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Avidin Chase Can Reduce Myelotoxicity Associated with Radioimmunotherapy of Experimental Liver Micrometastases in Mice

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Myelotoxicity is the main factor which decides the maximum tolerated dose (MTD) in radioimmunotherapy (RIT). Since bone marrow is mostly irradiated from blood radioactivity, enhancing the clearance of unbound circulating radiolabeled antibody is important to reduce myelotoxicity and to increase the MTD. We applied the avidin chase method, which was devised to obtain high tumor-to-background ratios in tumor-targeting, to RIT of experimental liver micrometastases and evaluated its influence on the side effects and therapeutic outcome. Seven days after intrasplenic injection of human colon cancer LS174T cells, nude mice were intravenously injected with biotinylated ^{131}I-labeled anti-CEA monoclonal antibody (MAb) (24–38 μg, 11.1 MBq). Mice of the chase group then received an intravenous injection of avidin twice (24 and 30 h, 72–115 μg each). Biodistribution, side effects (white blood cell counts and body weight change), and short- and long-term therapeutic effects were determined. Avidin chase markedly accelerated the clearance of radiolabeled MAb from the blood ($P<0.0001$) and normal tissues, resulting in milder leukocytopenia and body weight loss, both of which recovered earlier than in the non-chase group ($P<0.01$). The tumor uptake of radiolabeled MAb was also decreased by avidin chase, but the metastases-to-background ratios were increased. Avidin chase gave the therapeutic gain ratio of 1.89. Treated groups with and without avidin chase showed significant therapeutic effects compared to the non-treated group. There was no significant difference in the therapeutic effects between the two treated groups. Avidin chase effectively reduced the side effects of RIT and should increase the MTD.

Key words: Avidin chase — Radioimmunotherapy — Myelotoxicity — Liver metastasis — Antibody

The radioimmunotherapy (RIT) of solid cancers has met with only limited success[1,2] and the target of RIT has recently been shifted from large primary and recurrent lesions to smaller-sized tumors such as metastases and minimal residual disease after surgery.[3,4] Radioactivity uptake by the smaller lesions is reported to be higher and more homogeneous than that by larger lesions,[5,6] and smaller lesions can be controlled by a lower radiation dose compared to larger tumors.[7]

We and others have recently shown that RIT is effective in controlling experimental liver micrometastases in nude mice,[4,8,9] and we have reported that an absorbed dose of less than several thousand centigrays can effectively control submillimeter tumors.[8] However, a very high dose of radioactivity is still required to control small-sized tumors. Potential damage to the bone marrow and other normal tissues is inevitable and the administration dose is limited to the maximum tolerated dose (MTD). Since bone marrow is mostly irradiated from the radioactivity in the blood,[10] enhancement of the clearance of the unbound radiolabeled monoclonal antibody (MAb) from the blood is important to reduce myelotoxicity. A second antibody against the tumor-specific radiolabeled antibody or the DOTA chelate of the radiolabeled primary MAb has been used as a clearing agent.[11–13] The administration of avidin or streptavidin after an intravenous (i.v.) injection of a biotinylated MAb was previously reported to facilitate the normal tissue clearance of radioactivity and to result in high tumor-to-background ratios in tumor-targeting.[14,15] Modifications of streptavidin,[16] and also extracorporeal absorption of biotinylated radiolabeled MAb through an avidin-gel column have been tried.[17] In the present study, the avidin chase method was applied to RIT to reduce the myelotoxicity. The effects of avidin chase on the biodistribution, side effects, tumor absorbed dose and therapeutic outcome of RIT were evaluated, using a liver micrometastases model of human colon cancer established in nude mice.

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MATERIALS AND METHODS

Experimental liver metastasis Experimental liver micrometastasis of mice were developed by injection of cells of the carcinoembryonic antigen (CEA)-expressing human colon cancer line LS174T into the spleen of female BALB/c *nu*/*nu* mice, followed by splenectomy, as described previously.[8, 18] One week later, all mice developed multiple liver metastases of less than 1 mm in diameter.[8] LS174T cells were obtained from the American Type Culture Collection (Manassas, VA).

MAb, biotinylation, and radiolabeling The murine IgG_1 MAb designated F33-104 recognizes the CEA-specific proteinaceous part of the CEA molecule[19] and was purified from the ascitic fluid of hybridoma-bearing mice, using protein A affinity chromatography (Bio-Rad, Richmond, CA). This MAb was mixed with sulfosuccinimidyl-6-(biotinamide) hexanoate (NHS-LC-biotin) (Pierce Chemical Co., Rockford, IL) in 1 *N* $NaHCO_3$ at 4°C for 2 h at a NHS-LC-biotin-to-MAb molar ratio of 6:1. The mixture was then applied to a Centricon 30 microconcentrator (Amicon, Inc., Beverly, MA) to remove unbound NHS-LC-biotin. The conjugation ratio of biotin to antibody, determined by the method of Green using 2-(4′-hydroxyazobenzene)benzoic acid,[20] was 3.

