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Radioimmunoscintigraphy of xenografted human thyroid carcinoma

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We developed monoclonal antibodies against human thyroid cancer-associated antigen by fusing mouse myeloma cells with mouse spleen cells immunized by insoluble fraction of homogenized thyroid papillary carcinoma cells. One monoclonal antibody (KTC-3, IgM) was selected to evaluate basic usefulness for radioimmunoscintigraphy in xenografted human thyroid carcinoma. KTC-3 was labeled with ^{131}I by Iodogen method of 20 to 1 Iodogen to IgM molar ratio. It was also labeled with ^{111}In by cyclic DTPA anhydride method of 20 to 1 DTPA to IgM molar ratio. The labeling efficiency and specific activity for ^{131}I labeling were 16.5% and 0.66 mCi/mg IgM respectively, and those for ^{111}In labeling were 12.7% and 1.6 mCi/mg IgM. Imaging and biodistribution of labeled KTC-3 were evaluated in nude mice bearing thyroid anaplastic carcinoma (THC-5-JCK). The tumors were well visualized 3 and 5 days after injection of ^{131}I KTC-3. Tumor uptake of ^{131}I KTC-3 on day 7 was $0.52\pm 0.27\%$ ID/g and tumor to blood ratio was 1.98 ± 0.76 (n=6). Those of ^{111}In KTC-3 were $0.88\pm0.09\%$ ID/g and 5.51 ± 3.36 (n=6). In conclusion, KTC-3 is promising for radioimmunoscintigraphy of thyroid cancer.

Key words: Radioimmunoscintigraphy, Monoclonal antibody, Thyroid cancer

INTRODUCTION

SINCE THE INTRODUCTION of monoclonal antibodies (MoAb), their use in radioimmunoscintigraphy (RIS) has been widely reported. Clinical applications of MoAb reported are mostly for patients with malignant melanoma, colorectal cancer, ovarian cancer, lymphoma, prostatic cancer, neuroblastoma, breast cancer and several other cancers.[1] For thyroid cancer, RIS using anti-thyroglobulin antibody has been reported.[2]

We developed MoAb against thyroid cancer-associated (near cancer-specific) antigen for RIS and radioimmunotherapy (RIT).[3] Thyroid cancer with multiple metastases is known to be treatable by administration of large doses of ^{131}I; however, quite a large number of patients become refractory to this therapy. We, nuclear medicine physicians, often encounter such occasions in daily clinical practice. The final goal of this project is to examine and treat these patients. The purpose of this paper is to evaluate the basic usefulness of our MoAb in xenografted human thyroid carcinoma.

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MATERIALS AND METHODS

Production of antibody

The insoluble lipoprotein fraction of human thyroid papillary adenocarcinoma cells growing *in vitro* (TPC-1), which had been established in our laboratory before, was extracted and purified.[4,5] The hybridoma was produced by fusing spleen cells from BALB/c mice immunized by this insoluble fraction with mouse myeloma NS-1 or SP-2 cells.[6] Four different types of MoAb (KTC-1 to 4) were obtained. Of the four, KTC-3 (IgM) was selected for this study because of its superior characteristics and easy availability. By enzyme-linked immunosorbent assay (ELISA), KTC-3 was confirmed to react with both papillary and anaplastic human thyroid carcinoma

and not to cross-react with normal thyroid tissue or non-thyroidal carcinoma cell lines such as stomach, colorectal, pancreatic, pulmonary, renal cancer and so on (13 different types of cancer, 21 cell lines). The isolation of the antibody from spent tissue-culture medium or mouse ascites was achieved by sedimentation in saturated ammonium sulfate or 0.005 M phosphate buffer of increasing pH and subsequent gel filtration through Sepharose 6B (Pharmacia) column with a solvent of 0.01 M Tris-HCl 0.15 M NaCl buffer pH 7.3 or TSK 4000 SWXL (Toyo Soda) HPLC column with a solvent of 0.1 M phosphate buffer pH 7.0.

Radiolabeling of antibody KTC-3

KTC-3 was labeled with ^{131}I by Iodogen method.[7] A glass tube was coated with 25 μg Iodogen (Pierce Chemical) by dissolving in 1 m*l* methylene chloride and evaporating to dryness. A mixture of 2.5 mg KTC-3 (20: 1 Iodogen to IgM molar ratio) and 370 MBq (10 mCi) $Na^{131}I$ (Amersham) raised to a final volume of 1 m*l* by 0.01 M phosphate 0.07 M NaCl buffer (PBS) pH 7.3 was incubated at room temperature for 1 hour. Free iodide was removed from the labeled antibody by filtration through Sephadex G25 column (Pharmacia).

