



Visualization and body distribution of [¹³¹I]-herceptin in nude mice with BT-474 breast carcinoma

Z.X. Yang*, H. Cao*, C.G. Xing, S.H. Wei, G.Q. Jiang and Z.L. Liu

Department of Surgery, The Second Affiliated Hospital of Soochow University, Suzhou, China

*These authors contributed equally to this study.

Corresponding author: G.Q. Jiang

E-mail: guoqinjiangcn@126.com

Genet. Mol. Res. 13 (3): 6804-6812 (2014)

Received June 6, 2013

Accepted September 25, 2013

Published August 29, 2014

DOI <http://dx.doi.org/10.4238/2014.August.29.1>

ABSTRACT. The study aimed to investigate the bio-distribution and radio-immuno-imaging features of [¹³¹I]-herceptin in nude mice with BT-474 breast carcinoma. [¹³¹I]-Herceptin was administrated by tail intravenous injection to the nude mice with BT-474 breast carcinoma. Radiocounting was performed at 4, 12, 24, 48, and 96 h after administration. The activity ratio in the tumor tissue and non-tumor tissue (T/NT) and the radiocounting percentage per gram tissue to the injected dose (%ID/g) were calculated. The nude mice with BT-474 breast carcinoma were also visualized continuously by single photon emission computed tomography at 2, 4, 8, 12, 24, 48, and 96 h after the injection of [¹³¹I]-herceptin. Nude mice with MDA-MB-231 used as the control group were subjected to the same analyses. Clear tumor images were obtained after the injection of [¹³¹I]-herceptin in nude mice with BT-474 breast carcinoma. The images were the clearest at 24 h after the injection and remained clear even at 96 h. The T/NT ratio and %ID/g in the tumor tissues of nude mice with BT-474 were both significantly higher than those of the control group (P < 0.01). [¹³¹I]-

Herceptin displays tumors clearly in the nude mice with BT-474 and accumulates well in the tumor tissues.

Key words: Breast carcinoma; [¹³¹I]-Herceptin; Biodistribution; Radio-immuno-image; Nude mice

INTRODUCTION

Her-2 receptor (Her-2R) is a member of epidermal growth factor receptor family. The human *Her-2r* gene is located on the long arm of chromosome 17, on 17q21, encoding an 185 kDa transmembrane glycoprotein, which has the activity of protein tyrosine kinase (PTK). Her-2R is over-expressed in about 25% of breast carcinoma tissues (Schechter et al., 1984; Menard et al., 2000), indicating poor biological characteristics, therapeutic efficacy, and prognosis and drug resistance to chemotherapeutics (Nahta et al., 2004; Rae and Lippman, 2004). Herceptin, the molecular drug targeting Her-2, can inhibit the growth of the breast cancers overexpressing Her-2 in several ways (Pegram et al., 1999; Vogel et al., 2001; Cho et al., 2003; Pegram et al., 2004a,b); however, the total effective rate is only 12-24% if herceptin is administrated alone to the late phase of breast carcinoma (Albanell et al., 2003). Some of the Her-2-positive breast carcinomas can develop resistance to herceptin via many mechanisms (Mucohara, 2011; Ali Nahit Sendur et al., 2012). The combination of herceptin with other targeted drugs, such as lapatinib, can somewhat improve herceptin's therapeutic efficacy; however, its application in the treatment of breast cancer is still limited (Brandes et al., 2010).

[¹³¹I] can not only emit β rays that are used to kill malignant tumors but also emit detectable γ rays; hence, it is widely used in the treatment of malignant tumors and radio-immuno-imaging (Goldenberg, 2002). Herceptin-labeled [¹³¹I] has been reported to maintain its immunological activity (Blend et al., 2003). Owing to the limitation of herceptin administration alone in breast carcinoma, combining other targeted drugs with herceptin, especially the radio-immunity therapy targeting Her-2, is gaining wide interest. Combining nuclide with herceptin can accumulate nuclides in tumor tissues and increase the specificity of herceptin to improve the curative effect of nuclides' internal exposure to tumor cells on one hand, and suggest the tumor's location and size by using single photon emission computed tomography (SPECT) on the other hand. It not only reveals micrometastasis that is difficult to be observed by other imaging methods but also can be used to determine the therapeutic effectiveness by using contrast imaging. The activity ratio of tumor tissue and non-tumor tissue (T/NT) is a valid evaluation standard for radio-immuno-imaging and therapeutic efficacy.

