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Localization of a Radiolabeled Monoclonal Antibody to Calcitonin in Rat Medullary Thyroid Carcinoma Allografts

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A radiolabeled anticalcitonin monoclonal antibody (MoAb) was tested for tumor-localizing capability in WAG/Rij rats allografted with cultured medullary thyroid carcinoma cells (line 44-2). ^{131}I-labeled anticalcitonin IgG was taken up by the tumors in a time-dependent and dose-dependent manner. Tumor tissue uptake of the injected MoAb was 0.7 to 1.3%/g of tumor tissue when tested with a dose range of 40 to 250 μg/kg of body weight. Distribution ratios of the radiolabeled MoAb were 1.2:1 for tumor-to-blood and 2-20:1 for tumor-to-organs. In rats injected with control IgG, distribution ratios of radioactivity were 0.4:1 for tumor-to-blood, and 0.6-2:1 for tumor-to-organs. These results suggested a specific uptake of MoAb by the tumor. Some nonspecific uptake was seen by the visceral organs. This may represent the metabolic clearance of MoAb, presence of cross-reacting antigens, or metastasis of tumor to these organs. (Henry Ford Hosp Med J 1987;35:153-6)

Antibodies against tumor markers are potentially powerful tools for detecting metastatic malignancies, particularly small metastatic tumors for which methods of detection are still lacking. Antibodies against tumor cell-surface antigens or secretory products have been shown to localize in various types of human neoplasms, both in animals (1-7) and man (8-12). For medullary thyroid carcinoma (MTC), however, immunolocalization of antibodies against tumor markers of this type has rarely been demonstrated. Using polyclonal antibodies against human synthetic calcitonin (CT), Gautvik et al (13) reported a preferential uptake and localization of the ^{131}I-labeled immunoglobulins in rat MTC tissues. In contrast, Guilloteau et al (14) found that radiolabeled anti-CT immunoglobulin fragments prepared from ewes' antisera were unsuitable for MTC scintigraphic imaging. In theory, a monoclonal antibody (MoAb) has the advantage of greater specificity, but its restricted epitope specificity could have the disadvantage of limiting localizing efficacy. The suitability of monoclonal anti-CT antibodies for detecting MTC needs to be tested. We report here our radiotargeting studies in WAG/Rij rats allografted with rat MTC using ^{131}I- or ^{125}I-labeled MoAb to CT.

Materials and Methods

Antibody preparations and ^{131}I- or ^{125}I-labeled antibodies

MoAb to CT (produced by Hybritec, San Diego, CA) was purchased from Boehringer Mannheim Biochemicals (Indianapolis, IN). Mouse IgG was obtained from Sigma Co (St Louis, MO). The MoAb and mouse IgG were labeled with ^{131}I or ^{125}I using a modification of the chloramine-T method (15) of Cambridge Medical Diagnostics (Cambridge, MA). The labeled MoAb or mouse IgG has a specific activity of 12 to 16 μCi/μg.

Rat MTC

MTC allografts were produced in WAG/Rij rats by subcutaneous injection of cells of an established rat MTC line, 44-2 (rMCT 44-2) (16). These cells were generously provided by Dr. Robert F. Gagel, Veterans Administration Medical Center, Baylor College of Medicine, Houston, Texas, and were maintained in our laboratory as monolayer cultures. Flasks of rMTC 44-2 cells were washed with sterile saline and the cells were scraped off the flasks with a rubber policeman. The cells (about 5×10^6) were resuspended in 1 mL of saline and injected into the back of the rats' necks. Tumors about 1 cm in diameter usually formed in three to four weeks.

Measurement of serum immunoreactive CT

Serum immunoreactive CT (iCT) levels in sex- and age-matched control and tumor-bearing rats were measured by radioimmunoassay using antiserum against human CT (17).

Testing of biological activity of the ^{131}I-MoAb

A ^{131}I-labeled MoAb was tested by ELISA procedures for its ability to bind CT in comparison with the binding ability of unlabeled MoAb. Human CT was pipetted into the 96 wells of a polystyrene plate (Costar). The antigen was attached to the plate by overnight evaporation at 37°C. Before application of the primary antibody, the plate was treated with 1% bovine serum