KTC-3 was also labeled with ^{111}In by cyclic DTPA anhydride method.[8] Conjugation of DTPA to the antibody was performed by mixing 0.25 mg KTC-3 dissolved in 100 μl 0.1 M bicarbonate buffer pH 8.2 and 2 μg cyclic DTPA anhydride (Dojin Chemical) dissolved in 5 μl dimethyl sulfoxide (20: 1 DTPA to IgM molar ratio). After incubating for 1 hour, 2 m*l* 0.1 M citrate buffer pH 5.5 and 3 mCi/2 m*l* $^{111}InCl_3$ solution (Nihon Medi-physics) were added and incubated for an additional 1 hour. Unbound ^{111}In was removed by centrifugal microconcentrator, Centricon 30 (Amicon).

Labeling efficiency was determined by trichloroacetic acid precipitation for ^{131}I labeling and direct counting of the residue and filtrate after the centrifugal separation for ^{111}In labeling.

Evaluation for immunoreactivity

Immunoreactivity after radionuclide labeling was evaluated by live cell assay using TPC-1; 1×10^6 cells/100 μl culture medium were suspended in a test tube. Radiolabeled KTC-3, 10 ng/50 μl PBS containing 0.5% BSA was added and incubated for 1 hour at 4°C. Centrifugation and washing with 1 m*l* PBS containing 0.5% BSA were repeated twice. After counting the radioactivity of the cell pellet, the result was expressed as % total applied dose.

Imaging and biodistribution studies

Established human thyroid anaplastic carcinoma (THC-5-JCK) growing in nude mice was implanted by trocar in the right flank of 4-to 6-week-old female BALB/c nude mice (nu/nu). This carcinoma had been already confirmed to react with KTC-3 by immunohistochemical staining. The tumors were allowed to reach a size of 1–2 cm in diameter before the experiment. Under light anesthesia by ether or pentobarbital, the mice were injected with 180 μCi/270 μg ^{131}I KTC-3 or 46 μCi/30 μg ^{111}In KTC-3 in each mouse through the tail vein. The mice were imaged by a gamma camera equipped with pinhole collimator 1, 3, 5, and 7 days after injection and killed by exsanguination just after the final imaging. Dissected tumor and organs were weighed and counted for radioactivity. The results were expressed as percentage of the injected dose per gram of tissue (% ID/g) and tumor to blood or muscle ratio.

Control studies

Because free iodide is known to accumulate in thyroid carcinoma (well differentiated type), biodistribution of $Na^{125}I$ (Amersham) was evaluated for a control study in this tumor model. Imaging and biodistribution study of ^{131}I labeled non-specific mouse IgM was another control study. The non-specific mouse IgM was separated by TSK 4000 SW HPLC column from mouse immunoglobulin (Cooper Biomedical). The purified mouse IgM was labeled by a similar method to that of KTC-3.

RESULTS

KTC-3 was adequately labeled by both ^{131}I and ^{111}In. The labeling efficiency and specific activity of ^{131}I KTC-3 were 16.5% and 0.66 mCi/mg IgM respectively, and those of ^{111}In KTC-3 were 12.7% and 1.6 mCi/mg IgM. Immunoreactivity after radiolabeling was 30% for ^{131}I labeling and 16% for ^{111}In labeling.

Sequential gamma camera images of a mouse bearing human thyroid anaplastic carcinoma after injection of ^{131}I KTC-3 are shown in Fig. 1. Though the blood pool and tissue background activity were dominant on the image one day after injection, the tumor in the right flank was well visualized in the later images after the non-tumor activity had been cleared. Thyroid uptake, which was not blocked, was also seen.

Sequential gamma camera images of a mouse bearing human thyroid anaplastic carcinoma after injection of ^{111}In KTC-3 are shown in Fig. 2. Compared to the images of ^{131}I KTC-3, those of ^{111}In KTC-3 showed high uptake of radioactivity into liver, spleen, and kidneys from the initial image. Uptake of ^{111}In to the tumor was faintly seen in the later images.

Table 1 shows the biodistribution of ^{131}I KTC-3, ^{111}In KTC-3, ^{131}I mouse IgM, and Na^{125} seven days

