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Comparison of the Chase Effects of Avidin, Streptavidin, Neutravidin, and Avidin-Ferritin on a Radiolabeled Biotinylated Anti-tumor Monoclonal Antibody

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Injection of avidin can decrease the background radioactivity due to a radiolabeled biotinylated monoclonal antibody. We compared the chase effects of avidin, streptavidin, neutravidin, and avidin-conjugated ferritin on a radiolabeled antitumor monoclonal antibody in tumor-bearing nude mice. A radioiodine-labeled biotinylated monoclonal antibody (OST7) was administered to athymic mice bearing osteogenic sarcomas. After 24 h, an avidin, streptavidin, neutravidin or avidin-conjugated ferritin chaser was intravenously injected into the mice. At 2 h after the chase, the biodistribution of the radiolabeled monoclonal antibody was determined. Clearance from the blood was dose-dependently accelerated by avidin and its effect was 10-fold stronger than that of neutravidin or avidin-ferritin. Streptavidin did not promote clearance of the biotinylated antibody. Avidin was the most effective chasing agent for improving the biodistribution of the radiolabeled biotinylated monoclonal antibody among the four avidin derivatives tested.

Key words: Monoclonal antibody — Avidin — Biotin — Chase — Radioimmunoimaging

Several kinds of avidin derivatives and avidin-conjugated proteins are used in immunohistochemical and immunoscintigraphic studies. Immunoscintigraphy using avidin-biotin systems is usually performed clinically by a 2-step or 3-step method.[1-8] These methods employ a biotinylated antibody or an avidin-conjugated antibody for pretargeting and use radiolabeled biotin or avidin, which are cleared rapidly, for detecting the disease focus.[2,6,8,9] A new immunoscintigraphic method using a radiolabeled biotinylated monoclonal antibody and subsequent injection of avidin as a chaser has been shown to achieve a better antibody biodistribution for both immunoscintigraphy and radioimmunotherapy when compared with conventional methods.[10-12] In this study, we compared the chase effect of avidin and various avidin derivatives, i.e., streptavidin, neutravidin, and avidin-conjugated ferritin.

MATERIALS AND METHODS

Cells KT005 human osteosarcoma cells[13] were grown in RPMI 1640 medium (Nissui, Tokyo) containing 10% fetal calf serum (GIBCO Laboratories, Grand Island, NY) and 0.03% L-glutamine at 37°C in 5% CO_2. Subconfluent cells were removed using calcium- and magnesium-free phosphate-buffered saline (PBS) containing 0.02% EDTA to preserve their antigenicity.

Monoclonal antibodies The OST7 antibody (IgG_1 isotype) was raised against a human osteogenic sarcoma[14] and has been shown to react with an alkaline phosphatase-related substance on human osteogenic sarcoma cells.[15,16] The antibody was purified from the ascitic fluid of hybridoma-bearing mice using Protein A column chromatography (Bio-Rad, Richmond, CA). A monoclonal antibody 56C (IgG_1), which recognizes human chorionic gonadotropin, was used as the control antibody.[17]

Biotinylation of monoclonal antibody Monoclonal antibodies were biotinylated as reported previously.[10] Two to three molecules of NHS-LC-biotin complex were conjugated to each antibody, and more than 90% of the antibodies retained both antigen-binding and avidin-binding activity, as determined by the 2-(4′-hydroxyazobenzene)benzoic acid method of Green[18] and by binding assay with avidin-Sepharose gel (Pierce Chemical Co., Rockford, IL), respectively.[10,12]

Radiolabeling and quality control Monoclonal antibodies were radioiodinated using the chloramine-T method.[19,20] Purified monoclonal antibodies (40 μg) in 0.3 *M* phosphate buffer (pH 7.5), and ^{125}I (11.1 MBq) (Amersham International, Buckinghamshire, UK) or ^{131}I (7.4 MBq) (DuPont, Billerica, MA) for protein labeling were mixed with 3.0 μg of chloramine-T (Nakarai Chemicals, Kyoto) dissolved in 0.3 *M* phosphate buffer. After 5 min, the radiolabeled antibodies were separated from free iodine by PD-10 gel chromatography. The specific activity of the ^{125}I-labeled antibodies was about 222 MBq/mg, and that of ^{131}I-labeled antibody was 111 MBq/mg.

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Radiolabeled antibodies were analyzed by size-exclusion high-performance liquid chromatography using a TSKG3000SW column (Tosoh Co., Tokyo). More than 95% of the radioactivity was associated with the IgG fraction for radioiodinated antibodies.

