

Radioimmunotherapy of mice bearing breast tumors with ^{177}Lu -labeled trastuzumab

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Aim: In the present study, we investigated the therapeutic efficacy of trastuzumab labeled with lutetium-177 (^{177}Lu) in mice bearing breast tumors.

Materials and methods: Trastuzumab was labeled with ^{177}Lu via DOTA as a chelator. In vitro and in vivo quality control tests of incorporated activity, immunoreactivity, stability of ^{177}Lu -trastuzumab, biodistribution, and imaging studies were performed. Therapeutic efficacy and dosimetry studies were carried out in mice with breast tumors.

Results: The good in vitro and in vivo quality control results suggested that ^{177}Lu -trastuzumab could be used as a radioimmunotherapy agent. Reductions of 81% and 98% in the mean tumor volume for the group that received ^{177}Lu -trastuzumab [7.4 MBq] were observed at 42 and 45 days after treatment, respectively.

Conclusion: The results showed that ^{177}Lu -trastuzumab could be considered as a possible new radiopharmaceutical agent for use in radioimmunotherapy of breast cancer in humans.

Key words: Radioimmunotherapy, breast cancer, lutetium-177, trastuzumab, gamma camera imaging

Introduction

The development of antibodies for cancer therapy has emerged as one of the most promising areas in oncology. Trastuzumab (trade name: Herceptin) is a humanized IgG1 monoclonal antibody directed against the extracellular domain of the human epidermal growth factor receptor 2 (HER2) (1). This receptor is overexpressed in several epithelial tumors, including 35%–45% of all pancreatic adenocarcinomas, 25%–30% of ovarian cancers, and 4%–83% of colorectal adenocarcinomas (2–4).

HER2 is overexpressed in 20%–30% of early-stage breast cancers. Patients with HER2-overexpressing breast cancer may be candidates for trastuzumab treatment (5). However, due to the cardiotoxicity of trastuzumab, some patients cannot tolerate the

treatment due to preexisting heart conditions (6). Trastuzumab is an expensive antibody and the cost of cancer therapy is very high (7).

Radioimmunotherapy (RIT) is a targeted treatment that has the potential to augment the efficacy of conventional monoclonal antibodies (8). In RIT, a radioisotope is coupled to a monoclonal antibody to form a tumor-specific target agent (9).

We demonstrated that trastuzumab can be an effective carrier of RIT because it requires a smaller amount of antibody and therefore can reduce both side effects and cost (10–12).

In this study, the therapeutic efficacy of ^{177}Lu -trastuzumab was investigated in mice with breast tumors.

Received: 15.05.2011 – Accepted: 23.01.2012

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Materials and methods

Materials

Trastuzumab (Herceptin) was purchased as a 150-mg vial from Genentech (San Francisco, USA). The $^{177}\text{LuCl}_3$ was produced by bombarding $^{176}\text{Lu}_2\text{O}_3$ (74%; Campro Scientific, Berlin, Germany) dissolved in 0.05 M HCl at a flux of $2.6 \times 10^{14} \text{ n cm}^{-2} \text{ s}^{-1}$ for 10 days. All chemical reagents used in this study were prepared with deionized (DI) water. To prepare a $10\times$ conjugation carbonate buffer, NaHCO_3 (40.22 g), Na_2CO_3 (2.25 g), and NaCl (87.66 g) were dissolved in 1000 mL of DI water.

Radiolabeling of trastuzumab with ^{177}Lu

Trastuzumab was conjugated to DOTA as previously described (11,12). Briefly, trastuzumab (5 mg, 2 mL) in carbonate buffer (pH 8.6) was slowly added to NHS-DOTA (1 mg, 2.5 mL) and gently mixed for 24 h at room temperature. After purification, the number of chelates per antibody was determined using a spectrometric assay based on the titration of the Cu(II)–Arsenazo(III) complex (13). The final protein concentration was determined by the Lowry method (14).

For radiolabeling, 5 mCi of $^{177}\text{LuCl}_3$ with a typical specific activity of 20 Ci mg^{-1} was added to the trastuzumab–DOTA conjugation (150 μg) and heated for 3 h at 37°C .