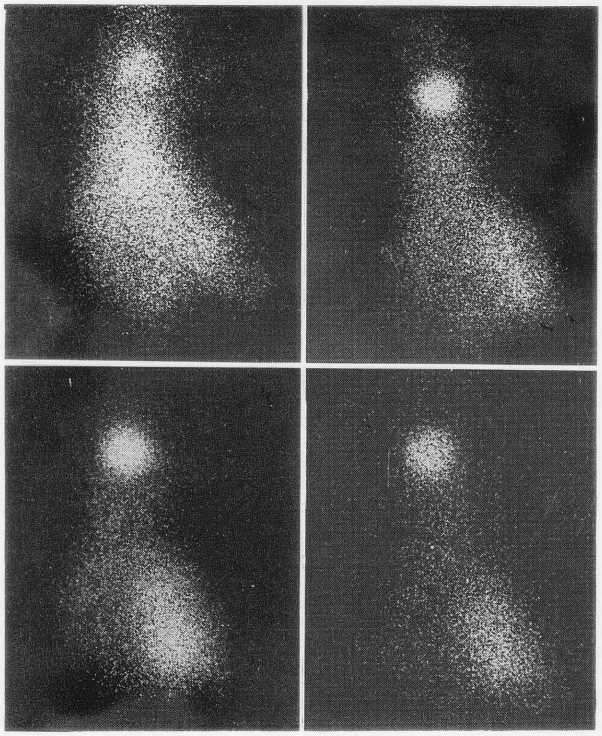

Fig. 1 Sequential gamma camera images of a mouse bearing human thyroid anaplastic carcinoma implanted in the right flank 1 day (left top), 3 days (right top), 5 days (left bottom), and 7 days (right bottom) after injection of ^{131}I KTC-3. The tumor was well visualized 3 and 5 days after injection.

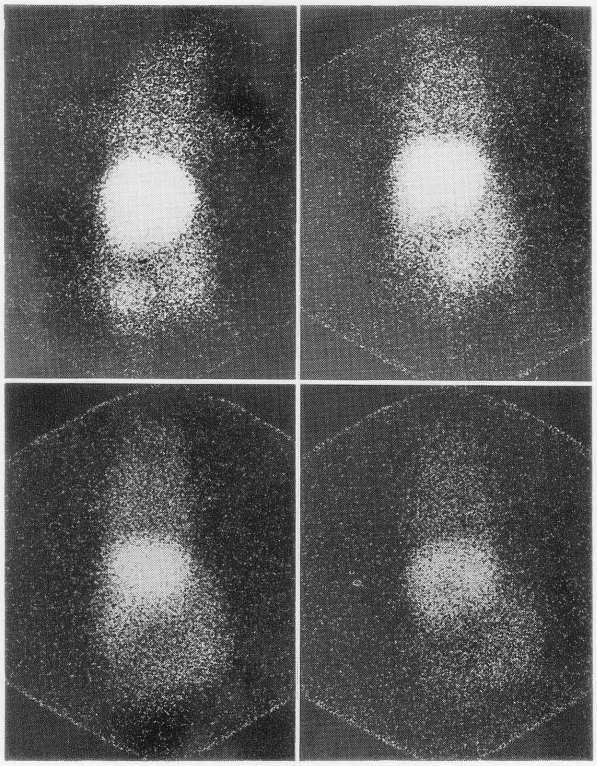

Fig. 2 Sequential gamma camera images of a mouse bearing human thyroid anaplastic carcinoma implanted in the right flank 1 day (left top), 3 days (right top), 5 days (left bottom), and 7 days (right bottom) after injection of ^{111}In KTC-3. The uptake to the liver, spleen, and kidneys was high though the tumor was only faintly visible.

Table 1 Biodistribution of ^{131}I KTC-3, ^{111}In KTC-3, ^{131}I mouse IgM, and Na ^{125}I seven days after administration of each preparation to a separate group of six tumor bearing mice

	^{131}I KTC-3	^{111}In KTC-3	^{131}I mouse IgM	Na^{125}I
Tumor	0.52±0.27	0.88±0.09	0.11±0.01	0.013±0.008
Blood	0.25±0.05	0.16±0.06	0.10±0.01	0.004±0.006
Muscle	0.07±0.03	0.67±0.30	0.02±0.003	0.017±0.019
Liver	0.10±0.03	7.92±0.81	0.22±0.07	0.009±0.004
Spleen	0.07±0.02	4.43±1.41	0.14±0.03	0.006±0.003
Kidney	0.10±0.02	10.9±3.10	0.11±0.02	0.011±0.007
Lung	0.11±0.02	0.68±0.24	0.12±0.07	0.010±0.006
Heart	0.08±0.02	0.93±0.18	0.03±0.004	0.005±0.003
Stomach	0.12±0.06	0.84±0.23	0.06±0.03	0.061±0.068
Thyroid	155±17.4	—	313±47.4	252±33.8
Bone	—	1.37±0.22	—	—

Mean±SD % ID/g n=6

Table 2 Tumor to blood and tumor to muscle ratios of ^{131}I KTC-3, ^{111}In KTC-3, ^{131}I mouse IgM, and Na ^{125}I seven days after administration of each prepartion to a separate group of six tumor bearing mice

