Radioimmunoimaging of colorectal cancer using ^{99m}Tc-labeled monoclonal antibody *

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Abstract

AIM To determine whether Hb3 and its fragment F(ab')$_2$ have practical value in radioimmunoimaging of colorectal cancer.

METHODS Intact Hb3 was purified by hydroxylapatite chromatography. The fragment F(ab')$_2$ was prepared by cold digestion and purified as intact Hb3. Hb3 and its fragment F(ab')$_2$ were labeled with 99mTc by direct labeling method using SnCl2 as reducing agent. The radioactive doses ranged from 15 to 40 mCi. The imaging was accomplished by single photon emission computered tomograph (SPECT) with imaging time ranging from 2.5 to 48 hours. In this study, 10 patients were selected. Among them, 7 were administered with intact Hb3, and 3 with F(ab')$_2$ fragment. All the patients were diagnosed as having colorectal adenocarcinoma.

RESULTS After purification, intact Hb3 and its fragment F(ab')$_2$ were fit for radioimmunoimaging. The percentage of labeling of ^{99m}Tc to Hb3 or F (ab')$_2$ was 80.6%-91.5%. Among the 10 patients, 3 of 7 patients administered with intact Hb3 had positive scans, the other 4 had negative scans, and 2 of 3 patients administered with F(ab')$_2$ had positive scans, the other 1 had negative scans.

CONCLUSION The results showed that both intact Hb3 and its F(ab')$_2$ have some practical value in radioimmunoimaging of colorectal cancer, and the effects of imaging with F(ab')$_2$ was better than that with intact Hb$_3$.

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INTRODUCTION

Colorectal cancer is a common malignant disease. In recent years, there have been many reports about radioimmunoimaging used to diagnose the colorectal cancer, but antibodies used were mainly the anti-CEA monoclonal antibodies. Hb3 is an anti-colorectal cancer monoclonal antibody produced by our laboratory, its sensitivity and specificity are superior to that of anti-CEA[1]. The positive rate of radioimmunoimaging with 131I labeled Hb3 in nude mice model of colorectal cancer was 92.4% (13/14)[2]. This result shows that Hb3 may be useful in clinical diagnosis of colorectal cancer. Based on the preparation of intact Hb3 and its fragments, clinical radioimmunoimaging has been done in 10 patients, the results showed that Hb3 is valuable in clinical practice.

MATERIALS AND METHODS

Preparation and purification of Hb3

The Hb3 ascites was prepared routinely and collected under aseptic condition. Hb3 was purified by hydroxylapatite chromatography (Sigma)[3]. In the process of purification, aseptic condition and pyrogen free condition were maintained.

Digestion of Hb3 to prepare F(ab')$_2$

Cold digestion was performed by modified Ballou method. Briefly, intact Hb3 solution(5 μg/L-10 μg/L), was adjusted to pH 4.0-4.2 with 0.3N acetate buffer, and pepsin (Sigma) in 0.3N acetate buffer (pH 4.0) was added to a final ratio of 1:10 (pepsin: Hb3, Wt/Wt). The digestion was allowed to proceed for 24-34 hours at 4℃. The reaction was terminated by adding 1M NaOH to bring the pH to 7.8-8.0. The F(ab')2 fragment was purified using hydroxylapatite chromatography, and the yield of F(ab')2 was calculated (Wt/Wt).

Detection of purity of Hb3 and F(ab')$_2$

The purity of Hb3 and its fragment F(ab')$_2$ were detected by SDS-PAGE using Multiphor II Electrophoresis System (Pharmacia). The concentration of stacking gel was 3%, and resolving gel 7.5%. The constant current was 50mA, the temperature 15℃, and the running time 2 hours. After electrophoresis, the gel was stained immediately using Coomassie Blue and dried in vacuum condition and reserved in room temperature. Before electrophoresis, 2-

mercaptoethanol was added to the sample solution, and the samples then boiled in water bath for 3 minutes.

Labeling of Hb3 and its fragment F(ab')$_2$ with ^{99m}Tc

The modified Paik's direct labeling method was used[5]. Briefly, monoclonal antibody Hb3 and its F(ab')$_2$ (2mg-8mg) were adjusted to pH 4.0-4.2 with 0.3N acetate buffer (pH 4.0), then added with freshly prepared SnCl2/HCl and mixed, the antibody-SnCl2 mixture was incubated for 15-30 minutes at room temperature, then freshly prepared 15mCi-40mCi ^{99m}Tc was added, reaction of antibody with technium was maintained for 0.5-1.0 hour at room temperature.