In this study, we used herceptin-labeled [¹³¹I] as a radio-immunological drug. By comparing the nuclide's body distribution features and tumor imaging in nude mice with BT-474 breast carcinoma over-expressing Her-2 and those in nude mice with MDA-MB-231 breast carcinoma low-expressing Her-2, we found that [¹³¹I]-herceptin displays tumors clearly in the nude mice with BT-474 and accumulates well in the tumor tissue.

MATERIAL AND METHODS

Animals

In this study, 6-week-old female BALB/c-neu nude mice were used; the animals

weighed about 20 g and were provided by the Experimental Animal Center of Shanghai. All mice were housed in a SPF room with constant temperature of 25°C and humidity of 45%. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The animal use protocol was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Soochow University.

Cell culture

Breast carcinoma cell lines BT-474 and MDA-MB-231 were both propagated in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS; Luo et al., 2000).

Tumor animal model

Cells in the logarithmic growth phase were collected and suspended in FBS-free 1640 medium. The BT-474 cells concentration was adjusted to 2×10^7 cells/mL. Next, each mouse was subcutaneously injected 0.2 mL cells on the right dorsal back. The tumor size was recorded every 3 days. When the diameter of tumors reached 0.8 cm, the breast carcinoma model was considered to be ready for use. The control group including MDA-MB-231 mice model was established in the same way.

Preparation of [¹³¹I]-herceptin

First, 50 µL 21 mg/mL herceptin was dissolved in an Iodogen column and incubated in a 15°C water bath. Next, 25 µL (1 mCi) Na[¹³¹I] (1.48 GBq/mL) was added into the column. The mixture was placed on an electromagnetic stirrer for 15 min, and 300 µL 0.1 M, pH 7.5, PBS buffer was immediately added to the column to stop the reaction. Subsequently, the labeled herceptin was isolated and purified by Sephadex G50, and its purity was detected using Xinhua No.1 chromatography filter paper.

Detection of the immunobinding rate of [¹³¹I]-herceptin

Cell binding test was used to detect the immunological activity of herceptin-labeled [¹³¹I]. BT-474 cells in the logarithmic growth phase were collected and divided into 0.2 mL/tube (1×10^5 cells/tube). Next, 20 µL [¹³¹I]-herceptin diluted with PBS was added to each tube and incubated for 4 h at 4°C. The cells' total radiation activity (T) was measured. After centrifugation at 2000 rpm for 5 min at 4°C and wash with PBS for 3 times, radiation activity (B) of the cell deposition was measured. The immunobinding rate was defined as IB rate = $B / T \times 100\%$. The immunobinding rate of the control MDA-MB-231 cells was measured in the same way. Each test was repeated 4 times.

Visualization

When the diameter of tumor reached 0.8 cm, 5 nude mice with BT-474 tumor were used to obtain tumor images. In order to block the action of thyroid gland, 0.1% KI dissolved

in 25% glucose was administered to the mice 3 days before detection. Tail intravenous injection of 0.1 mL/3.7 MBq [¹³¹I]-herceptin was administered, and BT-474 tumor images were obtained at 2, 4, 8, 12, 24, 48, and 96 h by using the pinhole high-energy collimator of SPECT (PHILIPS, USA). In the same manner, MDA-MB-231 tumor images were obtained.

Analysis of the nuclide body distribution

Each mouse was administrated 0.1 mL/0.74 MBq [¹³¹I]-herceptin by tail intravenous injection. At 4, 12, 24, 48, and 96 h after the injection, mice were killed. Each time point had 5 mice in this study. Blood, heart, liver, spleen, lung, kidney, skeleton, muscles, and tumor tissues of each mouse were collected. After the organs or tissues were flushed with purified water and dried, their weights were recorded. The radiocounting per minute (cpm) of each organ or tissue was measured using a counting-meter (SN-6958). The activity ratio (T/NT) and the radiocounting percentage per gram tissue to the injected dose (%ID/g) were calculated. The control group was subjected to the same tests as the test group.

Statistical analysis

Results are reported as the means \pm SD or means \pm SE and analyzed by the *t*-test of 2 sample mean comparison. All the statistical analyses were performed using the SPSS16.0 software for Windows. $P < 0.05$ was considered to be statistically significant.