	^{131}I KTC-3	^{111}In KTC-3	^{131}I mouse IgM	Na ^{125}I
Tumor/blood	1.98±0.76	5.51±3.36	1.06±0.17	2.82±0.95*
Tumor/muscle	7.65±3.63	1.31±0.76	6.50±0.70	2.55±3.07**

Mean±SD n=6 (*n=4, **n=5)

after administration of each preparation to a separate group of six tumor-bearing mice. Table 2 shows the tumor to blood and tumor to muscle ratios of these agents seven days after administration. Absolute tumor uptake (0.88% ID/g) and tumor to blood ratio (5.51) of ^{111}In were both greater than those (0.52% ID/g and 1.98 respectively) of ^{131}I ($P<0.05$) though the gamma camera images showed poorer tumor visualization by ^{111}In KTC-3. Though the absolute tumor uptake of ^{111}In KTC-3 was high, other organs such as liver, spleen, and kidney also accumulated the agent. The difference of the uptake into liver, spleen, and kidney between ^{111}In and ^{131}I was apparent. Low absolute tumor uptake of Na^{125}I (one-fortieth of that of ^{131}I KTC-3) verified that xenografted thyroid carcinoma in our experiments does not accumulate free iodide. Absolute tumor uptake and tumor to blood ratio of ^{131}I labeled non-specific mouse IgM were significantly lower than those of ^{131}I KTC-3; that is, localization index[9] was calculated to be 1.87. These results reflected the specific localization of the labeled KTC-3 in the tumor.

DISCUSSION

Many anti-human cancer MoAb have been produced and reported; however, quite a large number of them are known to cross-react with other types of cancer than the cancer used for immunization probably due to the existence of common antigens among different types of cancer. KTC-1 to 4, anti-thyroid cancer MoAb produced in our laboratory, are considered to be exceptionally quite specific for thyroid cancer based on intensive evaluation for cross-reactivity to other types of cancer using established human cancer cell lines. More evaluation for cross-reactivity is now being done using surgical specimens of different types of cancer. Interestingly, KTC-3 reacts with not only thyroid differentiated papillary carcinoma but also thyroid anaplastic carcinoma. The antigen recognized by KTC-3 was determined to be a protein of molecular size of 61.500 dalton and might be a common antigen among thyroid cancer of different histological types. Thyroid differentiated cancer sometimes shows anaplastic transformation during its course. Effectiveness of RIS and RIT using KTC-3 might not be affected even in such a case.

Immunoglobulin class of KTC-3 was determined to be IgM. Application of IgM for RIS reported to date is rather rare. One reason might be the difficulty involved in its purification.[10] There are many established purification methods for IgG but not so many for IgM. Our method, however, especially sedimentation in a solution of low ionic strength following separation by TSK 4000 SWXL column was very effective for the purification of IgM. Another reason for the rare utility of IgM might be its poor labeling efficiency. In this study, indeed, labeling of KTC-3 with ^{131}I or ^{111}In was not perfect. However, our preliminary evaluation of labeling conditions disclosed that the attainable labeling efficiency was near 30% for ^{131}I and 50% for ^{111}In (data not shown) by increasing Iodogen to IgM molar ratio[11] or antibody concentration in the reaction mixture for DTPA conjugation.[12] In spite of these demerits of IgM, we cannot help using IgM for RIS on certain occasions. That is, IgM is often the class of human monoclonal antibodies[13] most ideal for application in human studies to avoid hazardous anaphylactic effect. Moreover, IgM is known to be excreted more rapidly than IgG.[14,15] This might be a good point for early tumor visualization in RIS.

Preservation of immunoreactivity after labeling is also a key to success in RIS. Immunoreactivity after iodination in this study was fairly preserved although that after indium labeling was not so good. Indium labeling has yet to be evaluated for better immunoreactivity; the preliminary evaluation disclosed that the attainable immunoreactivity after indium labeling was near 30% (data not shown) by decreasing DTPA to IgM molar ratio.[16] Low immunoreactivity might be a cause of poor visualization of the xenografted tumor by ^{111}In KTC-3. However, it showed better absolute tumor uptake than ^{131}I KTC-3 as generally reported.[17] Whether an implanted tumor is well visualized or not is known to depend on not only the absolute tumor uptake of the agent but also tumor to blood or muscle ratio. Biodistribution of ^{111}In KTC-3 showed a better tumor to blood ratio but poorer tumor to muscle or tumor to other organs ratio compared with that of ^{131}I KTC-3. This might be a more convincing cause for poor visualization of the tumor by ^{111}In KTC-3. Tumor to muscle ratio of ^{131}I mouse IgM was rather high probably due to rapid clearance of the agent from the whole body. However, this did not contribute to good tumor visualization probably because both absolute tumor uptake and tumor to blood ratio of the agent were rather low.

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