	III	1.5	1.9	2.3
RC2		0.3	0.4	0.6
RC4		0.5	0.5	0.4

* Refers to the three spots in Figure 2B

results with RC4 antibody may either be due to the fact that only 10% of the tumor cells were RC4 positive or to the fact that RC4 is an IgM antibody. The data of Sears (*7*) indicate that it is difficult to accumulate IgM antibodies in tumor tissue. Possibly with much longer perfusions RC4 could also accumulate in RCC.

The kidney perfusion model as described in this study clearly does not reflect the in vivo situation in man, as the kidney is isolated from the systemic circulation and the antibodies therefore cannot interact with other organs. For instance the liver has no influence on the clearing of the Mabs tested. This results in different half lifes and biodistributions of the Mabs as compared to injection of similar amounts of Mab in patients. Also the extracorporeal circuit cannot be compared with the systemic circulation. So different ways of administration of Mabs, such as intraperitoneal or intravenous administration can not be studied.

Despite these disadvantages, this model in some respects resembles more closely the in vivo situation than the nude mouse model, e.g., with respect to vascularization of the tumor and relative sizes of tumor and normal kidney compartments. Also in the perfusion model a large number of human antigens not related to the tumor are exposed to the antibody. In the nude mouse xenograft model it is known that a higher uptake of radiolabeled Mabs is seen in the tumor as compared to the uptake in patients (*5,6*). This may be because the behavior of murine antibodies is different in man and mouse, respectively, and also because of different antigenic makeup of these two species. Some Mabs can cross-react with human tissues but not with tissues of mice, e.g., in some studies cross reactivity with circulating human white blood cells was observed in patients, whereas no such reaction was seen in mice (*14,15*). Although not studied yet, this ex vivo model seems also to be suited for studying other variables, such as the choice of radionuclide or the labeling procedure or the imaging properties of fragments of Mabs. The effort involved in performing organ perfusion studies in a well-organized clinical setting in a hospital with access to imaging equipment is quite acceptable. It requires rapid flushing of the organ removed to prevent blood clotting. The perfusion systems commonly used for storing kidneys for kidney transplants are relatively inexpensive and can easily be transported. The labeling of antibody is no problem. One-day advance notice that an operation is pending is sufficient for preparative measures. In our hospital (700 beds), about 20 tumor-bearing kidneys are removed per year.

It is concluded that this model is useful for evaluating Mabs for RIS in patients and that this perfusion model presents additional data as compared to immunohistochemistry and the nude mouse xenograft model, in particular with respect to uptake of radiolabel by the tumor versus normal kidney tissue.

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