Relationship Between In Vitro Binding Activity and In Vivo Tumor Accumulation of Radiolabeled Monoclonal Antibodies

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The relationship between in vitro cell binding and in vivo tumor accumulation of radiolabeled antibodies was studied using ^{125}I- and ^{111}In-labeled monoclonal antibodies to human osteosarcoma, and a human osteosarcoma xenograft (KT005) in nude mice. Three monoclonal antibodies—OST6, OST7, and OST15—raised against human osteosarcoma recognize the same antigen molecule. Although the binding of both ^{125}I- and ^{111}In-labeled OST6 to KT005 cells was higher than that of radiolabeled OST7 in vitro, ^{125}I-labeled OST6 showed a faster clearance from the circulation and a lower accumulation in the transplanted tumor than ^{125}I-labeled OST7. In contrast to the radioiodinated antibodies, the in vivo tumor accumulation of ^{111}In-labeled OST6 was higher, although not significantly, than that of ^{111}In-labeled OST7. OST15 showed the lowest binding in vitro, and its in vivo tumor localization was also lower than the others. The discrepancy in tumor uptake between OST6 and OST7 labeled with either ^{125}I or ^{111}In may have been a result of differing blood clearance. These results suggest that binding studies can be used to exclude from in vivo use those antibodies which show very poor binding in vitro, while in vivo serum clearance may be a better test for choosing antibodies with similar binding.

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Success in radioimmunoimaging of tumors depends on the selection of monoclonal antibodies and radionuclides. The in vitro characterization of monoclonal antibodies, such as the antigens which they target, the number of antigenic sites per tumor cell, the affinity of the antibody, and the immunoreactive fraction of the radiolabeled antibody as a final product, is important (*1*). Antibodies which show a higher binding to cells are presumed to accumulate more in the tumor in vivo (*1–3*). However, there are many other factors in vivo which affect the tumor accumulation of radiolabeled antibodies, in addition to in vitro binding properties. Radioiodinated and indium-111- (^{111}In) labeled antibodies have been widely used for imaging or therapy of tumors, although the results of tumor localization with these radionuclides are different. In this study, in order to clarify how in vitro binding activities correlate with in vivo tumor accumulation, their relationship was investigated using three monoclonal antibodies to human osteosarcoma, labeled with iodine-125 (^{125}I) and ^{111}In, and a human osteosarcoma xenograft in nude mice. Monoclonal antibodies OST6, OST7, and OST15 raised against human osteosarcoma recognize the same antigen molecule, and have been specifically localized in human osteosarcoma xenografts in nude mice (*4–6*).

MATERIALS AND METHODS

Antibody Preparation

The monoclonal antibodies OST6 (IgG_1), OST7 (IgG_1), and OST15 (IgG_{2a}) were prepared by a standard hybridoma method using osteosarcoma cells freshly resected from an untreated patient (*4*). The antibodies were purified by Protein A affinity chromatography from ascitic fluid obtained from

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hybridoma-bearing BALB/c mice. These antibodies recognize the same 87,000 D glycoprotein, which is closely related to alkaline phosphatase (*7,8*).

Radiolabeling of Monoclonal Antibodies

The radiolabeling of the monoclonal antibodies was performed as reported previously (*6*). The monoclonal antibodies were labeled with ^{125}I by the chloramine-T method using limiting amounts of chloramine-T. OST6 and OST7 were also labeled with ^{111}In using diethylenetriaminepentaacetic acid (DTPA) as a bifunctional chelating agent. The antibodies contained 0.3 to 0.5 DTPA molecules per antibody molecule. The specific activities of the radiolabeled antibodies were between 3 and 8 mCi/mg for the ^{125}I-labeled antibodies and between 0.5 and 2 mCi/mg for the ^{111}In-labeled antibodies.

In Vitro Reactivity

Human osteosarcoma KT005 was maintained by serial subcutaneous transplantation in athymic nude mice. Tumor cell suspensions were prepared from nude mouse KT005 tumors by passing the tumor specimens through a stainless steel mesh. Red blood cells were removed by hemolysis (*6*).