Quality control of the trastuzumab–DOTA– ^{177}Lu complex

The number of chelates per antibody and the incorporated activity of ^{177}Lu was determined as described previously (10). The stability of the complex in human blood serum was analyzed by instant thin layer chromatography. The immunoreactivity was also tested in SKBr3 cells based on the method described by Lindmo et al. (15).

Biodistribution and imaging study of the complex in mice bearing tumors

Breast tumors were established by subcutaneous injection of murine mammary carcinoma cells (approximately 10^{12} cells) in the right flank region of inbred female BALB/c mice (20–25 g, 8–10 weeks old). The tissue distribution experiments were performed when the tumor volumes reached 40–60 mm^3 . The effect of trastuzumab on tumor cells was

assessed by immunohistochemistry using the rabbit antimouse peroxidase technique (16).

The mice ($n = 35$) were injected intravenously with ^{177}Lu –trastuzumab (7.4 MBq 0.1 mL^{-1} , approximately 30 μg trastuzumab) via the tail veins. At 1, 2, 3, 4, 5, 6, and 7 days after the injection, groups of 5 animals were killed and tissues were removed, checked for ^{177}Lu activity, and compared with the percentage of injected dose per gram of tissues ($\text{ID}\% \text{ g}^{-1}$). The biodistribution of ^{177}Lu –IgG was studied at 1 and 7 days after injection. Before being killed, the animals were anesthetized by a combination of xylazine hydrochloride and ketamine hydrochloride. Scintigraphic images (550,000 counts, 256×256 matrix) were recorded using a gamma camera (e.cam, Siemens Medical Systems, Erlangen, Germany) equipped with a low-energy, high-resolution collimator.

Radioimmunotherapy and tumor response

RIT was performed in the mice with murine breast tumors. Mice were randomly divided into 5 groups each containing 10 mice. Group 1 was injected with 7.4 MBq of ^{177}Lu –trastuzumab (30 μg trastuzumab, 0.1 mL), group 2 with 5.5 MBq of ^{177}Lu –trastuzumab (25 μg trastuzumab, 0.1 mL), group 3 with 7.4 MBq of ^{177}Lu (unspecific control), group 4 with unlabeled trastuzumab (30 μg trastuzumab, 0.1 mL), and group 5 with phosphate buffer saline (PBS). The sizes of the tumors were measured in 3 dimensions every 3 days with an electronic slide caliper until the tumor volumes exceeded 2000 mm^3 (50–100 days). Tumor volume was calculated as:

$$V = \frac{4}{3} \pi \times r_1 \times r_2 \times r_3,$$

where r_1 , r_2 , and r_3 are tumor radii in 3 dimensions, expressed in cubic millimeters. Tumor inhibition rate (TIR) was calculated as follows:

$$\text{TIR}(\%) = \frac{V_1 - V_2}{V_1} \times 100,$$

where V_1 is the average volume of the tumors in the PBS group and V_2 is the average volume of the tumors in the treatment group. The doubling and quintupling times of the tumors were calculated and compared in groups.

The percentage of weight loss in mice was considered as complex toxicity. The body weight was expressed as relative body weight compared with the body weight on the first day of treatment, and toxicity was defined as a 15% body weight loss in any animal. When the tumor volume exceeded 2000 mm³, the mice were killed for humane reasons. All animal experiments were performed in compliance with the regulations of our institution and according to accepted guidelines governing such work.

Dosimetry

The estimated radiation dose delivered to the tumor and normal organs was calculated from the biodistribution data. Briefly, the trapezoidal regions were defined by the time–activity data after correction for physical decay. The resulting integral was converted to Gy MBq⁻¹ using S values (MIRD0SE3; unit density sphere model) calculated for each isotope by assuming uniformly distributed activity in small unit-density spheres, which do not assume 100% absorption of β-particles (17–19).

For better comparison, the organ doses were estimated by the Monte Carlo technique as well. Briefly, the Moby phantom was used as a mathematical mouse model (20). A spherical tumor shape (approximately 2000 mm³) was inserted into the right flank of mouse phantom.

