Improved Clearance of Radiolabeled Biotinylated Monoclonal Antibody Following the Infusion of Avidin as a "Chase" without Decreased Accumulation in the Target Tumor

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The techniques of radioimmunoimaging and radioimmunotherapy suffer from prolonged high background radioactivity because intravenously injected antibodies remain in the circulation and in the organs far longer than necessary for effective binding to the target. To decrease background and increase radionuclide excretion without decreasing the dose of radioactivity delivered to the target tumor, we used radiolabeled biotinylated antibodies followed by a "chase" avidin injection. **Methods:** A mouse monoclonal antibody, OST7 (IgG1), which reacts with human osteosarcoma, was biotinylated and labeled with ^{125}I, ^{131}I or ^{99m}Tc. Radiolabeled biotinylated OST7 (10 μg) was administered intravenously into nude mice bearing human osteosarcomas and 30 μg of avidin was injected intravenously 6 or 24 hr later. **Results:** Following avidin injection in mice pretreated with radiolabeled biotinylated antibodies, radioactivity was promptly cleared from the blood and deposited in the liver and spleen, after which radioiodine was rapidly detached from the antibody and excreted in the urine. The tumor-to-blood ratios at 6 and 24 hr after the injection of ^{125}I-labeled biotinylated OST7 increased compared with the values before the avidin chase without any loss of tumor radioactivity. Furthermore, the tumor-to-background radioactivity ratio was improved and better images were obtained more rapidly after the injection of radiolabeled biotinylated antibodies than with conventional immunoscintigraphy. **Conclusions:** This method may find application in clinical radioimmunoimaging, especially using short half-life radionuclides such as ^{99m}Tc and ^{123}I.

Key Words: monoclonal antibody; avidin; biotin; chase; immunoscintigraphy

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Radioimmunoimaging and radioimmunotherapy suffer from a prolonged high background level of radioactivity because intravenously injected antibodies remain in the circulation and organs far longer than necessary for effective binding to the target. Various approaches, including the use of antibody fragments (*1,2*) and the injection of second antibodies (*3*), have been explored to overcome this problem, but with only limited success.

Immunoscintigraphy using an avidin-biotin system has already been performed clinically by "two-step" and "three-step" methods (*4–11*). These methods employed a biotinylated antibody or an avidin-conjugated antibody for pretargeting, as well as radiolabeled biotin or avidin which was cleared rapidly for detecting the tumor and obtained high tumor-to-normal tissue radioactivity ratios (*5,9,11*). As a result, images with good contrast were obtained more rapidly than with conventional immunoscintigraphy. However, the disadvantage in the case of radioimmunotherapy was a low percentage of the injected dose of radiolabeled biotin or avidin taken up by the tumor, so far higher doses of radiolabeled agents had to be injected to obtain a therapeutic effect when compared with the use of conventional radiolabeled monoclonal antibodies (Mabs).

In the present study, we injected radiolabeled biotinylated antibodies followed by an avidin injection as a "chase" to decrease the background radioactivity and speed up the excretion of radionuclides without decreasing the dose of radioactivity delivered to the target tumor, in order to improve the tumor-to-background contrast for better radioimmunoimaging and radioimmunotherapy. The basic principles of this method have been previously reported by two groups of investigators (*12,13*), but we studied its application to immunoscintigraphy by investigating the metabolism and excretion of radionuclides in order to determine the appropriate timing for chasing a radiolabeled biotinylated antibody using avidin.

MATERIALS AND METHODS

Cells

KT005 human osteosarcoma cells (*14*) were grown in RPMI 1640 (Nissui, Tokyo, Japan) containing 10% fetal calf serum

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(GIBCO Laboratories, Grand Island, NY) and 0.03% L-glutamine at 37°C in 5% CO_2. Subconfluent cells were removed from the culture dishes using calcium- and magnesium-free phosphate-buffered saline (PBS) containing 0.02% EDTA to preserve their antigenicity.

Monoclonal Antibodies

The OST7 antibody (IgG1 isotype) was raised against a human osteogenic sarcoma (*15*), and it has been shown to react with human osteogenic sarcoma cells with the antigen being an alkaline phosphatase-related substance (*16,17*). The antibody was purified from the ascites of hybridoma-bearing mice using Protein A column chromatography (Bio-Rad, Richmond, CA).

Mab 56C (IgG1), which recognizes human chorionic gonadotropin, was used as the isotype-matched control antibody (*18*).

Biotinylation of Monoclonal Antibodies

Mabs were mixed with sulfosuccinimidyl-6-(biotinamido) hexanoate (NHS-LC-biotin) (Pierce Chemical Co., Rockford, IL) in 0.05 *M* PBS (pH 7.4) for 30 min at room temperature at a NHS-LC-biotin-to-antibody molar ratio of 12:1–20:1, after which the biotinylated antibody was separated by PD-10 gel chromatography (Pharmacia, Uppsala, Sweden) and unconjugated NHS-LC-biotin was removed. Under these conditions, two to three NHS-LC-biotin complexes were conjugated to each antibody, as determined by the 2-(4'-hydroxyazobenzene) benzoic acid (HABA) method of Green (*19*).