One hundred microliters of radiolabeled antibody were incubated with various numbers of tumor cells (2×10^4–2×10^6), suspended in 100 μl of phosphate buffered saline in 5.7 × 46 mm microcentrifuge tubes for 2 hr at 4°C. After centrifugation, the supernatant was aspirated and the tube was cut. The percentage of radioactivity bound to cells was determined by subtracting the nonspecific binding of an irrelevant monoclonal antibody (IgG_1), which recognized human chorionic gonadotropin. The immunoreactive fraction of radiolabeled antibodies was determined according to the method of Lindmo et al. (*9*) by linear extrapolation to conditions representing infinite antigen excess.

The apparent affinity constant for each antibody was calculated by the Scatchard analysis (*10*). Fifty microliters of ^{125}I-labeled antibody, 50 μl of unlabeled antibody (0.05–50 μg), and 1×10^6 cells suspended in 100 μl of phosphate buffered saline were incubated together, and the percentage of bound radioactivity was determined as described above.

In Vivo Biodistribution

Nude mice bearing KT005 xenografts or normal ddY mice were injected into the tail vein with 20 μg of antibody which had been prepared by mixing unlabeled and radiolabeled antibody (*6*). Tumors of 0.5–1 g sizes at 2-3 wk after transplantation were used in the present study. The mice were killed at 6, 24, 48, and 96 hr after the injection. Distribution data were represented as a percentage of the injected dose per gram normalized to a 20 g mouse. Statistical analysis was performed using Student's t-test.

RESULTS

In Vitro Reactivity

Radiolabeled OST6 showed a higher binding to KT005 cells than radiolabeled OST7 in both the ^{125}I-labeled and ^{111}In-labeled forms (Fig. 1). The binding of ^{125}I-labeled OST15 ([^{125}I]OST15) was much lower than that of the other two, although the immunoreactive fractions of all preparations were calculated as about 0.75 according to the method of Lindmo et al. (*9*). The binding of radiolabeled antibodies to KT005 cells was inhibited dose-dependently by the addition of unlabeled antibodies (Fig. 2), and the apparent affinity constants for OST6, OST7, and OST15 were calculated as 3.9×10^8, 1.8×10^8, and 3.2×10^7 M^{-1}, respectively.

In Vivo Biodistribution

The tumor accumulation of ^{125}I-labeled antibodies is shown in Figure 3. Peak concentration of OST6 in the tumor was observed at 24 hr after the injection and then decreased. The tumor concentration of OST7 was as high as that of OST6 at 24 hr and was retained up to 48 hr. As a result ^{125}I-labeled OST7 showed a higher accumulation in the tumor than [^{125}I]OST6 at 48 and 96 hr postinjection. The blood clearance of [^{125}I]OST6 was slightly faster than that of [^{125}I]OST7 (Fig. 4). Thus,