The organ activity of the phantom was adjusted according to the calculated cumulated activities. The GATE Monte Carlo package (version 4.0.0) was used for dose calculation (Gy MBq⁻¹) in the organs of the mouse phantom (21).

Statistical analysis

SPSS (version 13.0, SPSS Inc.) was used for statistical analyses. For all of the tests, P < 0.05 was considered as statistically significant. The t-test was used to analyze the biodistribution data and differences in the growth of the tumors.

Results

The number of chelates per antibody (5.5–6), incorporated activity (81%), and immunoreactivity (87%) were optimal for the ¹⁷⁷Lu–DOTA–trastuzumab. On average, 86% and 81% of the complex was stable in the phosphate buffer and in human blood serum for up to 4 days, respectively. The results are shown in Figure 1.

Biodistribution and imaging study of the complex in mice bearing tumors

The immunohistochemistry results revealed that the murine breast tumor used in this study expressed moderate levels of HER2. The tissue distribution experiments were performed in mice with breast tumors at 1–7 days after administration of ¹⁷⁷Lu–trastuzumab and the results are presented in Table 1. In all organs, the activity of ¹⁷⁷Lu decreased with time, excluding the tumor, which continued to accumulate radioactivity for up to 2 days after the injection. The maximum tumor uptake was 21.24 ± 2.6 ID% g⁻¹ at 2 days and then decreased gradually to 18.21 ± 5.9 ID% g⁻¹ at 7 days. The percentage of ID g⁻¹ for all normal organs was always less than 8%.

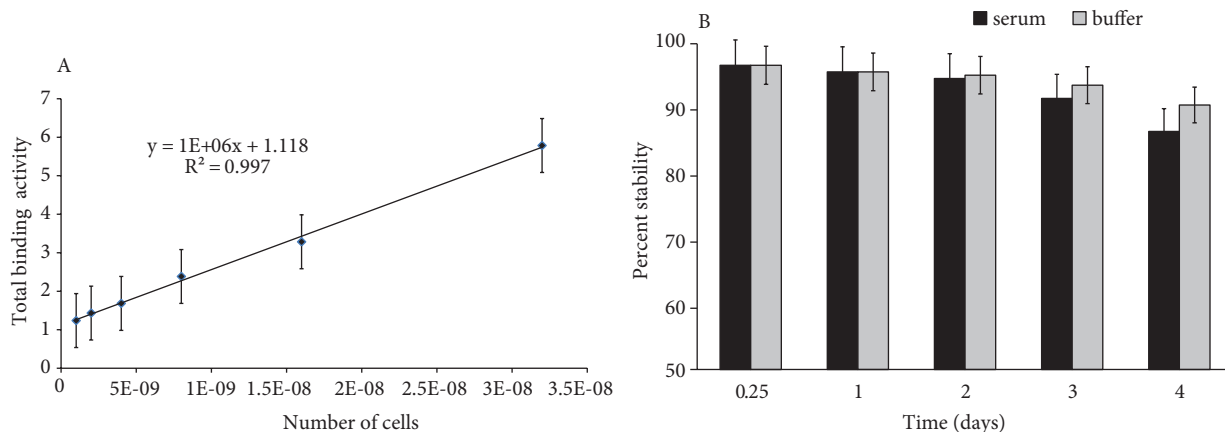


Figure 1. A) Immunoreactivity and B) stability in the phosphate buffer and in human blood serum for up to 4 days of ¹⁷⁷Lu–trastuzumab.

Table 1. Tumor-to-organ ratios for ^{177}Lu -trastuzumab in mice with breast tumors.