Radiolabeling and Quality Control

Unconjugated and biotinylated Mabs were radioiodinated using the chloramine-T method (*20,21*). Purified Mabs (40 μg) in 0.3 *M* phosphate buffer (pH 7.5), and ^{125}I (11.1 MBq) or ^{131}I (7.4 MBq) (DuPont, No. Billerica, MA) were mixed with 3.0 μg of chloramine-T (Nakarai Chemicals, Kyoto, Japan) dissolved in 0.3 *M* phosphate buffer. After reacting for 5 min, the radiolabeled antibody was separated from free iodine by PD-10 gel chromatography. The specific activity of the ^{125}I-labeled antibody was about 222 MBq/mg that of ^{131}I-labeled antibody was 111 MBq/mg.

Unconjugated and biotinylated Mabs were labeled with ^{99m}Tc by the direct method (*1,22,23*). A Mab solution (2.5 mg/ml) in 0.05 *M* PBS was incubated with 2-mercaptoethanol (2ME) at room temperature for 30 min at a molar ratio of 1:1000 and the reduced antibody was purified by PD-10 gel chromatography. Immediately afterwards, 100 μg of the reduced antibody was mixed with 5 μl of the solution from an HMDP bone-scanning kit (Mediphysics, Japan) reconstituted with 5 ml of 0.9% sodium chloride for injection and 14.8 MBq of pertechnetate eluted from a $^{99}Mo/^{99m}Tc$ generator (Daiichi Radioisotopes, Tokyo, Japan).

Radiolabeled antibodies were analyzed by size-exclusion, high-performance liquid chromatography equipped with TSKG3000SW column (Tosoh Co., Tokyo, Japan) and cellulose acetate electrophoresis. More than 95% of the radioactivity was associated with the IgG fraction, and no high molecular weight species indicating the presence of antibody aggregates were observed, as well as no free ^{125}I, ^{99m}Tc or ^{99m}Tc-HMDP (data not shown).

Cell-Binding Assay

The ^{125}I- and ^{99m}Tc-labeled Mabs (3–5 ng/100 μl) were incubated with increasing concentrations of KT005 ($10^4 - 5 \times 10^6$/100 μl) in 5.7 × 46-mm microcentrifuge tubes for 1 hr at 4°C. After centrifugation at 10,000 × g, the supernatant was aspirated and the tubes were cut. Then the radioactivity bound to the cells was counted in an auto-well gamma counter. Specific binding to the cells was calculated by subtracting the nonspecific binding of ^{125}I-labeled control biotinylated 56C from the binding of radiolabeled biotinylated and unbiotinylated OST7. The binding of 56C to OST7 was less than 3% of the added radioactivity. The immunoreactive fraction of the radiolabeled antibodies was determined by the method of Lindmo et al. (*24*).

Avidin-Biotin Binding Assay of Radiolabeled Biotinylated Antibodies

A 100-μl aliquot of each radiolabeled biotinylated antibody (100,000 cpm/7–10 ng) was incubated with about 0.5 ml of avidin-Sepharose gel (Pierce Chemical Co., Rockford, IL) for 10 min at room temperature, and then the gel was washed with 0.05 *M* PBS. The radioactivity of the gel and the supernatant was counted and the fraction of the radioactive biotinylated antibody which bound to avidin was calculated (*13*).

Biodistribution and Pharmacokinetic Study

For in vivo studies of the radiolabeled Mabs 5×10^6 KT005 cells were inoculated subcutaneously into female BALB/c-nu/nu mice. The tumors grew to be about 200 mg after 12 days. Potassium iodide solution was administered to the mice from 1 day before the injection of radioiodinated antibodies to inhibit radioiodine uptake by the thyroid. Nude mice bearing KT005 xenografts were injected via the tail vein with 37 kBq of radiolabeled biotinylated and unbiotinylated OST7. The antibody dose was adjusted to 10 μg per mouse in each assay.

Experiment One. To compare the biodistribution of biotinylated and unbiotinylated OST7 at 24, 48 and 96 hr after the intravenous injection of ^{125}I-labeled antibodies, groups of mice were killed, their organs and tumors were removed and weighed, and then the radioactivity was counted.

Experiment Two. Tumor-bearing mice were injected with 0.03–300 μg of avidin (Pierce Chemical Co., Rockford, IL) 24 hr after the injection of ^{125}I-labeled biotinylated OST7 to examine the relationship between the avidin dose and the clearance of biotinylated antibodies. Groups of mice were killed 2 hr after avidin injection and the biodistribution of the labeled antibody was examined.