RESULTS

Tumor formation

About one week after tumor cells were transplanted into the nude mice, mung bean size nodule formation was observed at the inoculation sites. At 28 days after transplantation, most tumors grew to approximate 0.8 cm in diameter. The tumor formation rate of the test group was 90%, and that of the control group was 96%.

Radioactivity measurement of the Sephadex G50 eluent

Sephadex G50 was used to purify the [¹³¹I]-herceptin label in this study. All tubes of eluent collected were measured using a counting meter. The third and fourth tubes of eluent were [¹³¹I]-herceptin, which appeared as the first peak (Figure 1). The labeling rate was 83.38%, and the purity was 94.53%.

Immunobinding rate of [¹³¹I]-herceptin

In order to determine whether the [¹³¹I]-herceptin label in this study could bind to cells, it was incubated with BT-474 cells and MDA-MB-231 cells. [¹³¹I]-Herceptin could bind well to BT-474 cells, and the immunobinding rate was $65.83 \pm 2.43\%$; however, it could barely bind to MDA-MB-231 cells, and the immunobinding rate was only $9.59 \pm 1.06\%$, which was significantly lower than that of BT-474 ($P < 0.05$).

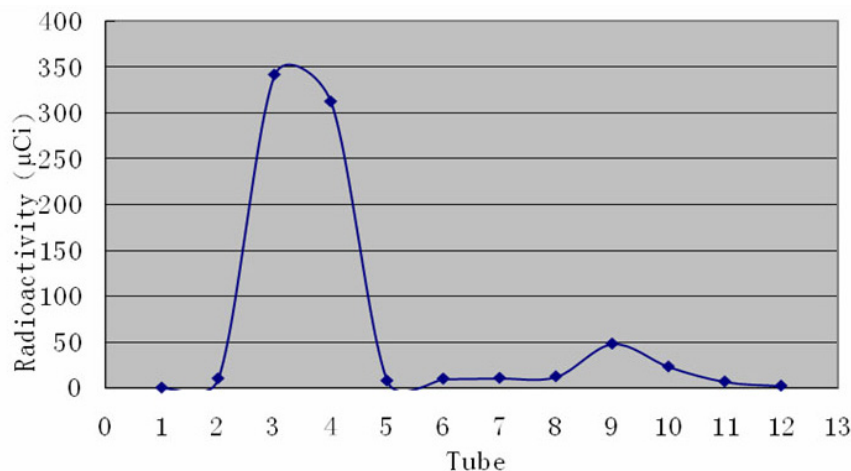


Figure 1. Elution curve of [¹³¹I]-herceptin eluent was measured by counting-meter. The first peak (the third and the fourth tube) was [¹³¹I]-herceptin.

Radio-visualization of tumors

To further confirm that [¹³¹I]-Herceptin could be used to view tumors *in vivo*, the tumor uptake rate of [¹³¹I]-herceptin of the 5 nude mice in both the groups was determined from images obtained at 2, 4, 8, 12, 24, 48, and 96 h by SPECT. The tumors of the test group were visible at 2 h after administration of [¹³¹I]-herceptin, and the tumor images became clearer with increasing time. The images were the clearest at 24 h after administration and remained clear at 96 h after administration (Figure 2). The 5 mice of the test group showed the same changing trend during the 7 time points. In contrast, the control mice showed no tumor images at any time point (Figure 3).

Analysis of nuclide distribution *in vivo*

In order to analyze how [¹³¹I]-herceptin was distributed in the body, the cpm of the organs and tumor tissues was detected using a counting meter. The T/NT ratio and %ID/g were calculated. The %ID/g of the tumor tissues of the test group increased gradually and reached the maximum of 13.85 at 24 h after the injection of [¹³¹I]-herceptin and then decreased to 8.69 at 96 h after the injection (Table 1). However, the %ID/g of the tumor tissues of the control group was only 2.05 and 1.17 at the respective time points. The %ID/g of the tumor tissues of the test group was significantly higher than that of the control group ($P < 0.05$). Irrespective of the group to which the mice belonged, they showed considerable radioactivity accumulation in the liver, spleen, kidney, and blood, but less accumulation in the skeleton and muscles. The T/NT ratios in all organs increased gradually and reached the highest level at 24 h after injection of [¹³¹I]-herceptin, but there was no significant decrease at 96 h, indicating that herceptin could guide [¹³¹I] to locate effectively the tumor tissues (Table 2). However, in the control group, the T/NT ratios were considerably lower than those of the test group ($P < 0.05$ or $P < 0.01$). In the control group, the ratios were always low and showed little variation.