Biotinylated MAb was labeled with ^{131}I using the chloramine-T method.[21] Sixteen hundred micrograms of biotinylated MAb in 690 μl of 0.3 *M* phosphate buffer, pH 7.5, and 740 MBq of ^{131}I (Du Pont, Wilmington, DE) were mixed with 20 μg of chloramine-T (Nacalai Tesque, Kyoto) dissolved in 0.3 *M* phosphate buffer. After 5 min, the radiolabeled MAb was separated from free iodine through PD-10 gel chromatography (Pharmacia LKB Biotechnology, Uppsala, Sweden). The specific activities of the ^{131}I-labeled biotinylated MAb ranged from 290.08 to 462.87 MBq/mg and the immunoreactive fractions measured by the method of Lindmo *et al*.[22] were more than 60%. More than 86% of the ^{131}I-labeled biotinylated F33-104 bound to Avidin-Sepharose gel (Pierce Chemical Co.) after a 30-min incubation.

Biodistribution study and monitoring of side effects One week after the intrasplenic injection of LS174T cells, mice received an i.v. injection of ^{131}I-labeled biotinylated F33-104 (11.1 MBq/24–38 μg), and the mice of the avidin chase group received an i.v. injection of avidin at 24 and 30 h (72–114 μg each, 3 times the dose of the injected MAb on a weight basis). Groups of mice were killed at 1 (24 h), 1.3 (31 h), 3, 6, and 10 days after MAb injection by ether inhalation. Livers with metastases were excised and quickly frozen in O.C.T. compound (Tissue Tec; Miles Inc., Elkhart, IN) from which 10-μm thick sections were made and processed for quantitative autoradiography (QAR). Blood and various organs were removed and weighed, and their radioactivity counts were determined. The percent of injected dose per gram of tissue (%ID/g) was determined by QAR for the normal liver and metastatic nodules as described previously,[8, 9] and by direct γ-counting for other organs and blood. The results were normalized to a 20-g mouse. Metastases-to-normal tissue uptake ratios of the radioactivity were also calculated.

The number of white blood cells (WBC) in blood taken from the hearts of mice was counted using a standard Bürker-Türk counting chamber (Erma Inc., Tokyo). Body weight changes were monitored in other groups of mice with and without avidin chase (n=5 each) from day 1 to 10.

All animal experiments were carried out in accordance with the Japanese regulations regarding animal care and handling. Statistical analyses of the effects of avidin chase on the biodistribution and myelotoxicity were done by two-factor analysis of variance with Scheffe's test, and on the body weight change by repeated measures analysis of variance with Bonferroni/Dunn test. All tests were two-sided and a probability value (P) of less than 0.05 was considered significant in analysis of variance and Scheffe's test, and 0.0011 in the Bonferroni/Dunn test.

Absorbed dose estimation The radiation-absorbed dose was estimated based on only the β-particle component of ^{131}I, because the β-particles represent 95% of the deposition within 0.99 mm. A dose estimation was done for metastases of three hypothetical diameters (1000, 500 and 300 μm) in chase and non-chase groups, using the conventional medical internal radiation dose (MIRD) schema. The mean absorbed dose in the target D (Gy) was calculated by use of the following formula; $D=A\Delta_\beta\phi/m$, where A is the cumulative radioactivity (Bq·s), Δ_β the energy emitted by disintegration (J/Bq·s^{-1}), ϕ the absorbed fraction and m the target mass. A was calculated as follows; tumor uptake (Bq/g) at various time points determined by QAR was corrected for the decay of ^{131}I and plotted against time. The area under the curve (AUC) (Bq·s/g) was calculated from this clearance curve. A was then obtained by multiplying by the hypothetical tumor weight. The 'S' factor ($=\Delta_\beta\phi/m$) was obtained from the table given by Bardies and Chatal.[23] Based on the results of autoradiography and our previous studies,[8, 9] the distribution of the radiolabel within the micrometastases was regarded as diffuse and the S factor of volume distribution was applied.

The radioactivity in the blood at various time points was also plotted against time, and the AUC was calculated. The therapeutic gain ratio of avidin chase was calculated; the ratio of the AUC of the tumor with chase to that without chase was divided by the ratio of the AUC of the blood radioactivity with chase to that without chase.