Experiment Three. To assess the time course of the chase effect of avidin injection, a "chase" study was done at 6 or 24 hr after the injection of ^{125}I- and ^{99m}Tc-labeled biotinylated OST7 or the control antibody (56C), as well as before (6 or 24 hr) and 30 min (6.5 or 24.5 hr), 2 (8 or 26 hr) and 6 (12 or 30 hr) hr after the intravenous injection of 30 μg of avidin (times after the injection of antibodies are shown in parentheses). Groups of mice were killed and their organs were removed, weighed and counted for radioactivity.

Results were expressed both as the percentage of the injected dose per gram of tissue and as the tumor-to-normal tissue ratio. The localization index was calculated as the tumor-to-blood ratio of OST7 divided by that of the control antibody (*18*). A chase effect index was also determined from the tumor-to-organ ratio of the antibody after chasing divided by that before chasing.

Experiment Four. Two groups of mice were placed in metabolic cages and the cumulative ^{125}I and ^{99m}Tc excretion in the urine and feces was determined from 24 to 32 hr after the intravenous injection of 10 μg of ^{125}I- or ^{99m}Tc-labeled biotinylated antibody. One group of mice was administered 30 μg of avidin in 150 μl of 0.05 *M* PBS, and the other control group was administered 150 μl of 0.05 *M* PBS alone 24 hr after injection of the antibody. Three mice were tested in each group. Statistical analysis was done with the Student's t-test.

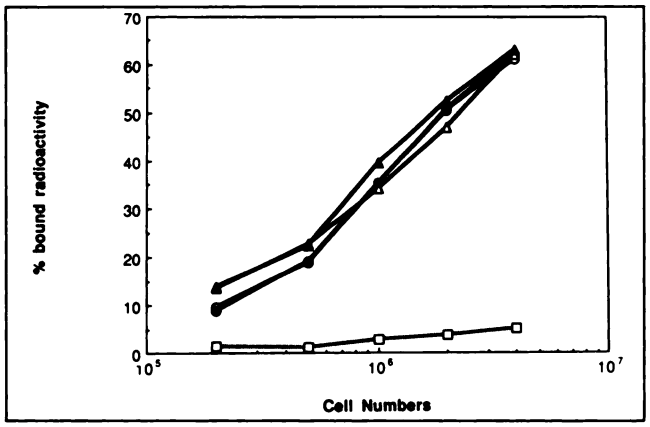

FIGURE 1. Binding of ^{125}I-labeled unbiotinylated OST7 (●), ^{99m}Tc-labeled unbiotinylated OST7 (▲), ^{125}I-labeled biotinylated OST7 (○), ^{99m}Tc-labeled biotinylated OST7 (△) and ^{125}I-labeled control biotinylated 56C (□) to KT005 cells. The percentage of bound radioactivity is plotted against the number of cells.

Immunoscintigraphy

For the imaging of tumor-bearing nude mice, 2.22 MBq per 20 μg of ^{131}I-labeled biotinylated OST7 or 4.44 MBq per 20 μg of ^{99m}Tc-labeled biotinylated OST7 was administered intravenously via the tail vein. At 24 hr after injection of the ^{131}I-labeled biotinylated antibody or 6 hr after the ^{99m}Tc-labeled biotinylated antibody, as well as before and 30 min and 6 hr after the injection of 60 μg of avidin, mice were anesthetized with intraperitoneal sodium pentobarbital and scintigrams were obtained using a gamma camera equipped with a pinhole collimator (*1,2,18*).

All animal experiments were carried out in accordance with the Japanese regulations regarding animal care and handling.

RESULTS

In Vitro Reactivity of ^{125}I- and ^{99m}Tc-Labeled Biotinylated or Unbiotinylated OST7

Biotinylation did not affect the immunoreactivity of OST7 and there was no difference in the binding to KT005 cells of ^{125}I- and ^{99m}Tc-labeled biotinylated or unbiotinylated antibodies (Fig. 1). Iodine-125-labeled biotinylated OST7 and ^{99m}Tc-labeled biotinylated OST7, respectively, showed 96.3% and 98.7% binding to avidin-Sepharose gel, but ^{125}I-labeled unbiotinylated OST7 and ^{99m}Tc-labeled unbiotinylated OST7 showed only 0.05% and 0.12% binding to avidin-Sephalose gel, respectively. The immunoreactive fraction and the avidin-binding reactive fraction of all the radiolabeled antibodies was more than 70% and more than 95%, respectively.

Biodistribution and Pharmacokinetic Studies

Experiment One. There were no significant differences between the biodistribution and tumor uptake of ^{125}I-labeled biotinylated OST7 and those of ^{125}I-labeled unbiotinylated OST7 (Table 1).

Experiment Two. As the injected dose of avidin was increased, the blood radioactivity level decreased and the levels in the liver, spleen and stomach all increased (Fig. 2). At 2 hr after the injection of 300 μg of avidin, the blood radioactivity was only 23.0% of that in the control mice.

