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Rhenium-186-mercaptoacetyltriglycine-labeled Monoclonal Antibody for Radioimmunotherapy: *In vitro* Assessment, *in vivo* Kinetics and Dosimetry in Tumor-bearing Nude Mice

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Stability and immunoreactivity of ^{186}Re-labeled monoclonal antibody were examined, and its *in vivo* kinetics was investigated in tumor-bearing Balb/c *nu*/*nu* female mice to assess the feasibility of using it in radioimmunotherapy (RIT). A murine IgG_1, A7, against a 45 kD glycoprotein in human colon cancer was radiolabeled with ^{186}Re by using a chelating method with a mercaptoacetyltriglycine (MAG3). ^{186}Re-MAG3 complex was conjugated to A7 after esterification of ^{186}Re-MAG3 with tetrafluorophenol (TFP). The efficiency of ^{186}Re-MAG3-TFP production and the labeling efficiency of A7 were 51–59% and 57–60%, respectively. Immunoreactivity of purified ^{186}Re-MAG3-A7 was 68.2% at infinite antigen excess. In 0.9% NaCl at 4°C, the radioactivity (12.7 MBq/mg, 3.55 MBq/ml) dissociated with time from ^{186}Re-MAG3-A7 as a small molecular weight moiety because of autoradiolysis. The addition of ascorbic acid, 5 mg/ml, as a radioprotectant or storage at –80°C could effectively prevent the radiolysis of ^{186}Re-MAG3-A7 for 7 days. Immunoreactivity of ^{186}Re-MAG3-A7, 6.70 MBq/mg (6.66 MBq/ml), stored in the presence of ascorbic acid was well retained up to 8 days after the preparation. In colon cancer xenografted mice, 31.0% of the injected dose/g of ^{186}Re-MAG3-A7 had accumulated in the tumors at 24 h postinjection. Estimated radiation dose to tumors was 14.9 cGy/37 kBq up to 8 days postinjection which was 12-fold greater than the whole-body radiation dose. These *in vivo* characteristics were superior to those of A7 labeled with radioiodine, affording greater therapeutic ratios than ^{131}I-A7. Because of the better image quality of ^{186}Re-MAG3-A7 as well as more favorable dosimetry, ^{186}Re-MAG3-A7 would be a better choice for RIT of colon cancer than ^{131}I-A7. These results indicated the feasibility of RIT with ^{186}Re-MAG3-A7, though the prevention of radiolysis of the labeled antibody should be considered.

Key words: Monoclonal antibody — Rhenium-186 — Autoradiolysis — Biodistribution — Dosimetry

Radioimmunotherapy (RIT) using monoclonal antibodies (mAbs) labeled with β emitters has been proven to be a good option for the management of cancer patients.[1,2] ^{131}I is the radionuclide that has been most widely used for this purpose.[1,2] One of the major shortcomings of ^{131}I is that ^{131}I radioactivity is rapidly cleared from target tissues after internalization and intracellular metabolism.[3,4] Another disadvantage of ^{131}I is high-energy γ emission, 364 keV, that is not ideal for γ detection and exposes patients to unnecessary radiation. With the development of chelating methods, radioimmunoconjugates labeled with radiometals such as ^{90}Y have been investigated.[5–7] The chemistry of ^{90}Y is similar to that of ^{111}In, and it is well known that ^{111}In-mAbs are more stable than ^{131}I-mAbs *in vivo*, and the radioactivity remains inside the cells after intracellular metabolism.[3,4] However, bifunctional chelates used for ^{111}In labeling are usually not rigid enough to retain ^{90}Y, and ^{90}Y radioactivity released *in vivo* would accumulate in bone, resulting in an increase of the radiation dose to the bone marrow, a critical organ for RIT.[6,8] Although the stability of ^{90}Y-mAbs could be improved by using macrocyclic chelates such as 1,4,7,10-tetraazacyclododecanetetraacetic acid, DOTA,[7] such chelates would stimulate an immune reaction and anti-chelate antibody could be produced *in vivo* in addition to human anti-mouse antibody.[9] Furthermore, because ^{90}Y does not have γ emission, imaging is difficult with ^{90}Y-mAbs and

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dosimetry should be performed with ^{111}In-mAb, which may behave differently from ^{90}Y-mAbs especially as regards the marrow dose.[6, 9]