Radioimmunotherapy The effects of avidin chase on the short- and long-term therapeutic outcome were examined. Mice with liver micrometastases were divided into three groups; the treated mice with avidin chase, the treated

mice without avidin chase, and the non-treated mice. Seven days after the intrasplenic injection of LS174T cells, the treated mice received an i.v. injection of ^{131}I-labeled biotinylated-MAb (11.1 MBq/24–38 μg). The group of mice with chase received an i.v. injection of avidin twice 24 and 30 h later (72–114 μg each).

For the evaluation of the short-term therapeutic effect, treated mice with and without chase (n=8 each) and non-treated mice (n=5) were killed 23 days after an injection of ^{131}I-labeled MAb, and the formation of the liver metastases was examined. Analyses of the results were performed by use of the Mann-Whitney test with the Bonferroni correction, based on the following grading of the metastasis: small tumor, multiple small metastases less than 2 mm in diameter; large tumor, metastases occupying more than 40% of the liver surface; intermediate tumor, between small tumor and large tumor; and non-visible tumor. A corrected P of less than 0.05 was considered significant.

The long-term survival was followed up to 120 days for the three groups of mice (mice treated with chase: n=20, without chase: n=21, and non-treated mice: n=14). The survival of the mice was analyzed by the Kaplan-Meier method. The logrank test with the Bonferroni correction was used for statistical analysis of the survival curve.

RESULTS

Biodistribution of ^{131}I-labeled biotinylated F33-104 The biodistribution data in the groups of mice with and without avidin chase are summarized in Table I. The clearance of the radioactivity from the blood was very slow in the non-chase group, in accordance with our previous biodistribution study using a therapeutic dose of ^{131}I-labeled MAb[8]; the radioactivity levels in the blood were 16.10 %ID/g on day 1 and still over 10 %ID/g on day 6. In contrast, avidin chase markedly accelerated the clearance of radioactivity from the blood; the blood radioactivity fell to 2.91 %ID/g (P<0.0001) 1 h after the second avidin chase (day 1.3). Most of the normal organs also showed a significant decrease of radioactivity. As an exception, the stomach showed a significantly higher uptake in the chase group (P<0.01) on day 1.3. This reflects the excretion from the stomach of free iodine, which was formed by the metabolism of radiolabeled MAb-avidin complexes after accumulation in the liver.

The tumor uptake of radiolabeled MAb was also decreased by the avidin chase; 15.71 and 24.07 %ID/g on day 3 in the chase and non-chase groups, respectively. However, this decrease was less than that in the normal organs and blood, resulting in increased tumor-to-normal

Table I. Effect of Avidin Chase on the Biodistribution of ^{131}I-labeled Biotinylated F33-104 in Experimental Liver Micrometastasis of Nude Mice[a)]

	Day 1	Day 1.3 (31 h)	Day 3	Day 6	Day 10
With chase					
%ID/g					
Blood	16.10±2.75	2.91±0.84[b)]	2.03±0.41[b)]	1.66±0.71[b)]	1.20±0.29
Kidney	2.96±0.86	1.11±0.39[b)]	0.50±0.11[b)]	0.34±0.14[b)]	0.24±0.04
Stomach	2.08±1.21	5.18±3.16[b)]	0.45±0.15	0.13±0.07	0.09±0.03
Lung	4.20±0.61	1.18±0.33	0.65±0.16[b)]	0.45±0.18[b)]	0.36±0.08
Muscle	0.76±0.08	0.52±0.05	0.17±0.03[b)]	0.10±0.03	0.08±0.01
Bone	0.89±0.44	0.54±0.09	0.29±0.07	0.17±0.09	0.10±0.02
Liver	2.08±1.74	1.35±0.59	0.35±0.27	0.23±0.13	0.24±0.14
Tumor	29.14±6.59	23.82±6.31	15.71±8.09	7.51±3.23	5.56±4.51
Tumor/Blood	1.86±0.55	8.39±2.09	8.48±5.47	6.13±5.19	4.77±3.85
Without chase					
%ID/g					
Blood	16.10±2.75	17.62±0.21	11.89±3.67	10.32±4.01	6.05±2.89
Kidney	2.96±0.86	3.28±0.34	2.07±0.18	1.68±0.41	0.90±0.38
Stomach	2.08±1.21	1.11±0.46	1.04±0.22	0.45±0.09	0.26±0.10
Lung	4.20±0.61	3.40±0.13	2.60±1.54	2.20±0.67	1.41±0.70
Muscle	0.76±0.08	0.73±0.04	0.76±0.39	0.40±0.11	0.21±0.09
Bone	0.89±0.44	1.00±0.30	0.90±0.32	0.81±0.29	0.44±0.24
Liver	2.08±1.74	1.36±0.46	1.08±0.16	1.12±0.46	0.54±0.27
Tumor	29.14±6.59	22.61±2.98	24.07±6.51	14.94±5.03	13.37±10.46
Tumor/Blood	1.86±0.55	1.29±0.17	2.12±0.95	1.86±1.77	2.21±1.01

a) Results represent mean±standard deviation of %ID/g and tumor-to-non-tumor ratios. n=4–7.
b) P<0.05 compared to the group without chase.