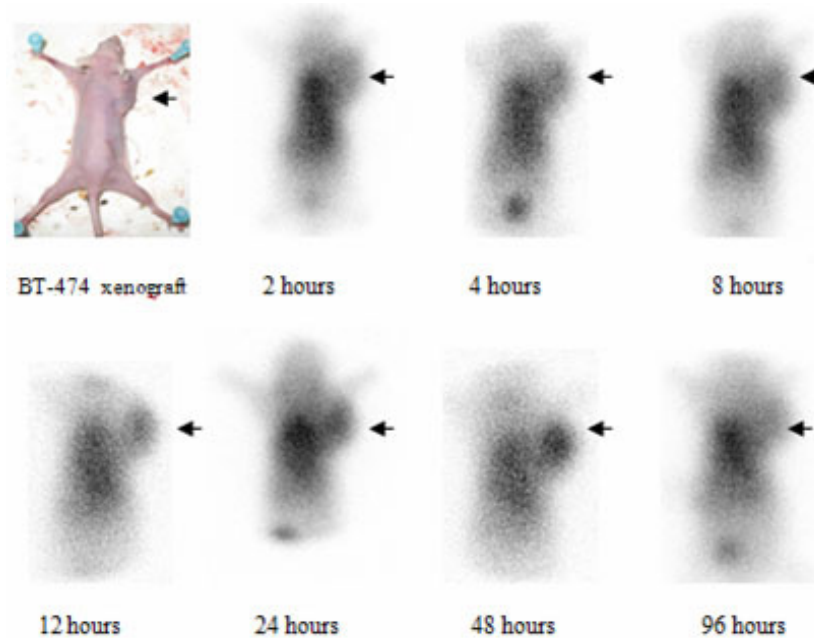


Figure 2. Whole-body images of the nude mice transplanted with HER2/neu-positive BT-474 human breast cancer at 2, 4, 8, 12, 24, 48, and 96 h after the injection of [¹³¹I]-herceptin. Tumor uptake (arrow) was visualized.

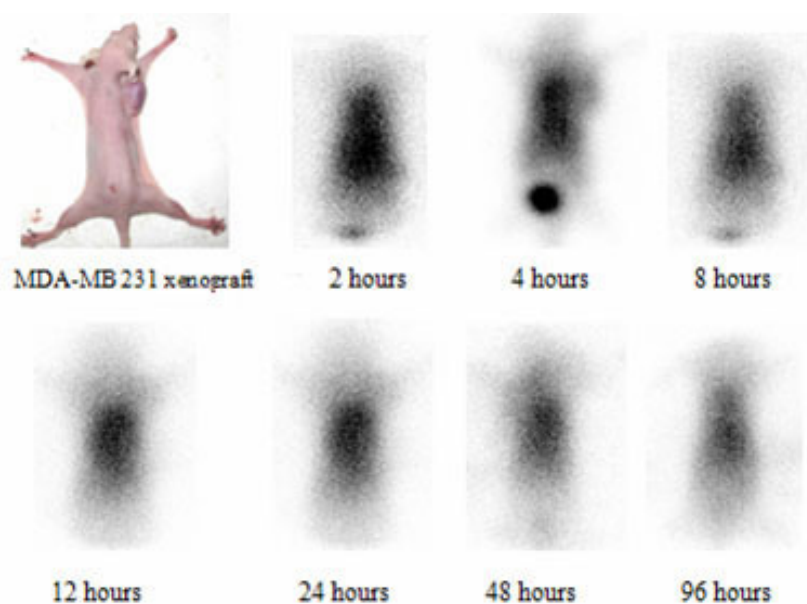


Figure 3. Whole-body images of the nude mice transplanted with HER2/neu-negative MDA-MB-231 human breast cancer at 2, 4, 8, 12, 24, 48, and 96 h after the injection of [¹³¹I]-herceptin. Tumor uptake was not visualized.

Table 1. Radiocounting percentage of per gram tissue to the injected dose (%ID/g) in both groups (N = 5).