^{186}Re appears to be a suitable radionuclide for RIT with an appropriate physical half-life of 3.7 days; this is long enough for a mAb to be localized in tumors and short enough to minimize toxicity to the whole body. Its abundant intermediate-energy β emission (71% of 1.07 MeV and 21% of 0.94 MeV) is comparable to that of ^{131}I, and its γ emission of 137 keV (9%) is suitable for external detection with γ cameras and produces a lower nonspecific radiation dose than ^{131}I. ^{186}Re has similar chemical properties to ^{99m}Tc. Although ^{99m}Tc-mAb is now widely used for radioimmunoscintigraphy (RIS),[10, 11] radiolabeling is performed by a direct labeling method that is not ideal for ^{186}Re because of the instability of directly labeled ^{186}Re-mAb.[12] Thus, indirect methods using ligands such as N_2S_2,[13] N_2S_4[14] and N_3S[15, 16] compounds have been investigated. Among them, a prechelating labeling method using S-benzoyl-mercaptoacetyltriglycine (MAG3), a N_3S ligand, seems to be a good choice because it can afford high *in vivo* stability and high specific activity of the labeled mAb.[15]

In the present study, we tried to label a mAb A7, a murine IgG_1, against 45 kD tumor associated glycoprotein expressed on colon cancer cells with ^{186}Re by using an S-benzoyl-MAG3 prechelating method. Although the reported response rates to RIT were not high for solid tumors such as colon cancer,[17] small tumors would respond to RIT better than large ones[18] and thus RIT for recurrent masses at the early stage or adjuvant RIT in patients at high risk of recurrence (e.g., Dukes' C patients) would be beneficial.[17, 18] Since RIT would follow the confirmation of recurrence by RIS and/or other modalities, the image quality of RIS should be good enough for postoperative assessment, especially in patients whose anatomical images could not differentiate recurrence from fibrous or granulomatous tissues. The scintigraphic counterpart of ^{186}Re-mAb is ^{99m}Tc-mAb, which is superior to ^{111}In-mAb or ^{131}I-mAb not only in obtainable image quality, but also in the availability and cost of the radionuclide.

For mAbs labeled with β emitters, we should pay attention to the possibility of autoradiolysis during the storage before the injection.[19, 20] Therefore, we also observed the stability and immunoreactivity of ^{186}Re-MAG3-A7 for a week, and examined the effect of a radioprotectant or cryopreservation on the radiolysis. Furthermore, the *in vivo* kinetics of ^{186}Re-MAG3-A7 was investigated in tumor-bearing nude mice, and the feasibility of RIT was tested from a dosimetric viewpoint. These results were compared with those for radioiodine-labeled A7 to see if there would be an advantage in the use of ^{186}Re-MAG3-A7 over ^{131}I-A7 in terms of tumor dose and nonspecific normal tissue doses. This study was performed as a part of the Working Group on Radioactive Rhenium supported by the Consultative Committee of Research on Radioisotopes and the Subcommittee for Production and Radiolabeling in the Japan Atomic Energy Research Institute (Tokai-mura).

MATERIALS AND METHODS

mAb and radiolabeling A7, an IgG_1 murine mAb that recognizes the 45 kD glycoprotein in human colon cancer, was used.[21] This mAb reacts with most colorectal cancers.[21] A drug immunoconjugate, A7-neocarzinostatin, has been examined for the treatment of colorectal cancer and encouraging results were reported.[22] Radioimmunoconjugates of this mAb labeled with radioiodine or ^{111}In were shown to localize well in tumors.[23, 24] ^{186}Re-perrhenate ($^{186}ReO^{4-}$) was produced by the ^{185}Re(n, γ) reaction at the Japan Atomic Energy Research Institute at a specific activity of 18.0–19.0 TBq/g (0.45–0.55 TBq/ml) on the day of assay, and supplied to us 2 days later. S-Benzoyl-MAG3 was a gift from Dr. Y. Arano (Faculty of Pharmaceutical Sciences, Kyoto University, Kyoto). The prechelated ^{186}Re-MAG3 was conjugated to the A7.[15] Briefly, $^{186}ReO^{4-}$ was reduced with 100 μl of $SnCl_2$ (1 mg/ml) in the presence of 150 μl of 1 *M* Na_2CO_3, 150 μl of Na_2SO_3 (100 mg/ml) and 25 μl of S-benzoyl-MAG3 (1 mg in 1 ml of acetonitrile/H_2O, 9:1). After evaporation under an N_2 stream, the mixture was further heated for another 15 min. The mixture was reconstituted with 500 μl of water, and incubated with 200 μl of 2, 3, 5, 6-tetrafluorophenol (TFP) (Nacalai Tesque, Kyoto) (100 mg in 1 ml of acetonitrile/H_2O, 9:1) and 50 mg of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (Wako Pure Chemical Industries, Ltd., Osaka) for 30 min after adjustment of the pH to 6 with 1 *N* H_2SO_4. The active ester, ^{186}Re-MAG3-TFP, was purified on a preconditioned C18 cartridge (Waters, Milford, MA) with 2.5 ml of acetonitrile. The solution was evaporated under an N_2 stream, the residue was dissolved in 500 μl of 0.9% NaCl, and the solution was reacted with 4 mg of A7 in a reaction volume of 2.5 ml for 30 min at room temperature after adjustment of the pH to 9.5 with 0.05 *M* Na_2CO_3. The ^{186}Re-MAG3-A7 was purified on a PD-10 column (Pharmacia LKB Biotechnology, Uppsala, Sweden) with 0.9% NaCl as an eluant. The analysis of ^{186}Re-MAG3 and ^{186}Re-MAG3-TFP was performed by thin layer chromatography (TLC) (Merck Art 5553, Darmstadt, Germany) with acetone as a solvent; the Rf values of ^{186}Re-MAG3, ^{186}Re-MAG3-TFP and $^{186}ReO^{4-}$ were 0.55, 0.70 and 1.0, respectively. The number of Re-MAG3 groups per mAb molecule was calculated from the specific activity of ^{186}Re and the observed specific activity of the immunoconjugates. ^{186}Re radioactivity was measured by the use of a dose calibrator set for ^{99m}Tc. In this