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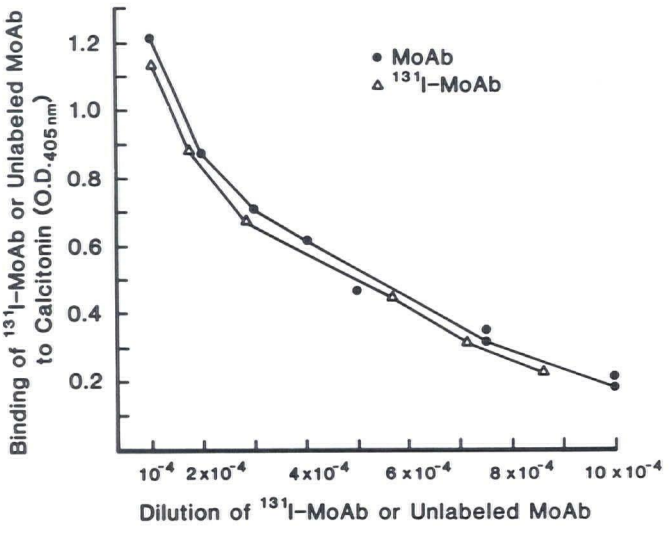

Fig 1—Comparison of binding of ^{131}I-labeled MoAb to calcitonin with that of unlabeled MoAb, as measured by ELISA procedures.

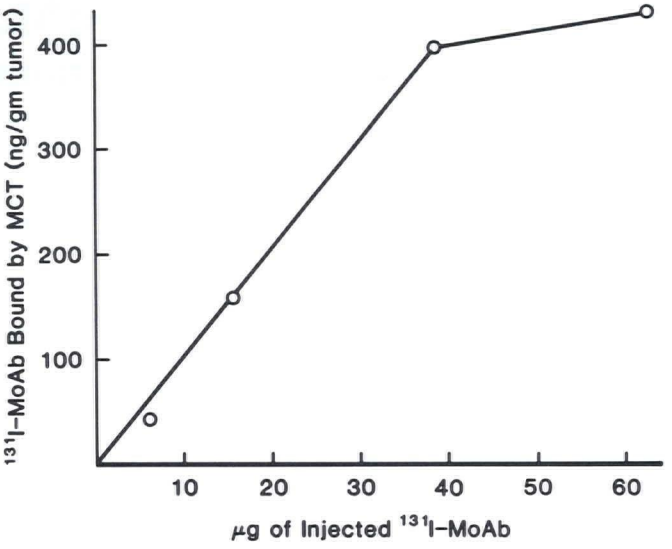

Fig 3—Uptake of ^{131}I-labeled MoAb by tumor on the sixth day after injection as a function of dose of injected ^{131}I-labeled MoAb.

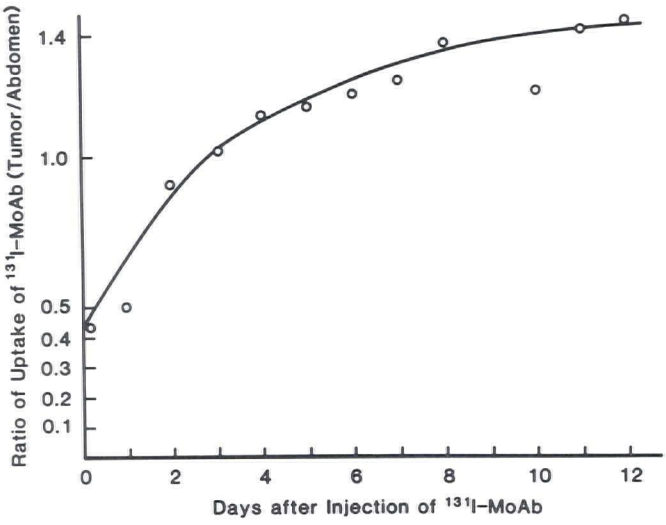

Fig 2—Tumor uptake of ^{131}I-labeled MoAb over time course of one hour to 12 days.

albumin solution to prevent nonspecific binding. MoAb, ^{131}I-labeled and unlabeled, was added and incubated for one hour at room temperature. After being washed with phosphate-buffered saline (PBS)-Tween, the plate was incubated with rabbit anti-mouse peroxidase conjugate for one hour at room temperature. After a final wash with PBS-Tween, peroxidase activity was visualized with a substrate solution containing azino-diethyl-benzthiazoline sulfonate and hydrogen peroxide. The color intensity of the reactants was measured at 405 nm.