Tissue	24 h	48 h	96 h	120 h	168 h
Blood	2.27 ± 0.54	5.15 ± 0.08	10.52 ± 0.07	17.28 ± 0.10	23.09 ± 0.09
Liver	2.82 ± 0.97	4.88 ± 0.52	9.65 ± 1.04	15.45 ± 1.28	19.59 ± 1.57
Spleen	4.03 ± 1.03	6.78 ± 1.0	10.42 ± 1.09	17.11 ± 1.43	20.79 ± 0.97
Kidney	3.47 ± 0.97	4.22 ± 1.11	8.29 ± 1.08	15.91 ± 0.99	21.81 ± 1.96
Bone	5.78 ± 0.97	4.26 ± 1.52	9.67 ± 1.08	13.9 ± 0.88	18.26 ± 0.97
Stomach	16.17 ± 2.61	20.99 ± 3.53	24.35 ± 4.10	26.88 ± 4.98	37.83 ± 5.17
Intestine	11.16 ± 2.34	17.49 ± 2.68	22.74 ± 3.74	28.92 ± 3.39	32.51 ± 5.20
Colon	11.21 ± 2.10	18.15 ± 2.06	22.32 ± 3.39	26.56 ± 3.54	24.48 ± 2.81
Muscle	15.81 ± 1.88	21.74 ± 1.95	23.63 ± 2.63	23.55 ± 2.49	25.07 ± 2.67
Lung	8.99 ± 1.05	15.41 ± 1.29	19 ± 1.47	23 ± 1.35	28 ± 1.43
Heart	5.62 ± 1.28	9.54 ± 1.56	11.87 ± 1.64	19.83 ± 2.55	25.69 ± 3.12

To demonstrate the specificity of ^{177}Lu -labeled trastuzumab uptake by tumors, ^{177}Lu -labeled IgG as a nonspecific antibody was also tested. The biodistribution studies were performed in mice with breast tumors on day 1 and day 7 postinjection and the results are shown in Figure 2. ^{177}Lu -trastuzumab was accumulated at very high concentrations in tumors compared with ^{177}Lu -IgG (about 5 times or more). ^{177}Lu was eliminated from the blood quickly (on the first day) after administration of ^{177}Lu -IgG and could be detected only in the liver, kidney, and bone after 7 days.

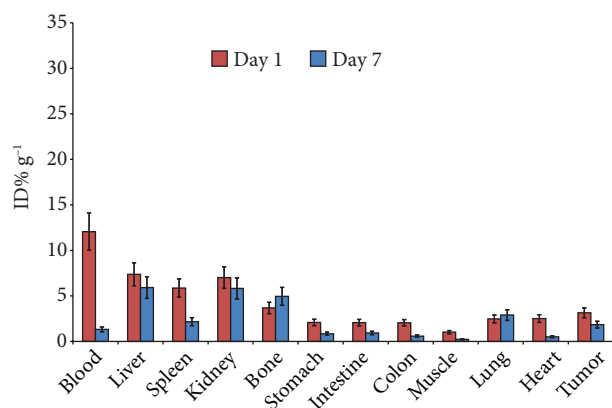


Figure 2. Tissue distributions of ^{177}Lu -IgG (7.4 MBq) in mice with breast tumors on days 1 and 7.

The gamma camera images from a representative mouse at day 1 and day 7 after injection of ^{177}Lu -trastuzumab are shown in Figure 3. The tumors are clearly evident in all images. A small amount of radioactivity was also observed in the liver area on day 1 postinjection.

Radioimmunotherapy and tumor response

The therapeutic efficacy of ^{177}Lu -trastuzumab (7.4 and 5.5 MBq), trastuzumab (30 μg), $^{177}\text{LuCl}_3$ (7.4 MBq), and PBS (as control) was evaluated in mice with breast tumors and the results are presented in

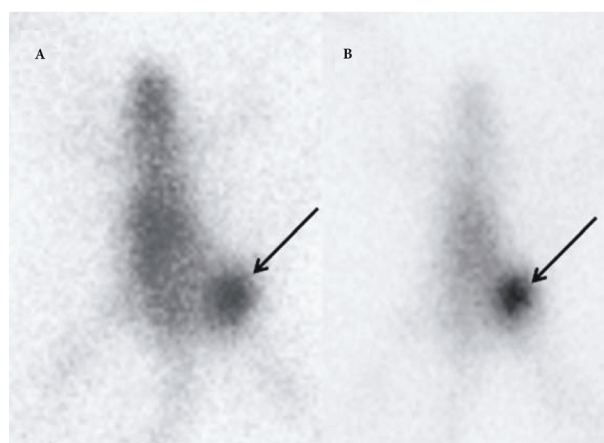


Figure 3. The gamma camera images acquired on A) day 1 and B) day 7 after injection of ^{177}Lu -trastuzumab.