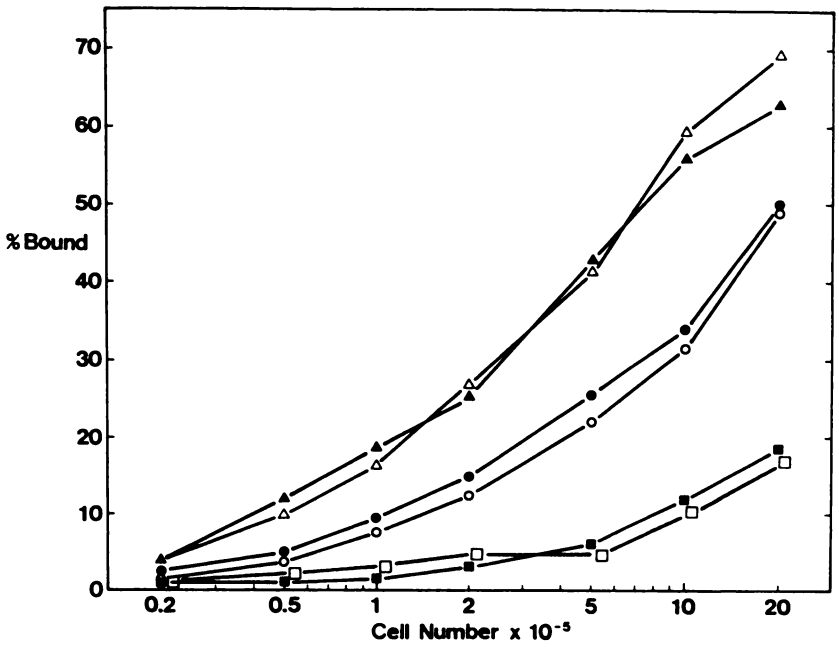

FIGURE 1
In vitro binding of radiolabeled monoclonal antibodies to KT005 cells. Percentage of bound radioactivity, after subtracting nonspecific binding of control antibody, was plotted against cell number. (▲) = [^{125}I]OST6, (●) = [^{125}I]OST7, (■) = [^{125}I]OST15, (△) = [^{111}In]OST6, (○) = [^{111}In]OST7, and (□) = [^{111}In]OST15.

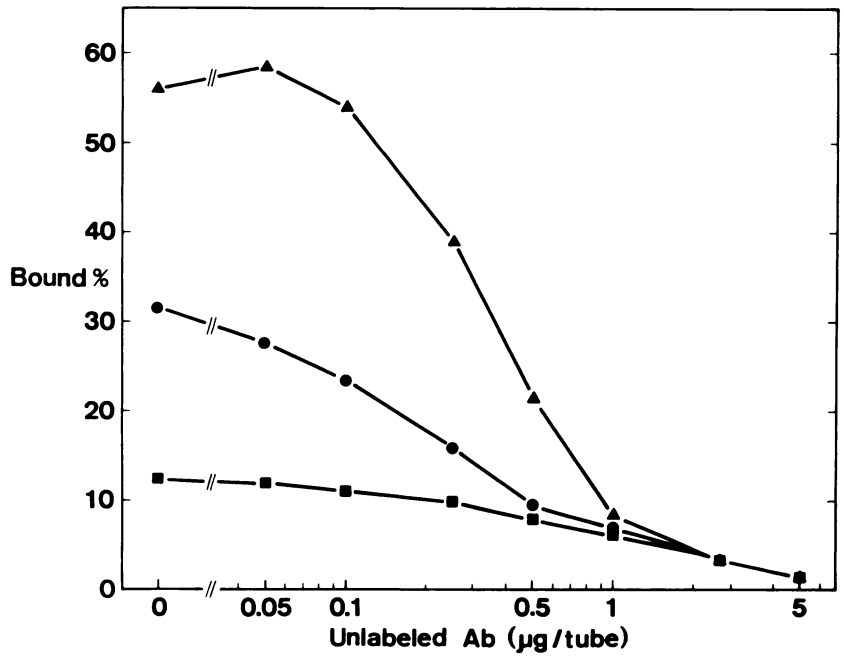

FIGURE 2
Inhibition curves for cell binding of ^{125}I-labeled monoclonal antibodies, following addition of unlabeled antibodies. (▲) = OST6, (●) = OST7, and (■) = OST15.