Biodistribution and pharmacokinetic studies For *in vivo* studies, 5×10^6 KT005 cells were inoculated subcutaneously into female BALB/c-nu/nu mice. After 12 days, the tumors reached about 200 mg in weight. Potassium iodide solution was administered to the mice from 1 day before the injection of radioiodinated antibodies to inhibit uptake of radioiodine by the thyroid gland. Tumor-bearing nude mice were injected with 37 kBq of ^{125}I-labeled biotinylated OST7 via the tail vein. The antibody dose was adjusted to 10 μg per mouse by adding unlabeled biotinylated OST7.

At 24 h after ^{125}I-labeled biotinylated OST7 injection, the mice were injected intravenously with avidin, streptavidin, neutravidin or avidin-conjugated ferritin (purchased from Pierce Chemical Co., Rockford, IL). The dose of avidin ranged from 0.03 to 300 μg per mouse and the dose of the other reagents was 30 μg per mouse. Two hours later, groups of mice were killed, their organs were removed and weighed, and the radioactivity was counted. The chemical characteristics of the avidin derivatives are shown in Table I.

Data were expressed as both the percentage of the injected dose per gram of tissue and tumor-to-normal

Table I. Characteristics of Avidin and Its Derivatives

	Avidin	Streptavidin	Neutravidin	Avidin-ferritin
Nature	glycoprotein	protein	protein	glycoprotein
Molecular weight	67,000–68,000	60,000	60,000	500,000
Number of biotin binding sites	4	4	4	4
Nonspecific binding	high	low	lowest	high
Isoelectric point	10.0	5.0	6.3	No data

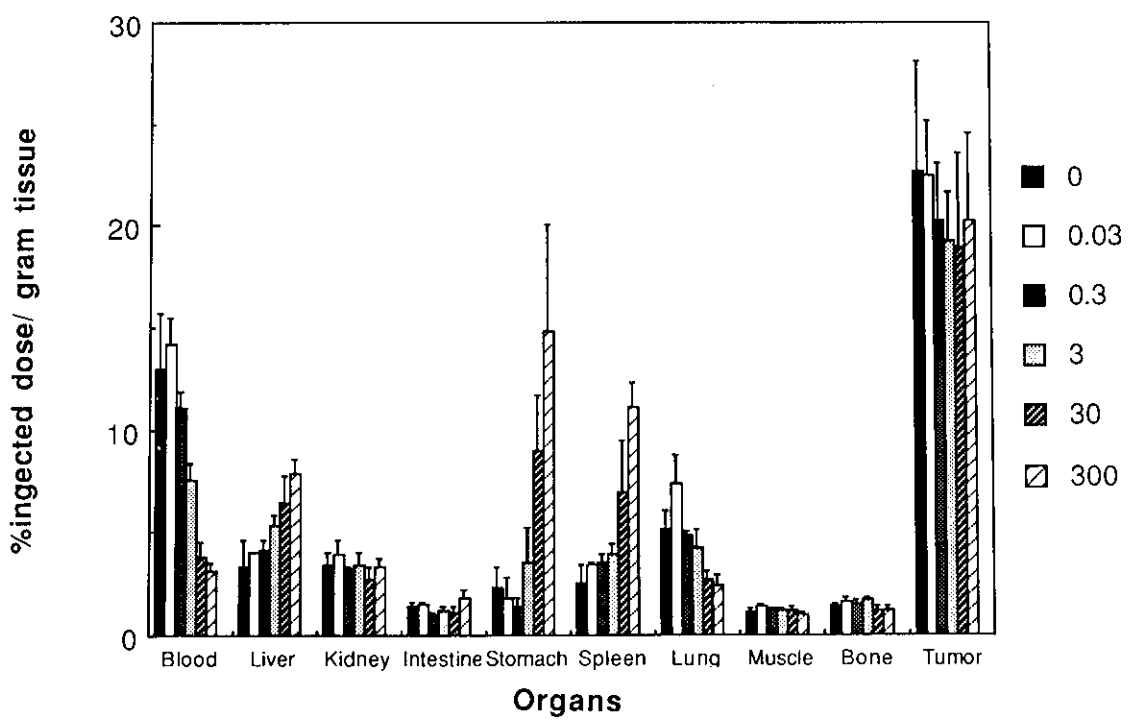

Fig. 1. Biodistribution of 37 kBq (10 μg) of ^{125}I-labeled biotinylated OST7 in KT005-bearing athymic mice at 2 h after the intravenous injection of increasing doses (μg) of avidin (26 h after OST7 injection). Four to six mice were tested in each group.

tissue radioactivity ratios. A chase effect index was also calculated by dividing the tumor-to-normal tissue radioactivity ratio after the chase by that before the chase.