Experiment Three. When a "chase" of avidin was injected at 6 hr after the injection of ^{125}I-labeled biotinylated OST7, the 30-min blood radioactivity level was only 14.3% of that before avidin injection. When avidin was injected 24 hr after OST7, the 30-min radioactivity level was 21.3% of that before avidin and it showed a slight increase after 6 hr. The radioactivity in the liver and spleen increased markedly immediately after avidin injection and then decreased rapidly, with the hepatic level at 6 hr after avidin injection being lower than that before avidin administration. The radioactivity in the stomach reached its peak at 2 hr after avidin injection and then rapidly decreased to become lower than before avidin administration. Although the radioactivity in all the other organs decreased rapidly, that of the tumor did not change in the 6-hr period after avidin injection (Figs. 3 and 4). In contrast, the radioactivity in the

TABLE 1
Biodistribution of OST7 and Biotinylated OST7 in Nude Mice Bearing KT005*

	Biotinylated OST7			OST7		
	24 hr (n=4)†	48 hr (n=5)	96 hr (n=4)	24 hr (n=4)	48 hr (n=5)	96 hr (n=4)
Blood	13.00 ± 2.67‡	7.36 ± 1.11	3.54 ± 2.21	12.30 ± 2.13	8.40 ± 1.58	4.99 ± 1.74
Liver	3.35 ± 1.28	1.85 ± 0.15	0.80 ± 0.59	3.41 ± 1.00	2.54 ± 0.47	1.47 ± 0.77
Kidney	3.40 ± 0.66	1.98 ± 0.28	0.95 ± 0.54	3.85 ± 0.72	2.39 ± 0.30	1.54 ± 0.75
Intestine	1.37 ± 0.23	0.70 ± 0.11	0.36 ± 0.20	1.35 ± 0.32	0.79 ± 0.16	0.48 ± 0.19
Stomach	3.31 ± 1.02	2.14 ± 0.73	0.93 ± 0.36	2.72 ± 0.65	1.29 ± 0.43	0.90 ± 0.05
Spleen	2.54 ± 0.84	1.22 ± 0.19	0.64 ± 0.48	2.28 ± 0.43	1.69 ± 0.38	0.94 ± 0.43
Lung	5.13 ± 0.86	2.25 ± 0.35	1.40 ± 0.73	4.97 ± 0.90	3.45 ± 0.68	2.10 ± 0.83
Muscle	1.10 ± 0.24	0.63 ± 0.15	0.39 ± 0.12	1.08 ± 0.15	0.84 ± 0.08	0.54 ± 0.18
Bone	1.37 ± 0.18	0.80 ± 0.11	0.27 ± 0.25	1.25 ± 0.10	0.96 ± 0.19	0.55 ± 0.17
Tumor	22.66 ± 5.42	21.34 ± 2.73	13.56 ± 5.60	19.72 ± 2.68	23.72 ± 4.15	18.12 ± 10.00

*Mean ± s.d.
†Numbers in parentheses show the number of animals.
‡Percentage of the injected dose per gram of tissue.

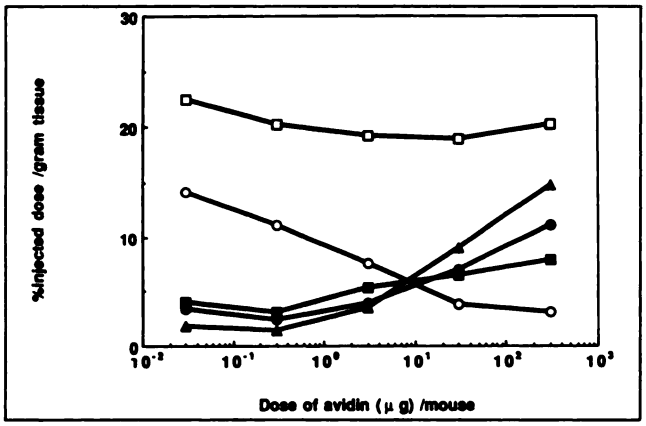

FIGURE 2. Radioactivity of ^{125}I in the blood (○), liver (■), stomach (▲), spleen (●) and tumor (□) in KT005-bearing athymic mice at 24 hr after the injection of 37 kBq (10 μg) of ^{125}I-labeled biotinylated OST7 and 2 hr after the intravenous injection of increasing doses of avidin. Three to six mice were tested in each group.

tumor as well as in all of the organs examined showed a decrease within 6 hr after the injection of 30 μg of avidin into mice preinjected with ^{125}I-labeled biotinylated 56C (Fig. 5).