tissue ratios in most of the organs and the blood after avidin chase (data of tumor-to-normal tissue ratios other than tumor-to-blood ratios are not shown).

Absorbed dose and therapeutic gain ratio The clearance curves of radioactivity from the blood and metastases are illustrated in Fig. 1. The AUC of the blood and metastases were decreased to 34.4% and to 65.0% by the avidin chase. The therapeutic gain ratio for the avidin chase was calculated to be 1.89.

The estimated absorbed doses of the metastases of three hypothetical diameters, 1000, 500 and 300 μm, after the injection of 11.1 MBq of ^{131}I-labeled MAb were 17.14, 10.75 and 7.29 Gy in the chase group and 26.37, 16.54 and 11.23 Gy in the non-chase group, respectively.

Side effects The results of the peripheral WBC counting are illustrated in Fig. 2. In both chase and non-chase groups, the number of peripheral blood WBC decreased after the i.v. injection of 11.1 MBq of ^{131}I-labeled MAb, reaching the nadir on day 6. Without avidin chase, the peripheral WBC number fell to 910/mm^3 on day 6 and the recovery was retarded, reaching only 1068/mm^3 on day 23. The WBC number of the chase group on day 6 was 1555/mm^3, which is higher than that of the non-chase group, although there was no statistical significance. The leukocytopenia recovered significantly earlier in the chase group (3260/mm^3 on day 10, $P<0.05$).

There was also a significant difference in the body weight change between the two groups (Fig. 3). The body weight decreased by 6.64% in the chase group and by 13.32% in the non-chase group ($P<0.0011$). The recovery of the body weight was also significantly faster in the chase group than in the non-chase group ($P<0.0011$).

Radioimmunotherapy The short-term therapeutic effect of RIT on the liver metastasis examined on day 23 is summarized in Table II. In the chase and non-chase groups, 6 of the 8 and 7 of the 8 mice had no visible metastasis, respectively, and the rest of the mice had very small metastases. All the non-treated mice showed progression of the tumor with hemorrhagic ascites. A significantly better therapeutic effect of the anti-CEA MAb was seen

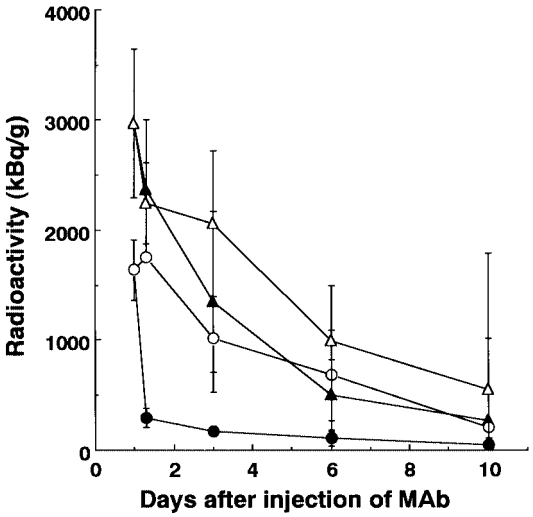

Fig. 1. Clearance of radioactivity (decay-corrected) from the blood (○, ●) and liver metastasis (△, ▲) in radioimmunotherapy with (●, ▲) and without (○, △) avidin chase (n=4–7, bars indicate standard deviation). Two-sided $P<0.0001$ for blood, compared between the chase and non-chase groups.

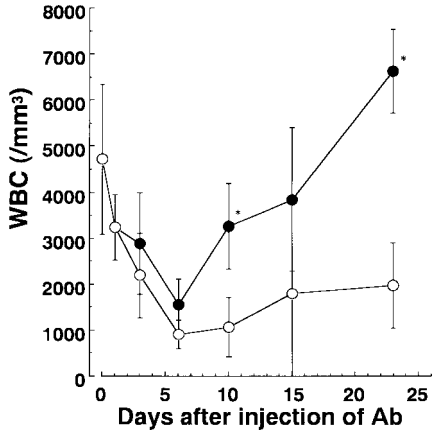

Fig. 2. Change of peripheral blood white blood cell numbers after the injection of ^{131}I-labeled F33-104 with (●) and without (○) avidin chase (n=4–10, bars indicate standard deviation). * Two-sided $P<0.01$.