Immunoscintigraphy For the imaging of tumor-bearing nude mice, 20 μg (2.22 MBq) of ^{131}I-labeled biotinylated OST7 was administered intravenously via the tail vein. At 6 h after injection of antibody, as well as 0.5 h after the injection of 60 μg of avidin or streptavidin, mice were anesthetized with intraperitoneal sodium pentobarbital and scintigrams were obtained using a gamma camera equipped with a pin-hole collimator.[10, 17]

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

RESULTS

The chase effect of avidin increased dose-dependently (Fig. 1) and a 3 μg dose clearly decreased the blood radioactivity at 2 h after injection. However, streptavidin at a dose of 30 μg had no effect on antibody biodistribution (Fig. 2). In contrast, the chase effect of 30 μg of neutravidin or avidin-ferritin was nearly equivalent to that of 3 μg of avidin (Table II).

The immunoscintigraphic findings supported the biodistribution data. At 0.5 h after avidin injection, ^{131}I-labeled biotinylated antibody accumulation could be seen in the liver and spleen, while the background radioactivity was markedly decreased. In contrast, after strep-

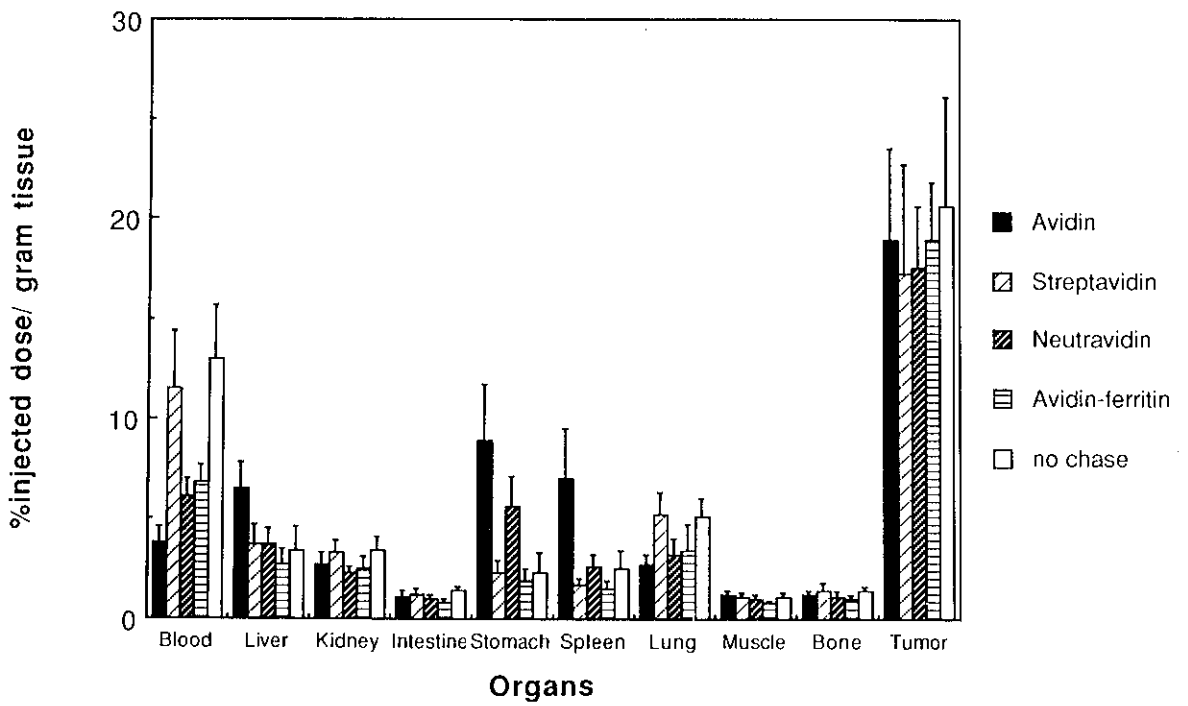

Fig. 2. Biodistribution of 37 kBq (10 μg) of ^{125}I-labeled biotinylated OST7 in KT005-bearing athymic mice at 2 h after the intravenous injection of 30 μg of avidin, streptavidin, neutravidin, or avidin-ferritin (26 h after OST7 injection). Four to five mice were tested in each group.