When avidin was injected at 6 hr after ^{99m}Tc-labeled biotinylated OST7, the blood radioactivity 30 min, 2 hr, and 6 hr postavidin was 7.4%, 6.9% and 14.0%, respectively, of the level before avidin administration. The radio-

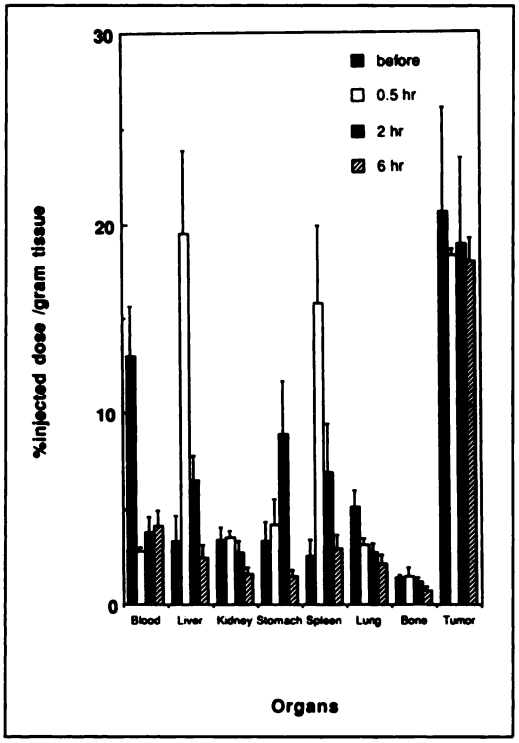

FIGURE 4. Biodistribution of 37 kBq (10 μg) of ^{125}I-labeled biotinylated OST7 in KT005-bearing athymic mice before, 0.5, 2 and 6 hr after the injection of 30 μg of avidin (24, 24.5, 26 and 30 hr after ^{125}I-labeled biotinylated OST7 injection, respectively). Four to six mice were tested in each group.

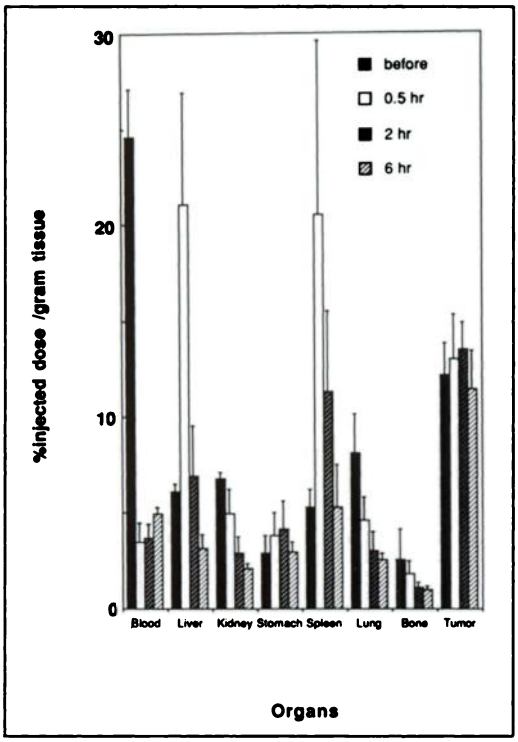

FIGURE 3. Biodistribution of 37 kBq (10 μg) of ^{125}I-labeled biotinylated OST7 in KT005-bearing athymic mice before, 0.5, 2 and 6 hr after the injection of 30 μg of avidin (6, 6.5, 8 and 12 hr after ^{125}I-labeled biotinylated OST7 injection, respectively). Four to six mice were tested in each group.

activity in the liver and spleen peaked immediately after avidin injection and then decreased more slowly in the case of ^{125}I-labeled biotinylated OST7. Although the radioactivity in the other organs decreased, the tumor radioactivity did not change over time (Fig. 6).

Avidin chase markedly increase the tumor-to-blood radioactivity ratio. In the case of ^{125}I-labeled biotinylated OST7, the tumor-to-blood ratio was improved from 0.5 ± 0.1 and 1.8 ± 0.3 to 3.8 ± 0.5 and 6.7 ± 0.6 when avidin was given at 6 hr and 24 hr after the antibody, respectively. The tumor-to-blood ratio of ^{99m}Tc-labeled biotinylated OST7 likewise increased from 0.6 ± 0.3 to 8.4 ± 2.2 when followed by an avidin chase at 6 hr after antibody injection.

The localization index did not change for the initial 2-hr period after the injection of avidin, and then it subsequently increased due to the decrease of ^{125}I-labeled control biotinylated 56C in the tumor.

The "chase effect" indices obtained at various times after avidin injection are shown in Table 2. For both ^{125}I- and ^{99m}Tc-labeled biotinylated OST7, the chase effect was very marked in the blood and all organs except the liver, spleen and stomach. At 6 hr after avidin injection, a chase effect on the ^{125}I-labeled biotinylated antibody was also noted in the liver. Although a chase effect on control biotinylated 56C was noted at all times, it was lower than that on ^{125}I-labeled biotinylated OST7 at 6 hr after avidin injection.