Figure 4. The tumor size significantly increased ($P < 0.05$) in PBS and native antibody groups compared with ¹⁷⁷Lu–trastuzumab. From day 30, significant ($P < 0.05$) differences were observed in tumor growth between the mice treated with 7.4 MBq and 5.5 MBq of the complex. No group of mice showed any significant ($P > 0.05$) weight loss after injection (data not shown). Reductions of 81% and 98% in the mean tumor volume for the group that received ¹⁷⁷Lu–trastuzumab (7.4 MBq) were observed at 42 and 45 days postinjection, respectively. The comparison between doubling and quintupling time of tumors after administration of ¹⁷⁷Lu–trastuzumab (7.4 MBq),

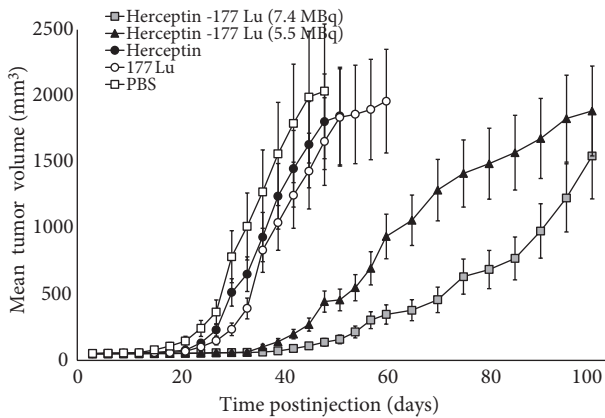


Figure 4. Comparison of the mean tumor volume in treatment groups. Each group is shown as a function of time and consists of 10 mice. The mice were killed when the tumor size exceeded 2000 mm³.

¹⁷⁷Lu–trastuzumab (5.5 MBq), trastuzumab, ¹⁷⁷Lu (7.4 MBq), and PBS is shown in Figure 5. The doubling time for the group that received ¹⁷⁷Lu–trastuzumab (7.4 MBq) was 45 days, whereas this time for the PBS group was 4.5 days.

Dosimetry

An estimation of mean tissue-absorbed dose calculated by MIRDOSE3 (19) and the Monte Carlo simulation using the Moby mouse phantom (20) and GATE simulator (21) are shown in Table 2. Some differences between the calculated dose with MIRDOSE3 and the Monte Carlo simulation were

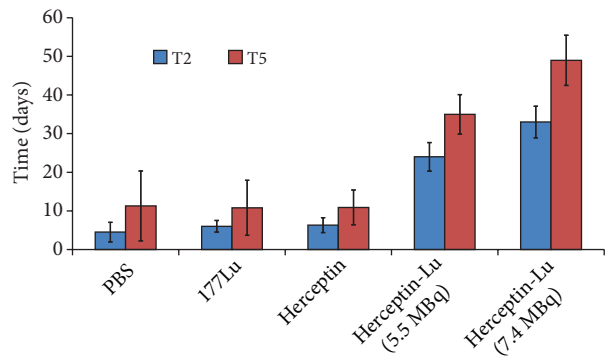


Figure 5. Comparison between doubling and quintupling time of tumors after administration of ¹⁷⁷Lu–trastuzumab (7.4 MBq), ¹⁷⁷Lu–trastuzumab (5.5 MBq), trastuzumab, ¹⁷⁷Lu (7.4 MBq), and PBS.

Table 2. A comparison between MIRDOSE3 and GATE simulators in the calculation of mean absorbed doses to the mice tissues treated with 7.4 MBq of the complex.