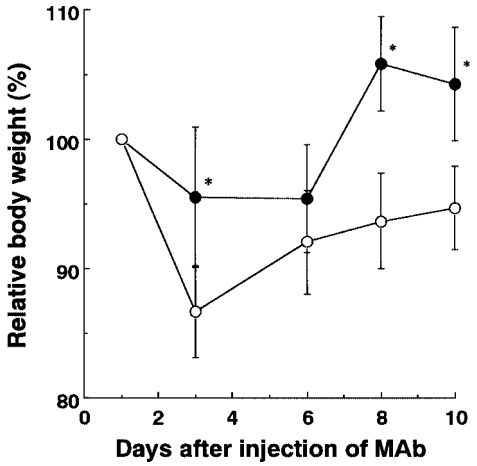

Fig. 3. Relative body weight change of mice after the injection of ^{131}I-labeled F33-104 with (●) and without (○) avidin chase (n=5, bars indicate standard deviation). * Two-sided $P<0.0011$.

Table II. Effect of Avidin Chase on the Short-term Therapeutic Effect of ^{131}I-labeled Biotinylated F33-104 on Liver Micrometastases in Nude Mice

Grade of metastases	Treated mice (n=8)		Non-treated mice (n=5)
	With chase[d]	Without chase[d]	
Non-visible	6	7	0
Small[a]	2	1	0
Intermediate[b]	0	0	2
Large[c]	0	0	3

a) Less than 2 mm in diameter.
b) Between small and large tumor.
c) More than 40% of liver surface with or without bloody ascites.
d) $P<0.01$ compared to the non-treated mice.

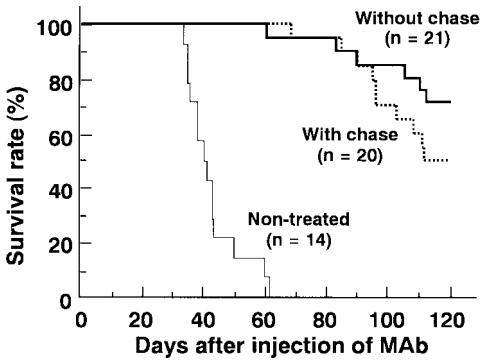

Fig. 4. Long-term survival of the mice. The treated mice with (·····, n=20) and without (——, n=21) avidin chase survived significantly longer compared to the non-treated group (——, n=14). Two-sided $P<0.0001$.

in both treated groups than in the non-treated group ($P<0.01$). There was no significant difference in the therapeutic outcome between the groups with and without chase.

The long-term survival curves of the three groups of mice are shown in Fig. 4. All the non-treated mice died of massive liver metastases with ascites from 33 to 61 days after the intrasplenic injection of the cancer cells (mean: 42 days). In contrast, 10 of the 20 mice treated with avidin chase died (68 to 112 days) and 6 of the 21 without chase died (60 to 112 days) of liver metastases. The rest of the mice, i.e., 10 of the 20 in the chase group and 15 of the 21 in the non-chase group, survived over 120 days. Then they were killed and showed no visible liver metastasis, excluding one in the non-chase group which had large liver metastases. Both treated groups survived significantly longer than the non-treated group ($P<0.0001$). Although the number of mice that survived in the chase group was less than that in the non-chase group, the difference of the survival was not significant.

DISCUSSION

For the success of RIT, high tumor uptake with high tumor-to-normal tissue ratios and a homogeneous accumulation of radiolabels in the tumors are essential. Small-sized metastasis has recently been reported to be a good target of RIT.[3, 4, 8, 9] With regard to tumor-to-normal tissue ratios, however, small-sized tumors have shown lower ratios compared to larger tumors.[9]

Kobayashi *et al*.[15] used an avidin chase method to obtain tumor-targeting with low background radioactivity. In this method, a radiolabeled biotinylated MAb is injected first, and after the optimal time for the MAb to accumulate to the tumor, avidin is administered. Since avidin has very high binding affinity with biotin,[14] it quickly forms complexes with the circulating unbound radiolabeled biotinylated MAb and the complexes are rapidly taken up by the liver and spleen. Radioiodine is quickly detached and released from these organs because of deiodination[24] and is cleared from the body, resulting in the rapid clearance of radioactivity from the blood and normal organs.