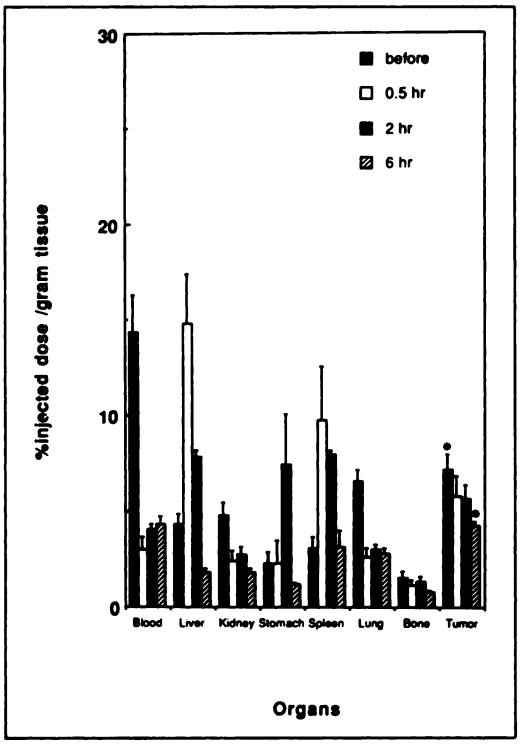

FIGURE 5. Biodistribution of 37 kBq (10 μg) of ^{125}I-labeled biotinylated 56C (the control antibody) in KT005-bearing athymic mice before, 0.5, 2 and 6 hr after the injection of 30 μg of avidin (24, 24.5, 26 and 30 hr after ^{125}I-labeled biotinylated OST7 injection, respectively). Four to five mice were tested in each group. There was a significant difference ($p < 0.01$) between the tumor radioactivity before and 6 hr (*) after avidin injection.

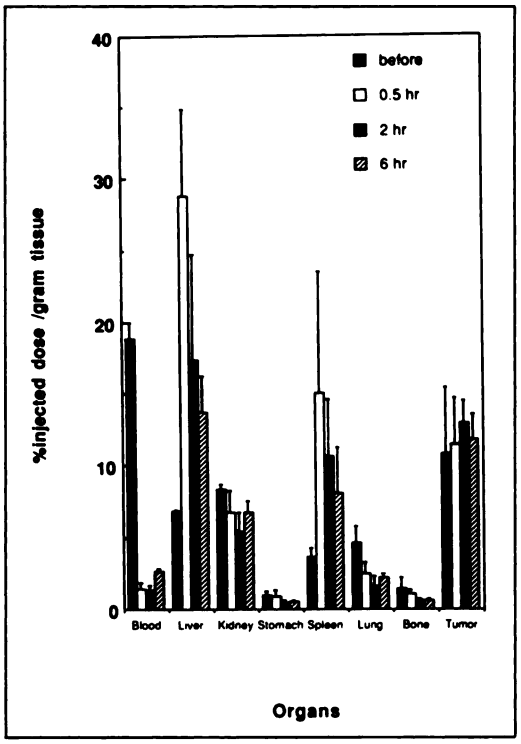

FIGURE 6. Biodistribution of 37 kBq (10 μg) of ^{99m}Tc-labeled biotinylated OST7 in KT005-bearing athymic mice before, 0.5, 2 and 6 hr after the injection of 30 μg of avidin (6, 6.5, 8 and 12 hr after ^{99m}Tc-labeled biotinylated OST7 injection, respectively). Four to six mice were tested in each group.

Experiment Four. Urinary excretion of the radioactivity of ^{125}I-labeled biotinylated OST7 accounted for 23.9% of the injected dose in the 8-hr period after intravenous avidin administration, which was 15.13-fold higher than in control mice. Urinary excretion of the radioactivity from ^{99m}Tc-labeled biotinylated OST7 was 11.59% of the injected dose in the same 8-hr period, being 4.62-fold higher than in control mice. Fecal excretion of radioactivity from ^{99m}Tc-labeled biotinylated OST7 was 2.89% of the injected dose, and was 6.42 times that noted in control mice (Table 3).

TABLE 2
Chase Effect Index Values for the Radiolabeled Biotinylated Antibodies

	Antibody											
	^{99m}Tc-biotin-OST7			^{125}I-biotin-OST7						^{125}I-biotin-56C		
	6 hr*			6 hr*			24 hr*			24 hr*		
	0.5 hr†	2 hr†	6 hr†	0.5 hr†	2 hr†	6 hr†	0.5 hr†	2 hr†	6 hr†	0.5 hr†	2 hr†	6 hr†
Blood	14.22	17.31	7.53	7.62	7.44	4.62	3.79	2.89	2.56	4.01	2.87	1.91
Liver	0.26	0.53	0.55	0.33	1.08	1.82	0.13	0.41	1.05	0.25	0.45	0.92
Kidney	1.34	1.88	1.34	1.52	2.76	3.10	0.79	1.00	1.66	1.65	1.31	1.51
Intestine	1.53	1.18	1.01	1.59	2.17	2.60	0.80	1.01	1.91	1.07	1.00	1.15
Stomach	1.45	2.68	2.36	0.83	0.78	0.91	0.65	0.31	1.73	1.06	0.25	1.04
Spleen	0.30	0.44	0.52	0.32	0.56	1.03	0.13	0.32	0.79	0.27	0.30	0.59
Lung	1.96	4.04	2.20	1.90	3.04	2.91	1.32	1.56	1.96	2.16	1.56	1.40
Muscle	1.50	2.47	1.47	1.44	1.46	1.33	1.09	1.00	1.67	1.14	1.03	0.89
Bone	1.01	1.86	1.75	1.20	1.99	1.92	0.93	1.07	1.93	1.10	0.83	1.13