In vivo uptake of ^{131}I-labeled MoAb and ^{131}I-labeled control IgG by tumor and normal tissues

WAG/Rij rats bearing tumors of 2 to 3 g (three to four weeks after transplantation) were used for localization experiments. About 200 μCi of ^{131}I-labeled MoAb or ^{131}I-labeled control IgG, representing 25 to 40 μg of protein, was injected intravenously via the tail vein. The rats' thyroid glands were blocked by the addition of sodium iodide to the animals' drinking water. Whole-body scintigrams of the rats anesthetized with ketamine hydrochloride were taken with a scintillation camera one hour after injection and every day for the next 12 days. Subsequently, day six was selected for dissection of the animals and direct measurement of the radioactivity in the tumor and organs. The rats were sacrificed by CO_2 asphyxiation. The tumors and various organs were dissected, weighed, and counted for radioactivity in a gamma counter (Nuclear Chicago, Model 4224). Blood was withdrawn by cardiac puncture.

In some experiments in which blood distribution in the tumor and organs was determined, this distribution was measured using intravenous injection of ^{111}InCl. In these experiments, a group of tumor-bearing rats were subjected to similar experimental manipulations, except that they received no ^{131}I-labeled MoAb or ^{131}I-labeled control IgG. The animals were injected with 200 μCi of ^{111}InCl and were sacrificed 15 minutes later. Blood was withdrawn and the organs were dissected, weighed, and counted for ^{111}In radioactivity.

Results

Serum concentration of immunoreactive CT in control and tumor-bearing rats

WAG/Rij rats that received transplanted rMTC cells had a serum iCT concentration of 4.0 ± 0.4 ng/mL at the time of the

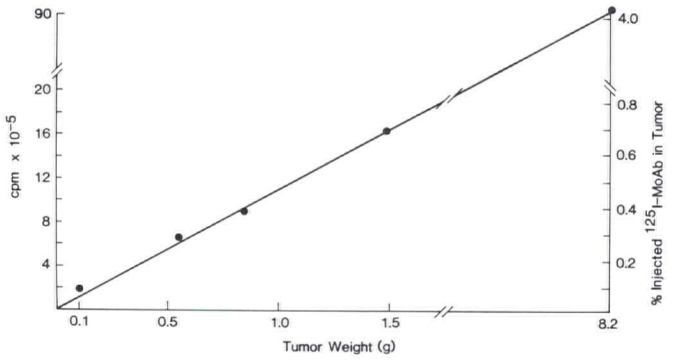

Fig 4—In vivo localization of the radiolabeled MoAb six days after injection in tumors of differing weights.

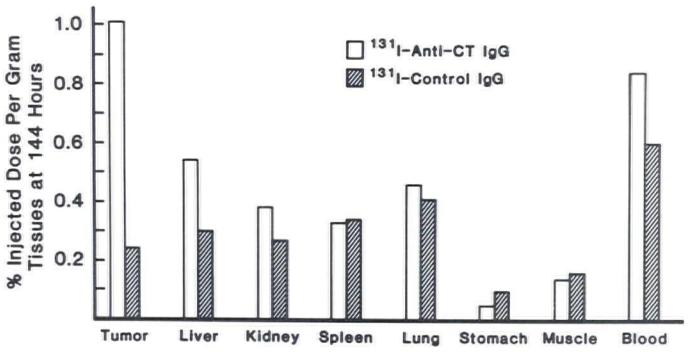

Fig 5—Distributions of radiolabeled MoAb and control IgG in tumor and normal tissues.

in vivo distribution experiments, whereas the age- and sex-matched control rats had a serum iCT concentration of 1.7 ± 0.6 ng/mL. The higher serum concentration of iCT indicated that the transplanted tumors synthesized and secreted iCT in vivo.

Biological activity of the iodinated MoAb

The ability of the ^{131}I-labeled MoAb to bind human CT was compared with that of unlabeled MoAb by the ELISA procedures. Fig 1 shows that the ^{131}I-labeled MoAb bound CT in a manner similar to that of the unlabeled MoAb, which suggested that the iodination procedures did not affect the ability of the MoAb to bind CT.

In vivo distribution studies

Fig 2 shows the ratio of ^{131}I-labeled MoAb counts over the tumor compared with that over the abdomen during one hour to 12 days postinjection. The uptake of the tumor increased gradually after radiolabeled MoAb administration and reached a plateau between the sixth and eighth days. We therefore selected the sixth day postinjection as the time for studying the distribution of the radiolabeled MoAb. Fig 3 shows the dose-uptake relationship of the ^{131}I-labeled MoAb in the tumor on the sixth day after injection. A linear increase of uptake by the tumor occurred between 6 and 38 μg (40 and 250 μg/kg), and localization of radiolabeled MoAb was proportionally related to the weights of tumors when these ranged from 150 to 8,250 mg (Fig 4). Consequently, no significant difference was seen in the uptake of MoAb per unit weight of MTC when the tumors were within this range of mass. We interpreted these results to mean that the tumors examined for this study contained no necrotic tissue.