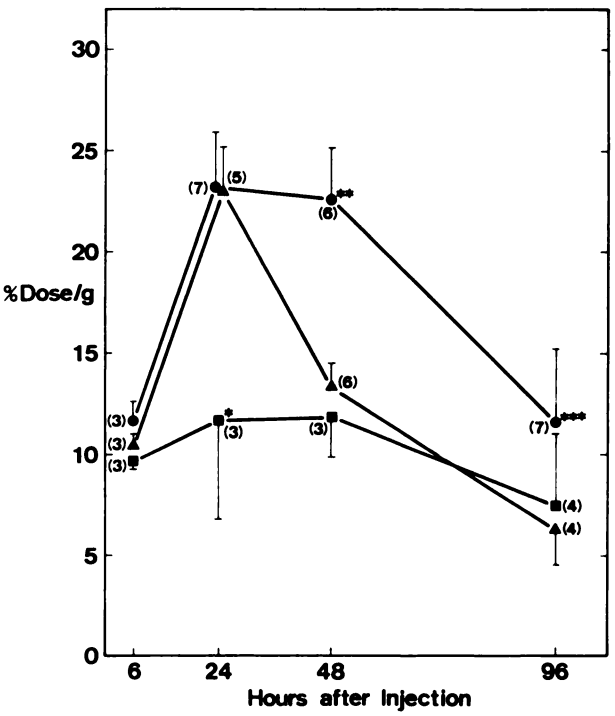

FIGURE 3
Accumulation of ^{125}I-labeled monoclonal antibodies in xenografted tumor. Mean ± s.d. Numbers in parentheses are numbers of animals. (▲) = [^{125}I]OST6, (●) = [^{125}I]OST7, and (■) = [^{125}I]OST15. *p < 0.01 compared with OST6 or OST7. **p < 0.001 compared with OST6 or OST15, and *p < 0.05 compared with OST6.

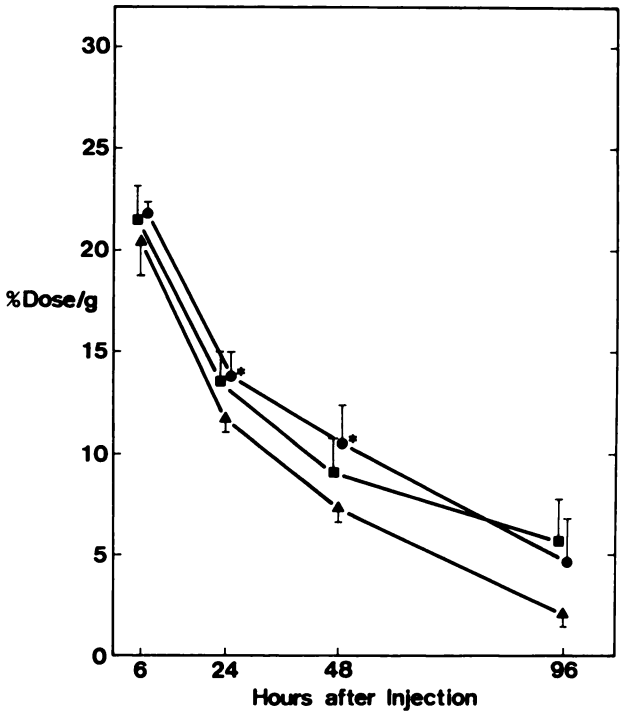

FIGURE 4
Blood clearance of ^{125}I-labeled monoclonal antibodies in nude mice bearing human osteosarcoma xenograft. Mean ± SD. Number of animals is shown in Figure 2. (▲) = ^{125}I-labeled OST6, (●) = ^{125}I-labeled OST7, and (■) = ^{125}I-labeled OST15. *p < 0.01 compared with OST6.

tumor-to-blood, tumor-to-liver, and tumor-to-muscle ratios of radioactivity were similar for both OST6 and OST7 (Table 1).

On the other hand, the tumor accumulation of [^{111}In] OST6 was higher, although not significantly, than that of [^{111}In]OST7 (Fig. 5), and the blood clearance of [^{111}In]OST6 was no faster than that of [^{111}In]OST7 (Fig. 6). The tumor-to-blood ratio for ^{111}In-labeled antibodies was higher than for ^{125}I-labeled antibodies, but significant differences in tumor-to-nontumor ratios were not observed between [^{111}In]OST6 and [^{111}]OST7 (Table 2).

Both the net concentration in the tumor, and the tumor-to-nontumor ratios of [^{125}I]- and [^{111}In]OST15 were much lower than the others.