*Antibody-to-avidin.
†Time after avidin.

TABLE 3
Excretion Ratios of the Radiolabeled Biotinylated Antibodies in the 8-hr Period after Avidin Injection

	^{125}I-labeled biotinylated OST7		^{99m}Tc-labeled biotinylated OST7	
	24 hr*		24 hr*	
	With avidin†	Without avidin†	With avidin†	Without avidin†
Urinary excretion	23.92‡	1.58	11.59	2.51
Fecal excretion	0.11	0.03	2.89	0.45

*Time from antibody to avidin.
†In 0.05 *M* phosphate buffer.
‡Percentage of injected radioactivity.

Immunoscintigraphy

The immunoscintigraphy findings supported the data obtained in the biodistribution studies. Immediately after avidin injection, both ^{131}I and ^{99m}Tc accumulated in the liver and spleen, while the background radioactivity was markedly decreased. At 6 hr after avidin injection, ^{131}I-labeled biotinylated OST7 was only localized in the xenografted KT005 tumors and the liver and spleen were not detected, but the ^{99m}Tc-labeled biotinylated antibody was still retained by the liver and spleen (Figs. 7 and 8).

In addition, we have studied five other ^{125}I- and ^{99m}Tc-labeled murine or chimeric, monoclonal or polyclonal antibodies in three other experimental mouse models and obtained similar results.

DISCUSSION

Avidin-induced blood clearance of biotinylated Mabs may have various applications in the fields of radioimmunoimaging and radioimmunotherapy, since it is extremely rapid (5–15 min) and produces up to a 10-fold increase in contrast between the blood and target tissues (except for the liver, kidney and stomach). Clearance may be started at any time, thus providing an opportunity to maintain an increased concentration of immunoreagents around the target and reduce it when necessary. In the present study, radioiodine-labeled biotinylated antibody-avidin complexes were trapped in the liver and spleen immediately after the injection of avidin. Then iodine was rapidly removed from the trapped complexes and excreted into the urine over the next few hours. Thus, the slight increase of radioactivity in the blood from 2 hr after avidin injection was probably caused by free radioiodine released from the metabolized biotinylated antibody-avidin complexes in the liver and spleen. The transient elevation of radioactivity in the stomach also supports this mechanism.

Technetium-99m-labeled biotinylated antibody-avidin complexes were also trapped in the liver and spleen immediately after avidin injection. However, ^{99m}Tc detached more slowly from the complexes than radioiodine. Thus, although the hepatic and splenic radioactivity of ^{99m}Tc was excreted more slowly than that of radioiodine, the circulating radioactivity remained lower at all the times after avidin injection. These findings suggest that, except in the upper abdomen, ^{99m}Tc-labeled biotinylated antibodies would be superior to ^{131}I-labeled biotinylated antibodies for radioimmunoimaging using this chase technique, in addition to the better physical properties of ^{99m}Tc. If a radioiodine-labeled biotinylated antibody is desired, ^{123}I would seem to be the most suitable radionuclide, because the present study showed that clear images could be obtained more rapidly after injection than with conventional immunoscintigraphy and because the photon energy of ^{123}I is suitable for imaging, especially SPECT scanning.

In this study, we used a dose of 10 μg (0.5 mg/kg) of the radiolabeled biotinylated antibody and 30 μg (1.5 mg/kg) of avidin in each mouse. Provided that the weight ratio of biotinylated antibody-to-avidin is kept at 1:3, the injected dose of both antibody and avidin could be decreased. With this 10-fold excess (300 μg) of avidin, we did not observe any side effects and others have supported this finding (*13*). Moreover, Paganelli et al. have reported on the use of 1–3 mg of biotinylated antibody and 4–6 mg of avidin in a