Detection of labeling efficiency

The free-form and combined ^{99m}Tc was detected with G-50 chromatography (0.9cm×40cm, Sephadex), and the radioactivity of free-form ^{99m}Tc (FT), combined ^{99m}TC (CT), and G-50 column absorbed ^{99m}Tc (AT) was measured with radioactivity meter. The percentage of labeling and radiochemical purity of antibody were calculated (because the labeled antibody was used directly without further purification, percentage of labeling was equal to radiochemical purity). The immunoreactivity of antibody before and after labeling and the fraction of immunoreactivity in G-50 chromatography were detected. Percentage of labeling = radiochemical purity = CT/CT+FT+AT

Indirect ELISA to detect specific antibody Hb3 and its F(ab')$_2$

Wells of plate were coated with 50 μg colorectal cancer crude antigen. The antibodies to be detected were Hb3 and its fragment F(ab')$_2$. The anti-Ig was rabbit anti-mouse IgG+IgM+IgA conjugated with horseradish peroxidase (diluted 1:1 000). The substrate was ABTS. OD_{405} value was obtained using Microplate Autoreader (EL309, BIO-TEK).

Clinical cases and imaging

Ten patients (6 males, 4 females, aged from 36 to 74 years, averaging 54.2) were selected (Table 1). Seven patients suffered from rectal cancer, and one of them was complicated with liver metastasis. Three patients had colonic cancer. All of the patients proved to have adenocarcinoma by histopathology and the results of immunohistochemistry of biospies with Hb3 were positive. The amount of antibodies used were 2mg-8mg, and the radioactivity of ^{99m}Tc used was 15mCi-40mCi.

Radioimmunoimaging was performed with GE STARCAM 3 000/4 000 SPECT system. ^{99m}Tc labeled antibodies were diluted in 100ml 0.9% NaCl solution, then injected into the patients iv. The anterior, posterior, left, and right side planar scanings were taken 3, 6, 24 and 48 hours later. Tomography was performed if the planar imaging is questionable. Thirty-two or 64 angles were recorded in each tomogram.

RESULTS

Preparation of Hb3 and its fragment

The hydroxylapatite chromatogram of Hb3 ascite showed three peaks. The third peak was IgM and possessed immunoreactivity[3]. After digestion, the chromatogram showed three peaks as well. The first was low molecular weight peptides, the second was F (ab')$_2$, and the third was intact IgM. Among them, the second and the third peaks possessed immunoreactivity (Figure 1). After digestion, the F(ab')$_2$ yield was 25. 0%-36.6%, and the immunoreactivity of F(ab')$_2$ was lower than that of intact Hb3.

SDS-PAGE

As showed in Figure 2, there were two bands in the lane of intact Hb3 denaturated and broken, their molecular weights were about *Mr* 72 000 and Mr 23 000, and they represented H chain and L chain, respectively (lane 2-5, the bands with molecular weight higher than *Mr* 94 000 were contaminated proteins). In the lane of F(ab')$_2$ (unbroken, lane 7), two bands were found, and their molecular weights were about Mr 130 000 and *Mr* 65 000, and represented F(ab')$_2$ and Fab' respectively. In the lane of F(ab')$_2$ (broken, lane 6), there were two bands as well, and their molecular weight were about *Mr* 43 000 and *Mr* 23000, and represented VH+CH1 and L chain respectively.

The labeling of antibody with ^{99m}Tc

99mTc Labeled Hb3 was analyzed by G-50 chromatography. The percentage of labeling and radiochemical purity was 80.4%-91.5%. In the chromatogram, the fractions of immunoreactivity of labeled antibody overlapped the fractions of radioactivity (Figure 3). After the antibodies were labeled, their immunoreactivity was 90% more than that of unlabeled antibodies.

Imaging

Radiommunoimaging was performed in 10 patients, 5 had positive images, and 5 negative images (Table 1), with no side effects. Patient No. 4 suffered from rectal carcinoma complicated with liver metastasis with positive images at 6 and 24 hours. Figure 4 (upper left) shows an unclear abnormal radiation concentration under bladder in 6 hour. In 24 hour scan (lower left), there was a clear abnormal radiation concentration in the left lobe of liver.