	Absorbed dose to different organs	
	MIRDOSE3	GATE simulator
Tissue	Gy MBq ⁻¹	Gy MBq ⁻¹
Tumor	6.35 ± 1.2	8.3
Liver	0.98 ± 0.3	1.49
Spleen	0.7 ± 0.1	1.4
Kidney	0.54 ± 0.09	0.49
Blood	1.62 ± 0.13	3.54
Colon	0.19 ± 0.03	0.14
Bone	0.41 ± 0.09	0.38

seen. This may be due to the fact that the organ mass of the Moby phantom at the standard settings differed from the experimental mice, but in general the mean absorbed dose with both methods was similar. The mean absorbed dose to tumor and select organs was recorded as gray per megabecquerel.

Discussion

Radiolabeled monoclonal antibodies have shown great promise for cancer therapy (22–26). Trastuzumab is a monoclonal antibody specific for the HER2 receptor and is approved for the treatment of metastatic breast cancer (27). HER2 is overexpressed in a variety of epithelial cancers and is being used as an ideal target in RIT research (2–4). Many radioisotopes are of potential use in RIT (22–26).

We selected ^{177}Lu for trastuzumab labeling due to its promising physical and chemical characteristics. ^{177}Lu displays low-energy β -emission (0.497 MeV, 78.7%) with minimal tissue penetration (0.04–1.8 mm), making it suitable for the therapy of small and metastatic tumors (28–31). The half-life (6.65 days) of ^{177}Lu allows the application of sophisticated procedures to synthesize and purify the radiopharmaceutical. ^{177}Lu also emits γ -photons (208 keV, 11% abundance) suitable for imaging with a conventional gamma camera, which may be used to image the distribution of radiopharmaceuticals in the patient's body. This technique is potentially very important for the determination of the dose delivered to tumors and normal tissues in patients. The other advantage of ^{177}Lu is the possibility for its production in reactors at a large scale at a low price and high specific activity.

In previous studies we labeled trastuzumab with ^{177}Lu by different methods, found the best labeling condition, and investigated its cytotoxicity in SKBr3 and MCF7 breast cancer cell lines (10–12).

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In the present study, the therapeutic efficacy of ^{177}Lu -trastuzumab was assessed in mice with breast tumors. The biodistribution study showed that ^{177}Lu -trastuzumab accumulated to a greater extent in tumors than in all other analyzed organs (less than 10%). After 2 days, the tumor uptake of ^{177}Lu -trastuzumab was $24.14 \pm 3.6 \text{ ID\% g}^{-1}$; in contrast, the value reported for ^{188}Re -SOCTA-trastuzumab was $7.23 \pm 1.12 \text{ ID\% g}^{-1}$ (22).

The main objective of RIT is the treatment of small metastases. Thus, in this experiment, ^{177}Lu -trastuzumab was injected into the mice 10 days after tumor implantation, when the mean sizes of the tumors were 40–60 mm³. The mice that received 7.4 and 5.5 MBq of ^{177}Lu -trastuzumab had significantly ($P < 0.05$) delayed tumor progression compared with native trastuzumab and control groups. The absorbed doses from ^{177}Lu -trastuzumab to tumor and liver were $6.35 \pm 1.2 \text{ Gy MBq}^{-1}$ and $0.98 \pm 0.3 \text{ Gy MBq}^{-1}$, respectively. In treatment with ^{90}Y -trastuzumab, the absorbed dose to tumor was 0.48 Gy MBq⁻¹, compared to the liver, which received 0.47 Gy MBq⁻¹ (32). We suggest that the treatment efficiency could be improved further by using higher amounts of ^{177}Lu -trastuzumab. It is probable that the maximum tolerable dosage was not reached in this study, because no adverse effects could be found from the treatment. Moreover, the spontaneous breast tumor that we used in this study expressed moderate levels of HER2. It is probable that ^{177}Lu -trastuzumab may show better results with tumors that overexpress HER2, but that needs more investigation.

^{177}Lu -DOTA-trastuzumab could be considered for further evaluation in humans as a new radiopharmaceutical for use in the RIT of breast cancer. ^{177}Lu -trastuzumab showed promising properties as an effective radioimmunoconjugate for RIT of HER2-overexpressing breast tumors. This complex can be considered as an alternative to ^{131}I -labeled antibodies for further investigation.

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