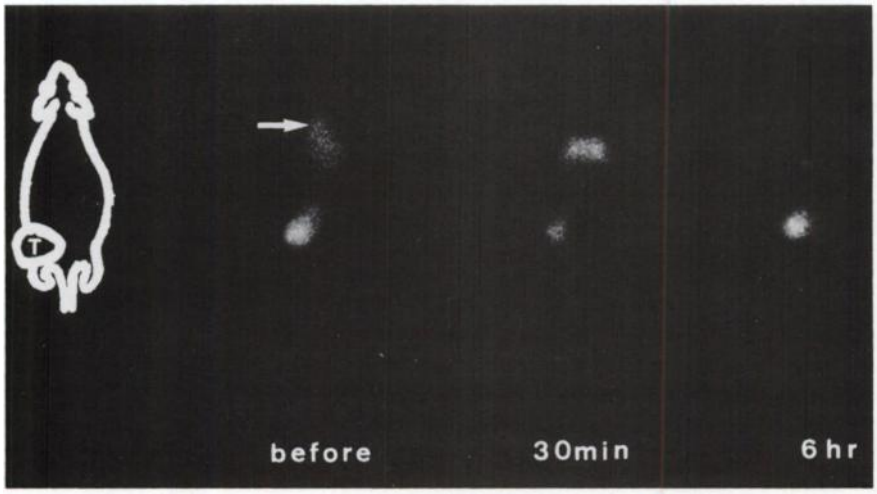

FIGURE 7. Scintigrams of a mouse bearing KT005 human osteogenic sarcoma. Images were obtained at 24 hr after the injection of ^{131}I-labeled biotinylated OST7, as well as 30 min and 6 hr after avidin injection as a chase (24.5 hr and 30 hr after OST7 injection, respectively). T indicates the site of the xenografted tumor. The blood pool in the heart (arrow) was not visualized at 30 min and 6 hr after avidin injection.

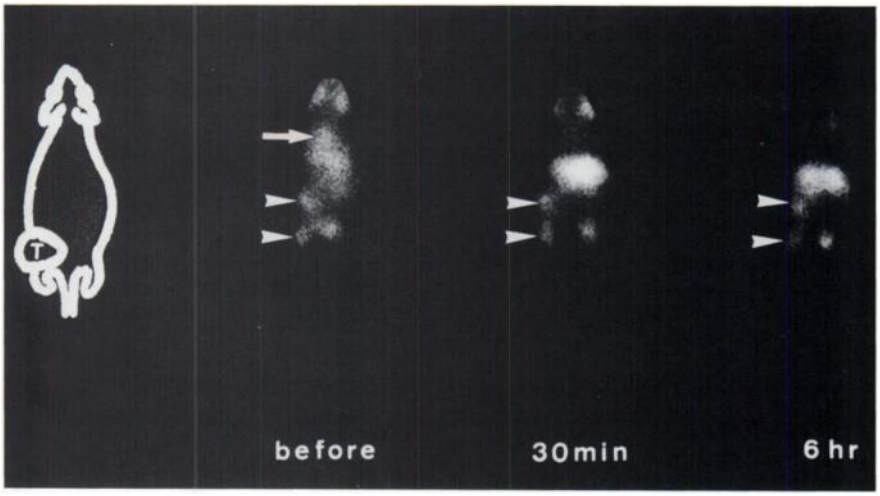

FIGURE 8. Scintigrams of a mouse bearing KT005 human osteogenic sarcoma. Images were obtained 6 hr after the injection of ^{99m}Tc-labeled biotinylated OST7, as well as 30 min and 6 hr after avidin injection as a chase (6.5 hr and 12 hr after OST7 injection, respectively). T and the arrowheads indicate the xenografted tumor. The blood pool in the heart (arrow) was not visualized at 30 min and 6 hr after avidin injection.

clinical study, with no major side effects being detected. Thus, our method may also be clinically applicable.

In addition, any sort of antibody can easily be biotinylated. The desired effect can be achieved by even slight modification of the antibody (incorporation of two to three biotin molecules per antibody molecule), and NHS-LC-biotin has a long spacer between the protein-binding site and biotin to reduce steric hinderance of the avidin-biotin reaction. Although different Mabs may show varying responses against biotinylation, we have also treated two Mabs for CA125 using the same method and found no loss of immunoreactivity due to biotinylation and radioiodination (data not shown). Moreover, even for immunoscintigraphy using the nonspecific control antibody, this method was able to enhance tumor radioactivity, so it may be applicable to scintigraphy for detecting sites of inflammation using polyclonal IgG.

When radioimmunotherapy is performed, the prolonged retention of background radioactivity leads to unwanted irradiation of the normal organs, with blood radioactivity providing a major source of irradiation to the bone marrow. Thus, avidin injection to decrease the blood radioactivity level at a time when the target tumor radioactivity is high enough for a therapeutic effect should be applicable to radioimmunotherapy using biotinylated antibodies and should increase the tolerable radiolabeled antibody dose.

In conclusion, our method should be of value for improving the biodistribution of antibodies and for reducing the time required to obtain images with a good contrast. Technetium-99m and ^{123}I are suitable for imaging studies but have a short half-life, while more specific targeting therapy using ^{131}I-labeled biotinylated Mabs may also be possible.

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