On the sixth day after administration of radiolabeled MoAb, we compared distribution of MoAb in the tumor and several visceral organs. A ^{131}I-labeled irrelevant mouse IgG was included in these studies to serve as control for the nonspecific uptake of ^{131}I in various tissues. Fig 5 summarizes the results. MTC tumors had an uptake of ^{131}I-labeled MoAb of slightly more than 1.0% of the injected dose per gram of tumor, whereas the major visceral organs had uptake values ranging from 0.05% to 0.54%. Distribution ratios of the radiolabeled MoAb were 1.2:1 for tumor-to-blood and 2-20:1 for tumor-to-organs. In the tumor-bearing rats injected with the ^{131}I-labeled control IgG, the radioactivity distribution ratios were 0.4:1 for tumor-to-blood and 0.6-2:1 for tumor-to-organs.

Table
Uptake of Radiolabeled MoAb in Allografted MTC and Normal Tissues in Rat Relative to Blood Concentration

Organ	^{131}I-labeled MoAb (cpm/g)	^{111}InCl (cpm/g)	^{131}I/^{111}In Ratio*
Blood	3.12×10^6	1.36×10^5	1.00
Muscle	5.25×10^5	0.60×10^4	3.82
Stomach	1.89×10^5	1.49×10^4	0.55
Spleen	1.22×10^6	2.23×10^4	2.39
Kidney	1.41×10^6	1.16×10^5	0.53
Liver	1.98×10^6	3.27×10^4	2.65
Lung	1.72×10^6	6.79×10^4	1.10
Tumor	3.79×10^6	1.08×10^4	15.30

*Tissue/blood ratio.

Since vascularization and blood flow in tumors and organs may differ, Gautvik et al (13) suggested that the difference in blood volume of tumor and organs should be considered in evaluations of the affinity of antibody to its antigen in vivo. We measured the blood distributions of the tumor and the organs using intravenous injections of ^{111}In. Uptake of ^{131}I-labeled MoAb in the tumor and organs was expressed as the ratio of ^{131}I/^{111}In, the blood ratio being set as equal to one. The data in the Table, which lists the relative uptakes of radiolabeled MoAb in tumor and organs in a typical experiment, demonstrate that MTC tumor had an uptake ratio 15 times above that of blood.

Discussion

CT is the most specific and sensitive marker of MTC (18). Our study of the in vivo distribution of a MoAb against CT in MTC-bearing rats showed that the MoAb localize in the tumor, and

that the tumor/blood ratio is about two times higher than the ratio of a polyclonal counterpart, as reported by Gautvik et al (13). One would expect a high degree of variation in antibody distribution among different monoclonal or polyclonal preparations. Some MoAbs may be capable of more effective tumor localization than other preparations. Further studies comparing the activities of other antibody preparations against various epitopes are required.

One striking difference in the results of our study and that of Gautvik et al (13) concerned the radioiodination of the antibodies. The Gautvik group found that radioiodination, using the chloramine-T method under conditions that were not specified, resulted in products that did not bind antigens. In our study, when the MoAb was iodinated by the chloramine-T method using mild conditions, the labeled antibody bound to the antigen in a manner similar to that of the unlabeled antibody, which indicated that the integrity of the antibody required for binding the antigen survives iodination. We speculate that differences in the nature of the proteins and in the conditions of iodination may have contributed to the discrepancy.

The distribution of antibodies in vivo is undoubtedly related to their binding characteristics, to blood supplies to the tumor and organs, and to metabolic clearance of the antibodies. As the Table shows, when ratios of blood supplies to the tumor and major organs were considered together, the affinity of the MoAb for the tumor seemed to be much higher than for blood or for normal tissues. The nonspecific uptake by the visceral organs may represent the metabolic clearance of MoAb, the presence of cross-reacting antigens in these organs, or microscopic metastasis to these organs, although distant metastases were not apparent in the animals studied. One reason for the relatively low proportion of MoAb localized to tumor (about 1% of injected ^{131}I-labeled MoAb per gram) may be that CT is a secretory antigen that is constantly released into the blood stream. Whether or not a MoAb against certain cell surface antigens of MTC would localize more efficiently remains to be investigated.

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