Table 1 Clinical data and imaging results

No.	Sex	Age (year)	Clinical diagnosis	Tumor size(cm)	Pathological diagnosis	Immunohisto chemistry	Quantity antibody (mg)	of Radioactivity of ^{99m} Tc (mCi)	Imaging
1	Male	74	Rectal carcinoma	3.0×5.0	Adenocarcinoma	+++	3.0	23	+
2	Female	47	Rectal carcinoma	1.8×2.0	Papilloadenocarcinoma	++	5.0	20	-
3	Male	35	Rectal carcinoma	3.0×4.5	Adenocarcinoma	++	7.5	19	-
4	Male	67	Rectal carcinoma	3.5×4.5	Low differentiated adenocarcinoma	+++	7.5	16	+
5	Female	54	Colonic carcinoma	4.0×5.0	Adenocarcinoma	++	8.0	15	-
6	Female	55	Rectal carcinoma	2.5×3.5	Adenocarcinoma stage II	++	5.0	19	+
7	Male	61	Colonic carcinoma	2.5×3.5	Adenocarcinoma	+	5.0	40	-
8**	Male	38	Rectal carcinoma	3.5×5.0	Adenocarcinoma	++	5.0	38	+
9**	Male	65	Colonic carcinoma	2.5×3.0	Adenocarcinoma stage II	++	2.0	15	-
10**	Female	44	Rectal carcinoma	2.5×3.0	Adenocarcinoma stage II	++	5.0	18	+

"+" partial weak staining; "++" partial strong staining or broad weak staining; "+++" broad strong staining.complicated with liver metastasis. The antibodies used were fragment F(ab')$_2$.

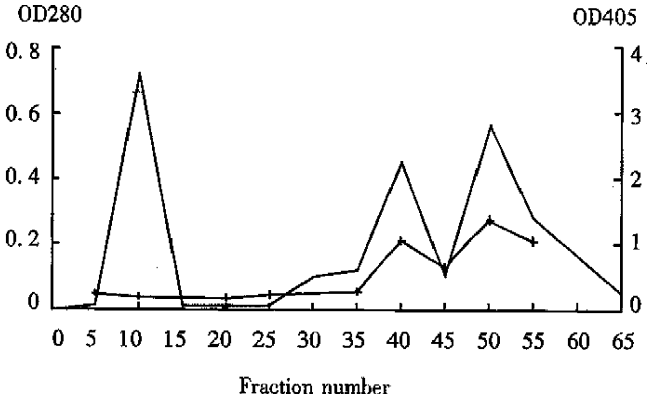


Figure 1 Chromatogram of digested Hb3. OD_{280} (i¤ - i¤) shows concentration of protein. OD_{405} (+ - +) shows immunoreactivity.

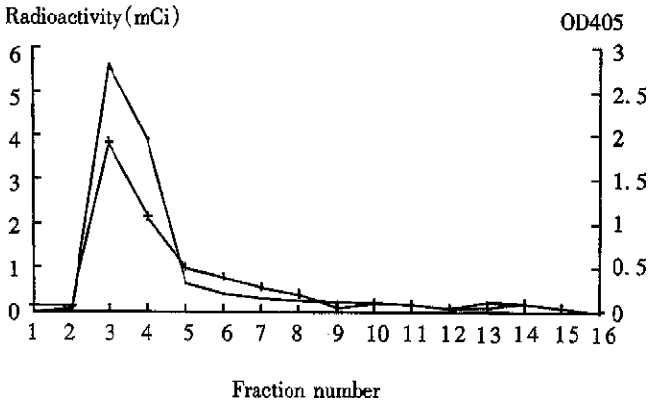
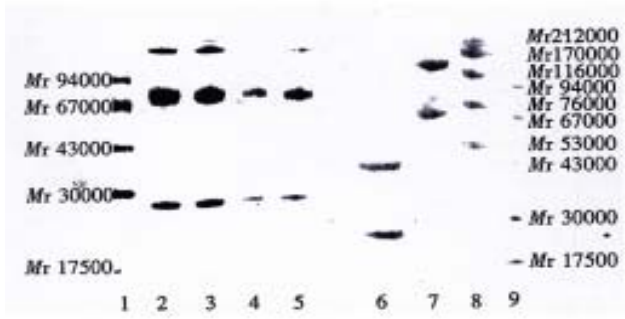


Figure 3 Chromatogram of ^{99m}Tc labeled antibody, OD_{405} shows immunoreactivity. i¤ - i¤ radioactivity, + - + immunoreactivity.



Lane 1: low molecular weight marker; lane 2-3: intact Hb3; lane 4-5: intact Hb3 (1/10 concentration); lane 6: Hb3 fragments F(ab')$_2$ (broken); lane 7: Hb3 fragments F(ab')$_2$ (unbroken); lane 8: high molecular weight marker; lane 9: low molecular weight marker.