Organ	Time points				
	4 h	12 h	24 h	48 h	96 h
BT-474					
Heart	2.31 ± 0.57	2.60 ± 0.19	3.57 ± 0.33	1.67 ± 0.28	0.95 ± 0.11
Liver	5.21 ± 1.22	6.02 ± 0.24	6.69 ± 0.41	7.30 ± 0.33	7.77 ± 0.83
Spleen	4.26 ± 1.01	5.01 ± 0.67	5.05 ± 1.90	6.19 ± 0.68	7.19 ± 0.38
Lung	2.59 ± 0.88	3.68 ± 1.37	1.98 ± 0.36	2.07 ± 0.47	1.17 ± 0.28
Kidney	33.07 ± 1.65	39.03 ± 0.87	26.13 ± 3.87	42.91 ± 2.21	51.19 ± 0.62
Skeleton	1.02 ± 0.39	1.29 ± 0.34	0.89 ± 0.11	1.12 ± 0.16	0.24 ± 0.08
Muscles	1.82 ± 0.21	1.80 ± 0.20	0.96 ± 0.20	0.92 ± 0.01	0.88 ± 0.07
Tumor	6.34 ± 1.17	9.17 ± 1.26**	13.85 ± 1.10**	12.97 ± 1.00**	8.69 ± 0.95
Blood	12.01 ± 2.30	9.33 ± 0.08	7.48 ± 0.51	4.97 ± 0.39	3.17 ± 0.56
MDA-MB231					
Heart	2.72 ± 1.85	2.97 ± 0.62	2.39 ± 0.66	2.25 ± 0.47	1.19 ± 0.28
Liver	8.43 ± 3.09	7.08 ± 1.13	7.14 ± 1.29	6.55 ± 1.37	6.13 ± 0.68
Spleen	9.29 ± 8.68	7.31 ± 1.26	7.24 ± 0.91	6.67 ± 1.92	4.12 ± 0.55
Lung	6.64 ± 1.83	4.78 ± 0.77	3.71 ± 1.76	1.61 ± 0.47	1.18 ± 0.27
Kidney	44.85 ± 8.26	37.37 ± 6.27	29.01 ± 3.47	36.81 ± 1.04	46.11 ± 0.69
Skeleton	1.48 ± 0.91	1.38 ± 0.37	0.83 ± 0.22	0.87 ± 0.06	1.13 ± 0.09
Muscles	2.04 ± 0.63	2.09 ± 0.30	2.13 ± 0.33	1.21 ± 0.18	1.09 ± 0.18
Tumor	1.55 ± 1.83	1.96 ± 0.68**	2.05 ± 0.59**	1.17 ± 0.31**	1.19 ± 0.15
Blood	13.87 ± 4.51	12.75 ± 3.88	8.42 ± 1.96	6.99 ± 0.10	4.09 ± 0.88

Data are reported as means ± SE. **P < 0.01 versus the respective control.

Table 2. Activity ratio of the tumor tissue and non-tumor tissue (T/NT) in both groups (N = 5).

Organ	Time points				
	4 h	12 h	24 h	48 h	96 h
BT-474					
Heart	2.74 ± 0.43	3.53 ± 0.9	3.88 ± 1.04	7.77 ± 0.64**	9.15 ± 1.12**
Liver	1.22 ± 0.07	1.52 ± 0.04	2.07 ± 0.09**	1.77 ± 0.13**	1.12 ± 0.13
Spleen	1.47 ± 0.27	1.83 ± 0.53	2.74 ± 0.12	2.09 ± 0.13	1.21 ± 0.14
Lung	2.45 ± 0.61	2.49 ± 0.32	6.99 ± 0.17**	6.27 ± 0.32**	7.43 ± 1.21
Kidney	0.19 ± 0.03	0.23 ± 0.05	0.53 ± 0.04**	0.30 ± 0.03	0.17 ± 0.08
Skeleton	6.23 ± 0.74**	7.10 ± 1.00**	15.58 ± 1.93**	11.58 ± 0.97	36.21 ± 3.34
Muscles	3.48 ± 0.65**	5.09 ± 0.82	14.47 ± 1.26**	14.10 ± 0.90	9.88 ± 1.98
Blood	0.53 ± 0.08	0.98 ± 0.12	1.85 ± 0.69	2.61 ± 0.10**	2.74 ± 0.67**
MDA-MB231					
Heart	0.57 ± 0.05	0.66 ± 0.08	0.86 ± 0.03	0.52 ± 0.05	1.00 ± 1.09
Liver	0.18 ± 0.04	0.28 ± 0.02	0.29 ± 0.05	0.17 ± 0.03	0.19 ± 0.06
Spleen	0.17 ± 0.08	0.27 ± 0.02	0.28 ± 0.05	0.18 ± 0.04	0.29 ± 0.12
Lung	0.23 ± 0.09	0.41 ± 0.05	0.55 ± 0.08	0.72 ± 0.02	1.01 ± 0.06
Kidney	0.04 ± 0.04	0.05 ± 0.01	0.07 ± 0.02	0.03 ± 0.03	0.03 ± 0.01
Skeleton	1.05 ± 0.26	1.42 ± 0.08	2.47 ± 0.19	1.34 ± 0.45	1.06 ± 0.07
Muscles	0.76 ± 0.17	1.93 ± 0.18	0.96 ± 0.14	0.97 ± 0.14	1.10 ± 0.08
Blood	0.12 ± 0.03	0.15 ± 0.02	0.24 ± 0.02	0.17 ± 0.02	0.29 ± 0.05