Table II. Chase Effect of Avidin and Its Derivatives at 26 h after Injection of 10 μg of ^{125}I-Labeled Biotinylated OST7 (2 h after Injection of the Chase Agent)

	Avidin					Streptavidin	Neutravidin	Avidin-ferritin
Dose (μg)	0.03	0.3	3	30	300	30	30	30
Blood	0.90[a)]	1.04	1.45	2.89	3.71	0.90	1.61	1.59
Liver	0.87	0.93	0.50	0.41	0.34	0.92	0.63	1.03
Lung	0.81	0.94	1.15	1.56	1.58	0.88	1.21	1.27

a) Chase effect index = (tumor to normal tissue ratio with chase)/(tumor to normal tissue ratio without chase).

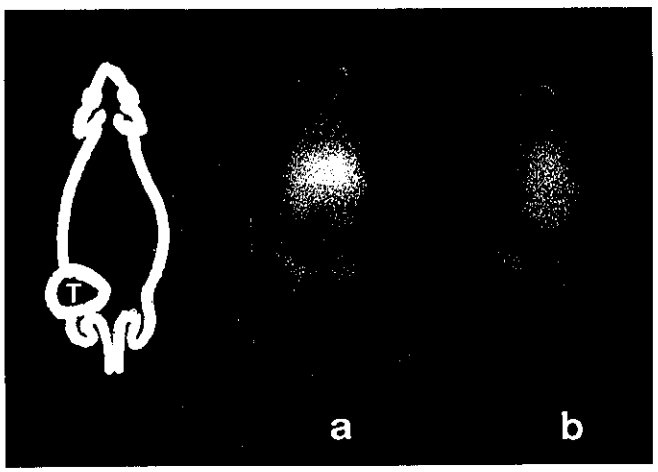

Fig. 3. Scintigrams of mice bearing KT005 human osteogenic sarcomas. Images were obtained at 6 h after the injection of 2.22 MBq (20 μg) of ^{131}I-labeled biotinylated OST7, as well as 0.5 h after the injection of 60 μg of avidin (a) or streptavidin (b) as a chaser (6.5 h after OST7 injection). "T" indicates the tumor. The antibody accumulated in the liver and spleen after avidin injection but not after streptavidin injection. The blood pool in the heart was still visualized in the mouse given streptavidin, but not in the mouse given avidin.

tavidin injection the ^{131}I-labeled biotinylated antibody still remained in the blood (Fig. 3).

DISCUSSION

The binding affinity constants for biotin of avidin and its derivatives all have the same value ($K_a = 1 \times 10^{15}$), which is the largest known for a biological interaction.[9-12] Avidin, streptavidin, neutravidin and avidin-conjugated ferritin have four biotin binding sites per molecule. Thus, if the same molar amount of each avidin derivative is injected into mice which have been administered a standard amount of a biotinylated antibody, the same amount of avidin-biotin complexes should theoretically be formed in each case. However, we found that avidin accelerated the clearance of a biotinylated antibody dose-dependently, whereas streptavidin had no such chase effect. Therefore, the antibody clearance achieved by avidin might not be due to the high molecular weight of avidin-biotin complexes, but instead may be due to affinity of avidin for the liver and spleen.[1, 9] Radiolabeled avidin is reported to be cleared from the blood and trapped by the reticuloendothelial system in the liver and spleen much faster than streptavidin.[9] Thus, avidin and streptavidin may bind to similar amounts of circulating biotinylated antibodies, after which the antibodies bound to avidin are trapped in the liver and spleen, while the antibodies bound to streptavidin remain in the blood.[9]

Avidin-ferritin also achieved a rapid decrease of radioactivity in the liver and spleen, but a ten-fold higher dose of neutravidin or avidin-ferritin was required to obtain the same chase effect as that of avidin. Thus, for immunoscintigraphy using a radiolabeled biotinylated monoclonal antibody followed by an avidin chase, the most effective chasing agent among the four tested in the present study appears to be avidin.

This rapid chase effect of avidin may not only be applicable to the rapid clearance of radiolabeled biotinylated antibodies from the blood, as well as the acceleration of antibody metabolism and radionuclide excretion, but may also be employed in the 2- or 3-step method using biotinylated antibodies, streptavidin or radiolabeled streptavidin, and/or radiolabeled biotin.[7, 8] For example, if an appropriate dose of avidin was injected before injection of streptavidin at the second step, it could decrease the levels of the biotinylated antibody and endogenous biotin, which competitively inhibit streptavidin binding to the biotinylated antibody in the target tumor. Accordingly, streptavidin administered after the avidin chase would bind almost exclusively to biotinylated antibodies in the target tumor.

In conclusion, the clearance of a biotinylated monoclonal antibody from the blood was most effectively accelerated by avidin. Streptavidin did not promote antibody clearance, probably because its affinity for the liver and spleen is very low. Thus, streptavidin may be a more useful radioactive tracer than avidin for pretargeting using biotinylated antibodies.

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