In this study, avidin was used as a clearing agent against the circulating unbound ^{131}I-labeled MAb to reduce the unnecessary irradiation of normal tissues. We used 11.1 MBq of ^{131}I-labeled MAb, which would control submillimeter tumors but would induce significant myelosupression, and evaluated how avidin chase would work in radioimmuotherapy, from the viewpoints of side effects, biodistribution and therapeutic outcome. As for the dose of avidin, we used 3 times the dose of injected MAb on a weight basis, which was sufficient to form a complex with biotinylated MAb in the circulation in a previous dose escalation study.[15] Avidin was injected twice with a 6-h interval to eliminate the additional biotinylated MAb that had reentered the circulation from the extravascular space.[25] In order to obtain a sufficiently high accumulation of radioactivity in the tumor, we waited 24 h after the MAb injection to inject avidin as the first chase.

As expected, the avidin chase rapidly cleared the circulating radiolabeled MAb and the AUC of the blood radioactivity decreased to less than 35% of that of RIT without chase. The bone marrow toxicity and body weight loss were milder in the avidin chase group than in the non-chase group. WBC number decreased by 48% in the group without chase, and by 28% in the chase group. Our results are similar to those of the previous report, in which a second antibody was used as a clearing agent. In RIT of tumor-bearing hamsters by Blumenthal *et al*.,[11] an injection of 37 MBq of ^{131}I-labeled antibody, which corresponds to 7.4 MBq for 20-g mice, the WBC number

decreased by 52% in the control group, but by 26% with a second antibody.

The radioactivity uptake by the metastasis was also decreased by the avidin chase, even though the avidin was injected after the tumor uptake of radiolabeled MAb reached the peak value. The catabolism and loss of radiolabeled MAb at the tumor are in equilibrium with the recruitment of unbound radiolabeled MAb from the circulation. A rapid drop in circulating radiolabeled MAb level broke the equilibrium and caused a decrease in tumor radioactivity. Reduction of tumor uptake was also reported in a second antibody method.[12] Tumor radioactivity was decreased to about one-third of that of the control group 24 h after the second antibody injection. In the case of avidin chase, the tumor uptake was decreased to about two-thirds at day 3. The reduction after avidin chase was milder compared to that after the second antibody injection.

The calculated tumor absorbed dose was consequently decreased by the avidin chase to about two-thirds of that without chase, which is equal to that obtained with 7.4 MBq of ^{131}I-labeled MAb without chase. Our previous study revealed that 1.85 to 9.25 MBq of ^{131}I-labeled MAb had a dose-dependent therapeutic effect on the liver micrometastases in nude mice.[8] In the present study, although the survival of the group with chase seems to be somewhat shorter than that of the group without chase, the difference was not significant. Despite the substantial decrease of tumor absorbed dose after the avidin chase, the initial high tumor uptake in the chase group was the same as in the non-chase groups. The tumor absorbed dose from day 0 to day 3 was decreased only to 91.5% by chase. After day 3, the absorbed dose of the chase group was reduced to as little as 47.7% of that of the group without chase. It is possible that the irradiation with the higher dose rate at earlier times is more cytotoxic and effective than the lower dose rate irradiation at later times.[10] We were able to reduce the AUC of the blood radioactivity to one-third with the avidin chase. Bone marrow toxicity and body weight loss, however, could not be avoided, although they were milder. These side effects may also depend on the high radioactivity in the blood at earlier times. Earlier recovery from these side effects may reflect the markedly decreased blood radioactivity after the avidin chase. Hematopoietic regeneration is possible during periods when the marrow dose rate is less than 1 cGy per hour.[26]

To overcome bone marrow toxicity induced by RIT, several other methods have been tried. Cytokines such as granulocyte-macrophage colony stimulating factor, interleukin-1 or both have accelerated the recovery from myelosuppression and could increase the MTD by 25%.[27, 28] Dose escalation has been achieved by employing bone marrow or peripheral blood stem cell transplantation.[26, 29] Another approach, fractionated RIT, has been reported to be effective.[30] Greater therapeutic effect with less bone marrow toxicity was achieved by injection of a larger total dose with fractionation.[30] Use of a clearing agent, such as avidin, is also beneficial in this fractionation RIT. The dose of each fraction can be increased by avidin chase and earlier recovery from the side effects makes the retreatment safer and easier.

In conclusion, the present investigation has shown that the side effects of RIT, bone marrow toxicity and body weight loss, were reduced by the use of avidin chase. The radioactivity uptake of liver metastasis was also reduced by the avidin chase, resulting in a decreased absorbed dose in the tumor, but the therapeutic outcome was not significantly affected. Avidin can be used as a clearing agent of circulating radiolabeled MAb in RIT, affording an increase of the MTD.