In normal mice, the blood clearance of [^{125}I]OST6 was slightly faster than that of [^{125}I]OST7 as well as in tumor-bearing mice, whereas [^{111}In]OST6 showed a slightly slower blood clearance than [^{111}In]OST7 (data not shown).

DISCUSSION

In vitro binding activity is usually related to the in vivo tumor accumulation of radiolabeled monoclonal antibodies, as shown by the lowest tumor uptake of OST15 in this study (*2,3*). Theoretically, if there are two antibodies, targeted to the same tumor antigen, the one with the higher affinity constant, will provide higher tumor-to-nontumor ratios and better images (*1*). However, in vivo tumor accumulation of radiolabeled antibodies is influenced by many more complicated factors than simply the in vitro binding activity, and they do not always correlate well. Vascular permeability may play an important role in antibody localization in the tumor. Antigen shedding, or the nature of an antigen to be targeted, may also cause a lack of correlation between the in vitro cell binding and accumulation in tumor xenografts of a radiolabeled monoclonal antibody (*11*). Furthermore, the radionuclide or labeling method alters the biodistribution and tumor targeting of a given monoclonal antibody (*6,12–15*). It has been reported that deiodination of radioiodinated antibody resulted in a marked difference in tumor localization

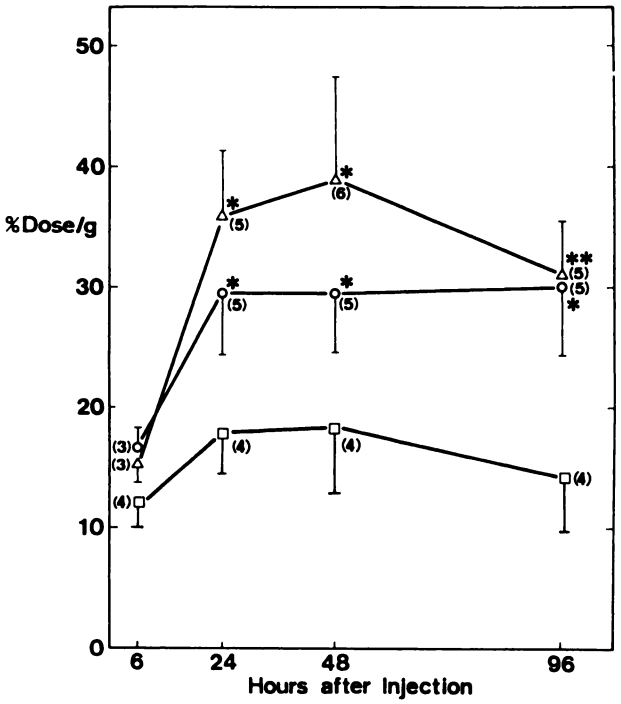

FIGURE 5
Accumulation of ^{111}In-labeled monoclonal antibodies in xenografted tumor. Mean ± s.d. Numbers in parentheses are numbers of animals. (△) = [^{111}In]OST6, (○) = [^{111}In]OST7 and (□) = [^{111}In]OST15. *$p < 0.01$ compared with OST15, and **$p < 0.001$ compared OST15.

of ^{131}I- and ^{111}In-labeled T101 antibody in patients with cutaneous T-cell lymphoma (*16*). In our study, using serially transplanted osteosarcoma xenograft as a tumor model, we have compared in vitro binding activity and