Data are reported as means ± SE. **P < 0.01 versus the respective control.

DISCUSSION

Herceptin is an anti-Her-2 mosaic antibody of human and mouse, which shows 95% humanization. It has high affinity to tumors over-expressing Her-2. It can also inhibit the proliferation and differentiation of tumors. In 1998, herceptin was licensed to be used in the treatment of breast carcinoma (Harris, 2004). It could strikingly increase the sensitization of breast cancer cells to radiation (Liang et al., 2003). In this study, [¹³¹I]-herceptin was used for radio-immuno-

imaging in breast carcinomas expressing *Her-2* gene. The T/NT ratio and %ID/g of nude mice with BT-474 breast carcinoma over-expressing *Her-2* and those of MDA-MB-231 breast carcinoma low-expressing *Her-2* were compared. Further, multi-time point imaging was performed on the tumor models to evaluate the accumulation characteristics of [¹³¹I]-herceptin in the tumor tissues. Our finding that herceptin-labeled [¹³¹I] retained its immunological activity corroborated the findings of a previous report (Blend et al., 2003). The tumor formation rates of animal models established using BT-474 cell line over-expressing Her2/neu and Her2/neu-negative breast carcinoma cell line (Caldas-Lopes et al., 2009) MDA-MB-231 were 90% and 96%, respectively.

Nude mice with BT-474 breast carcinoma cells showed the clearest tumor images at 24 h after the injection of [¹³¹I]-herceptin, and the images remained clear at 96 h after the injection. However, the nude mice with MDA-MB-231 breast carcinoma cells did not display any tumor images at any time point. Thus, [¹³¹I]-herceptin possesses good immunogenicity and tumor-affinity. Owing to its advantages to display tumors as small as 0.8 cm, [¹³¹I]-herceptin might be used to detect small-sized breast carcinoma and to evaluate the therapeutic effect. Both the test and control groups showed considerable nuclide accumulation in the liver, spleen, and kidney. This might be because of the rich blood flow and effective metabolism of [¹³¹I]-herceptin in the reticuloendothelial system of these organs.

The T/NT ratios in most organs were found to be higher than 1, and most were even higher than 3, except that of kidney. For example, the T/NT ratio of tumor to muscles was as high as 14.87 at 24 h after the injection of [¹³¹I]-herceptin, and it remained high at 96 h after the injection. However, in the control group, the T/NT ratios in most organs were lower than 1, except those in the skeleton and muscles. The radiocounting percentage per gram tumor tissue to the injected dose in the test group was higher than that in the control group, although there was no significant difference in organs. In both the test and control groups, there was considerable nuclide accumulation in the liver, spleen, and kidney, which was in accordance to our imaging results. Although herceptin is known to produce cardiotoxicity via mechanisms, such as lowering the ejection fraction of the left ventricular and causing pulmonary edema, peripheral edema, and cardiac hypertrophy (Anderson et al., 2005), considerable [¹³¹I]-Herceptin accumulation was not noted in the heart in this study, which is consistent with the finding that cardiac muscles express low Her2/neu (Oshiro et al., 2002).

CONCLUSION

Our results suggest that [¹³¹I]-herceptin can accumulate specifically in the tumor tissues and clearly reveal tumor profiles. Determining the ideal tumor uptake concentration and T/NT ratio might facilitate the diagnosis of small breast carcinoma lesions. We intend to study the positioning diagnosis and treatment of micrometastases in breast carcinoma in the future. This study only partly provides evidence that [¹³¹I]-herceptin can be used for the treatment of human breast cancer. Further studies are needed to explore the administration dose of [¹³¹I]-herceptin, its therapeutic frequency, and protective measures.

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