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REFERENCES

1) Meredith, R. F., Bueschen, A. J., Khazaeli, M. B., Plott, W. E., Grizzle, W. E., Wheeler, R. H., Schlom, J., Russell, C. D., Liu, T. and LoBuglio, A. F. Treatment of metastatic prostate carcinoma with radiolabeled antibody CC49. *J. Nucl. Med.*, **35**, 1017–1022 (1994).
2) Behr, T. M., Sharkey, R. M., Juweid, M. E., Dunn, R. M., Vagg, R. C., Ying, Z., Zhang, C.-H., Swayne, L. C., Vardi, Y., Siegel, J. A. and Goldenberg, D. M. Phase I/II clinical radioimmunotherapy with an iodine-131-labeled anti-carcinoembryonic antigen murine monoclonal antibody IgG. *J. Nucl. Med.*, **38**, 858–870 (1997).
3) Behr, T. M., Goldenberg, D. M. and Becker, W. S. Radioimmunotherapy of solid tumors: a review "of mice and men." *Hybridoma*, **16**, 101–107 (1997).
4) Vogel, C. A., Galmiche, M. C. and Buchegger, F. Radioimmunotherapy and fractionated radiotherapy of human colon cancer liver metastases in nude mice. *Cancer Res.*, **57**, 447–453 (1997).
5) Behr, T. M., Sharkey, R. M., Juweid, M. E., Dunn, R. M., Ying, Z., Zhang, C.-H., Siegel, J. A. and Goldenberg, D. M. Variables influencing tumor dosimetry in radioimmunotherapy of CEA-expressing cancers with anti-CEA and antimucin monoclonal antibodies. *J. Nucl. Med.*, **38**, 409–418 (1997).
6) Hagan, P. L., Halpern, S. E., Dillman, R. O., Shawler, D. L., Johnson, D. E., Chen, A., Krishnan, L., Frincke, J., Bartholomew, R. M., David, G. S. and Carlo, D. Tumor size: effect on monoclonal antibody uptake in tumor models. *J. Nucl. Med.*, **27**, 422–427 (1986).
7) Fowler, J. F. Radiobiological aspects of low dose rates in