TABLE 1
Tumor-to-Nontumor Ratios of ^{125}I-Labeled Monoclonal Antibodies*

	Hours after injection			
	6	24	48	96
Tumor-to-blood				
OST6	0.52 ± 0.07 (3)	1.95 ± 0.12† (5)	1.81 ± 0.23§ (6)	3.13 ± 1.01§ (4)
OST7	0.53 ± 0.11 (3)	1.69 ± 0.30‡ (7)	2.21 ± 0.61¶ (6)	2.90 ± 1.26 (7)
OST15	0.45 ± 0.06 (3)	0.86 ± 0.32 (3)	1.32 ± 0.12 (3)	1.28 ± 0.21 (4)
Tumor-to-liver				
OST6	1.72 ± 0.20	7.77 ± 1.37§	7.56 ± 1.01†	10.41 ± 1.05‡
OST7	1.95 ± 0.07	6.36 ± 1.31	7.48 ± 1.86	10.09 ± 3.20
OST15	2.08 ± 0.19	4.12 ± 2.04	3.57 ± 0.34	6.69 ± 1.70
Tumor-to-muscle				
OST6	13.00 ± 2.52	22.60 ± 3.85§	23.40 ± 1.56†	33.65 ± 8.27§
OST7	13.52 ± 3.36	22.27 ± 2.40‡	28.38 ± 7.76	32.64 ± 10.12
OST15	11.62 ± 0.27	13.23 ± 3.54	15.40 ± 1.55	17.52 ± 2.77

* Mean ± s.d.; numbers in parentheses are numbers of animals.
† $p < 0.001$ compared with OST15.
‡ $p < 0.01$ compared with OST15.
§ $p < 0.02$ compared with OST15.
¶ $p < 0.05$ compared with OST15.

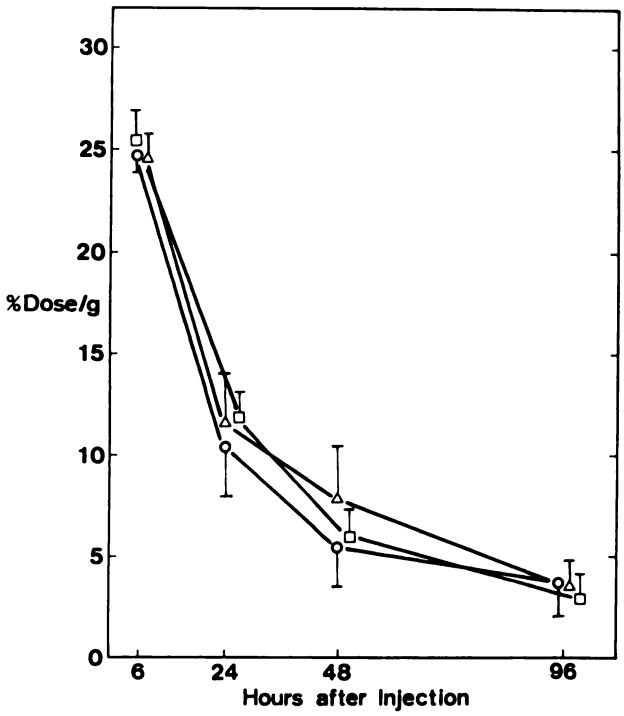

FIGURE 6
Blood clearance of ^{111}In-labeled monoclonal antibodies in nude mice bearing human osteosarcoma xenograft. Mean ± s.d. Number of animals is shown in Figure 4. (△) = [^{111}In] OST6, (○) = [^{111}In]OST7 and (□) = [^{111}In]OST15.

in vivo tumor accumulation of three radiolabeled monoclonal antibodies, which recognize the same antigen molecule (*7,8*).