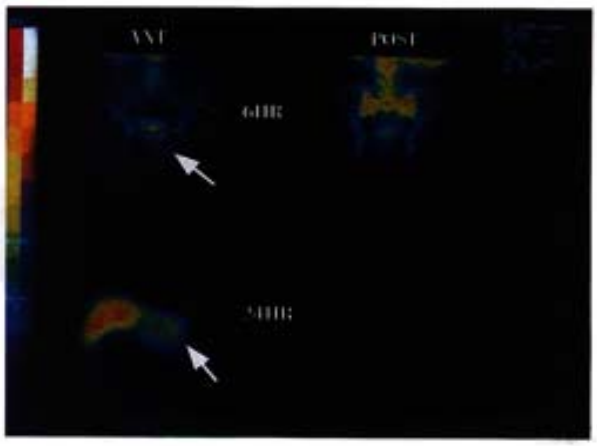


Figure 4 Images of Patient 4. Rectal cancer complicated with liver metastasis.
Anterior scan (6 hour) shows an unclear abnormal radiation concentration under the bladder (upper left). Anterior scan (24 hour) shows a clear abnormal radiation concentration in the left lobe of liver (lower left).

DISCUSSION

Purification of antibody and preparation of fragment

Hydroxylapatite chromatography is simple. The column is short and is convenient for aseptic and pyrogen free treatment. The purity of antibody after purification was fit for clinical usage. As reported, antibody can be digested to $F(ab')_2$ by cold and warm digestion. In our study we selected cold digestion to prepare $F(ab')_2$. The yield of $F(ab')_2$ varied from 25.0% to 36.6%, lower than that prepared by Ballou[4] (cold digestion, 40%-60%), but higher than that prepared by Kurkela[5] (warm digestion, 24%±11%). As a matter of fact the prepared fragments contained $F(ab')_2$ and Fab' (Figure 2), because pepsin digestion canproduce these two kinds of fragments, and it is difficult for hydroxylapatite chromatography to separate them.

Labeling of Hb3 with ^{99m}Tc

Direct labeling of antibody is to combine the reduced ^{99m}Tc with antibody through the disulfied linkage. Its advantage is that the labeling process can be finished in one step, which is beneficial for making a marketable radioimmunoimaging kit. In this study, we selected SnCl2 as reducing agent, and labeled antibody with ^{99m}Tc using direct method. The reaction was performed in one step.

Imaging

Hb3 is an IgM monoclonal antibody against CA-Hb3. The results of immunohistochemistry showed that the positive rate of Hb3 reacting with colorectal carcinoma was high, ant it did not react with normal colorectal tissue [1]. The results of radioimmunoimaging in nude mice model of colorectal carcinoma also showed a higher imaging efficiency[2]. Among the 10 patients in this study, five got positive images. The reasons why the positive rate is not high may be: ① The effect of antibody molecular weight: The molecular weight of intact Hb3 is Mr950 000, its half-life and optimum imaging time are longer than 48 hours, yet the half-life of ^{99m}Tc is 6 hours, and the imaging is required to be performed within 24 hours. In his study, one of the radioimmunoimagings was performed in a transverse colon cancer patient with intact Hb3, the imaging result at 24 hour was negative. Fourty-eight hours later, the resected tumor sample was scanned by SPECT. In the circumstance of prolonging the acquisition time (30 minutes), the radioactivity of tumor was higher than that of the surrounding normal tissues. This evidence showed that the inconsistency of half-life between antibodies and radionuclide was unfavorable to imaging. The molecular weight of $F(ab')_2$ is 1/7 that of intact Hb3, and its half-life is 26 hours, which is more consistent with that of ^{99m}Tc, and its imaging efficiency is higher than that of intact Hb3. ② Effect of free ^{99m}Tc: Free ^{99m}Tc (5.3%-8.6%, Figure 3) can pass through gastric mucosa accompanying the secretion of gastric juice, and get into the gastrointestinal tract through the peristalsis, resulting in false positiveness[7]. Among the 10 patients in this study, negative images were found in the intestinal tract of four patients. Accumulation of Tc-Sn colloids (absorbed by column G50, 3.2%-11. 0%, Figure 3) can also influence tumor imaging. ③ The effect of HAMA (human anti-mouse antibody): Antibody Hb3 is mouse-derived, it can cause HAMA after being injected into human bodies. This reaction will interfere with the combination of antibody with antigen, and then influence the imaging. In this experiment, the duration from antibody injection to imaging was short, so HAMA was not the major influencing factor.

In conclusion, we consider that Hb3 is a valuable monoclonal antibody in radioimmunoimaging of colorectal carcinoma. The imaging effect of $F(ab')_2$ is better than that of intact Hb3. Further study is needed to optimize the labeling condition, increase the labeling rate of Hb3 with ^{99m}Tc, and finally improve the diagnostic value of radioimmunoimaging.

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