radioimmunotherapy. *Int. J. Radiat. Oncol. Biol. Phys.*, **18**, 1261–1269 (1990).
8) Saga, T., Sakahara, H., Nakamoto, Y., Sato, N., Zhao, S., Iida, Y., Kuroki, M., Endo, K. and Konishi, J. Radioimmunotherapy for liver micrometastases in mice: pharmacokinetics, dose estimation, and long-term effect. *Jpn. J. Cancer Res.*, **90**, 342–348 (1999).
9) Sato, N., Saga, T., Sakahara, H., Nakamoto, Y., Zhao, S., Iida, Y., Kuroki, M., Matsuoka, Y. and Konishi, J. Intratumoral distribution of radiolabeled antibody and radioimmunotherapy in experimental liver metastases model of nude mouse. *J. Nucl. Med.*, **40**, 685–692 (1999).
10) Muthuswamy, M. S., Robersin, P. L. and Buchsbaum, D. J. A mouse bone marrow dosimetry model. *J. Nucl. Med.*, **39**, 1243–1247 (1998).
11) Blumenthal, R. D., Sharkey, R. M., Snyder, D. and Goldenberg, D. M. Reduction by anti-antibody administration of the radiotoxicity associated with ^{131}I-labeled antibody to carcinoembryonic antigen in cancer radioimmunotherapy. *J. Natl. Cancer Inst.*, **81**, 194–199 (1989).
12) Sharkey, R. M., Primus, F. J. and Goldenberg, D. M. Second antibody clearance of radiolabeled antibody in cancer radioimmunodetection. *Proc. Natl. Acad. Sci. USA*, **81**, 2843–2846 (1984).
13) Casey, J. L., King, D. J., Pedley, R. B., Boden, J. A., Boden, R., Chaplin, L. C., Dorning, M. and Begent, R. H. J. Clearance of yttrium-90-labeled anti-tumor antibodies with antibodies raised against the 12N4 DOTA macrocycle. *Br. J. Cancer*, **78**, 1307–1312 (1996).
14) Klibanov, A. L., Martynov, A. V., Slinkin, I. Y., Smirnov, M. D., Muzykantov, V. R., Danilov, S. M. and Torchilin, V. P. Blood clearance of radiolabeled antibody: enhancement by lactosamination and treatment with biotin-avidin or anti-mouse IgG antibodies. *J. Nucl. Med.*, **29**, 1951–1956 (1988).
15) Kobayashi, H., Sakahara, H., Hosono, M., Yao, Z., Toyama, S., Endo, K. and Konishi, J. Improved clearance of radiolabeled biotinylated monoclonal antibody following the infusion of avidin as a "chase" without decreased accumulation in the target tumor. *J. Nucl. Med.*, **35**, 1677–1684 (1994).
16) Marshall, D., Pedley, R. B., Melton, R. G., Boden, J. A., Boden, R. and Begent, R. H. J. Galactosylated streptavidin for improved clearance of biotinylated intact and F(ab′)$_2$ fragments of an anti-tumor antibody. *Br. J. Cancer*, **71**, 18–24 (1995).
17) Chen, J. Q., Atrand, S.-E., Tennvall, J., Lindgren, L., Hidorf, C. and Sjögren, H.-O. Extracorporeal immunoadsorption compared to avidin chase: enhancement of tumor-to-normal tissue ratio for biotinylated rhenium-188-chimeric BR96. *J. Nucl. Med.*, **38**, 1934–1939 (1997).
18) Warren, R. S., Yuan, H., Matli, M. R., Gillett, N. A. and Ferrara, N. Regulation by vascular endothelial growth factor of human colon cancer tumorigenesis in mouse model of experimental liver metastasis. *J. Clin. Invest.*, **95**, 1789–1797 (1995).
19) Kuroki, M., Arakawa, F., Higuchi, H., Matsunaga, A., Okamoto, N., Takakura, K. and Matsuoka, Y. Epitope mapping of the carcinoembryonic antigen by monoclonal antibodies and establishment of a new improved radioimmunoassay system. *Jpn. J. Cancer Res.* (Gann), **78**, 386–396 (1987).
20) Green, N. M. A spectrophotometric assay for avidin and biotin based on binding of dyes by avidin. *Biochem. J.*, **94**, 23c–24c (1965).
21) Hunter, W. N. and Greenwood, F. C. Preparation of iodine-131 labeled human growth hormone of high specific activity. *Nature*, **194**, 495–496 (1962).
22) Lindmo, T., Boven, E., Cuttitta, F., Fedorco, J. and Bunn, P. A., Jr. Determination of the immunoreactive fraction of radiolabeled monoclonal antibodies by linear extrapolation to binding at infinite antigen excess. *J. Immunol. Methods*, **72**, 77–89 (1984).
23) Bardies, M. and Chatal, J.-F. Absorbed dose for internal radiotherapy from 22 beta-emitting radionuclides: beta dosimetry of small spheres. *Phys. Med. Biol.*, **39**, 961–981 (1993).
24) Press, O. W., Farr, A. G., Borroz, K. I., Anderson, S. K. and Martin, P. J. Endocytosis and degradation of monoclonal antibodies targeting human B-cell malignancies. *Cancer Res.*, **49**, 4906–4012 (1989).
25) Kobayashi, H., Sakahara, H., Endo, K., Yao, Z., Toyama, S. and Konishi, J. Repeating the avidin "chase" markedly improved the biodistribution of radiolabelled biotinylated antibodies and promoted the excretion of additional background radioactivity. *Eur. J. Cancer*, **31**, 1689–1696 (1995).
26) Vriesendorp, H. M., Quadri, S. M., Andersson, B. S. and Dicke, K. A. Hematologic side effects of radiolabeled immunoglobulin therapy. *Exp. Hematol.*, **24**, 1183–1190 (1996).
27) Neta, R., Douches, S. and Oppenheim, J. J. Interleukin 1 is a radioprotector. *J. Immunol.*, **136**, 2483–2485 (1986).
28) Blummenthal, R. D., Sharkey, R. M. and Goldenberg, D. M. Dose escalation of radioantibody in a mouse model with the use of recombinant human interleukin-1 and granulocyte-macrophage colony-stimulating factor intervention to reduce myelosuppression. *J. Natl. Cancer Inst.*, **84**, 399–407 (1992).
29) Richman, C. M., DeNardo, S. J., O'Grady, L. F. and DeNardo, G. L. Radioimmunotherapy for breast cancer using escalating fractionated dose of ^{131}I-labeled chimeric L6 antibody with peripheral blood progenitor cell transfusions. *Cancer Res.*, **55** (Suppl.), 5916s–5920s (1995).
30) Schlom, J., Molinolo, A., Simpson, J. F., Siler, K., Roselli, M., Hinkle, G., Houchens, D. P. and Colcher, D. Advantage of dose fractionation in monoclonal antibody-targeted radioimmunotherapy. *J. Natl. Cancer Inst.*, **82**, 763–771 (1990).