There was no significant difference in the in vitro binding between the ^{125}I- and ^{111}In-labeled antibodies, and with both radionuclides OST6 showed the highest bound percentage. OST6 had the highest affinity constant to KT005 cells among the three antibodies tested. However, [^{125}I]OST6 showed a lower in vivo tumor accumulation than [^{125}I]OST7. This in vitro and in vivo discrepancy of tumor concentration of radioactivity was not observed when ^{111}In-labeled antibodies were used. The blood clearance of OST6 was slightly faster than OST7 when they were labeled with ^{125}I, while [^{111}In] OST6 did not show faster blood clearance than [^{111}In] OST7. This finding was seen in normal mice as well as in tumor-bearing mice. Several mechanisms could be responsible for the discordant clearance of [^{125}I]OST6 and [^{125}I]OST7. It is unlikely that the different distribution is secondary to the shed antigen or the modulation of antigen (*16*), since both antibodies are reactive with the same antigen molecule. The difference in blood clearance may have resulted from labeling change, although antibody activity seemed to be retained after labeling procedure. Another explanation is due to the dehalogenase activity present in tumor and organs such as liver, kidney and spleen. The rapid breakdown of some radioiodinated antibodies has been previously described (*12,13*), although Halpern et al. reported the in vitro and in vivo stability of ^{111}In-labeled antibodies against carcinoembryonic antigen (*14*). In addition, marked differences in apparant biodistribution have been suspected using a dual labeled (^{111}In and ^{125}I) antibodies against mammary tumors (*15*). Iodine-125-labeled OST6 may be more susceptible to deiodination or to labeling damage than [^{125}I]OST7. The difference in tumor uptake between OST6 and OST7 labeled with

TABLE 2
Tumor-to-Nontumor Ratios of ^{111}In-Labeled Monoclonal Antibodies*

	Hours after injection			
	6	24	48	96
Tumor-to-blood				
OST6	0.63 ± 0.12 (3)	3.32 ± 1.46¶ (6)	5.77 ± 2.95 (6)	9.44 ± 3.00‡ (5)
OST7	0.68 ± 0.07¶ (3)	2.92 ± 0.45‡ (5)	6.10 ± 1.94§ (5)	9.04 ± 2.40‡ (5)
OST15	0.50 ± 0.15 (4)	1.56 ± 0.46 (4)	2.96 ± 1.02 (4)	5.06 ± 1.57 (4)
Tumor-to-liver				
OST6	1.38 ± 0.16	4.39 ± 1.29†	4.69 ± 0.82†	4.16 ± 0.53†
OST7	1.52 ± 0.24¶	4.35 ± 1.02†	5.13 ± 0.74†	5.04 ± 0.72†
OST15	1.00 ± 0.24	1.49 ± 0.40	1.64 ± 0.87	1.19 ± 0.43
Tumor-to-muscle				
OST6	16.13 ± 1.71‡	36.46 ± 9.38†	46.84 ± 11.90†	41.78 ± 9.89‡
OST7	17.35 ± 1.05†	26.14 ± 4.82†	33.90 ± 8.32‡	40.26 ± 5.64†
OST15	8.32 ± 1.82	14.20 ± 3.21	13.60 ± 5.62	15.95 ± 4.92

* Mean ± s.d.; numbers in parentheses are numbers of animals.
† $p < 0.001$ compared with OST15.
‡ $p < 0.01$ compared with OST15.
§ $p < 0.02$ compared with OST15.
¶ $p < 0.05$ compared with OST15.

either ^{125}I or ^{111}In may have been a result of differing blood clearance (*17,18*).

Recently monoclonal antibodies labeled with radioiodine and ^{111}In have been clinically used for radioimmunoimaging or radioimmunotherapy of tumors (*1, 16,18–21*). The results will depend on the ratio of radiation delivered to tumor tissues versus radiation delivered to normal organs. Tumor-to-blood, tumor-to-liver, and tumor-to-muscle ratios of radiolabeled OST6 and OST7 were similar to each other, so that we would obtain a similar tumor image and similar therapeutic efficacy using OST6 and OST7. Present studies indicate that binding studies can be used to exclude from clinical trials those antibodies which show very poor binding in vitro. Also, there may be difference in susceptibility to deiodination or to labeling damage among different monoclonal antibodies. Finally, in vitro studies may be better able to predict tumor accumulation of ^{111}In-labeled than of radioiodinated monoclonal antibodies. The different results obtained for in vitro cell binding and in vivo tumor accumulation of radioiodinated antibodies suggests the importance of considering in vivo metabolism of radiolabeled monoclonal antibodies, as well as their in vitro antigen binding activities, in the selection of antibodies and radionuclides for in vivo use.

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