system, ^{186}Re activity was obtained by multiplying the result by a factor of 2.8.

***In vitro* stability and effect of radioprotectant or cryopreservation** ^{186}Re-MAG3-A7, 2.48–12.7 MBq/mg (2.78–3.55 MBq/ml, Re-MAG3:mAb 0.57–0.76:1), was stored in 0.9% NaCl at 4°C for 7 days, and its stability was assessed by a size-exclusion high-performance liquid chromatography (HPLC) using a TSK3000SW-XL column (Tosoh, Tokyo) with 0.1 *M* phosphate buffer, pH 7.4, at 1 ml/min. In this system, the retention times of IgG, ^{186}Re-MAG3 and ^{186}Re-perrhenate were 8 min, 13.5 min and 13.5 min, respectively. The effect of the presence of 5 mg/ml ascorbic acid[15, 20] and/or preservation at −80°C[19] was examined in the same manner. Stability in human plasma at 37°C, at a concentration of 13.2 μg/ml (168 kBq/ml, 0.1% NaN_3), was also assessed for 7 days. There was no evidence of bacterial growth during the 7-day period.

Determination of immunoreactivity Immunoreactivity of ^{186}Re-MAG3-A7, 6.70 MBq/mg (6.66 MBq/ml, Re-MAG3:mAb 0.43:1), at infinite antigen excess, was determined after the purification, and was observed during storage with ascorbic acid at 4°C for 8 days, using LS180 human colon carcinoma cells as described by Lindmo *et al*.[25] Briefly, 100 ng of labeled A7 was incubated with increasing concentrations of cells from 3.75×10^5 to 1.2×10^7 in 200 μl for 1 h at room temperature. Non-specific binding was determined by adding 50 μg of cold A7. Triplicate assay was performed for the determination. HPLC assessment was simultaneously performed as described above.

***In vivo* kinetic study and estimated dosimetry** Animal studies were performed in compliance with the regulations of our institution. Balb/c *nu*/*nu* mice (female, 20 g) were subcutaneously xenografted with 1×10^7 LS180 cells in the thigh and used for the experiments 8 days later when tumors weighed 0.2–0.3 g.

^{186}Re-MAG3-A7 (666 kBq/100 μg, 100 μl) was intravenously injected into the tail vein of mice. The animals were killed at 6 h and 1, 2, 5, 8 days after the injection (n=4–5), and the organs were weighed and counted for radioactivity. The biodistribution of ^{186}Re-MAG3-A7 was expressed as a percentage of the injected dose per gram of organ (%ID/g). For the dosimetry, we assumed a homogeneous distribution of radioactivity throughout the tissues, ignoring γ-ray absorption. The cumulative radioactivity, μCi*h/g, in various organs after injection of 1 μCi (37 kBq) was calculated by the trapezoidal method using the biodistribution data, from which the radiation dose was estimated by multiplying by a factor of cGy*g/μCi/h for ^{186}Re of 0.73.[26] In this calculation, the contribution of radiation after 8 days postinjection was omitted. The estimation of whole-body dose was performed as described by Gerretsen *et al*.[27]

To see if there would be an advantage in the use of ^{186}Re-MAG3-A7 over a radioiodine-labeled A7 in terms of *in vivo* kinetics, the biodistribution of ^{125}I-A7 (54.2 MBq/mg, labeled by chloramine-T method) in the same animal model (n=4) was compared with that of ^{186}Re-MAG3-A7. The dosimetry of ^{131}I-A7 was done by using the biodistribution data of ^{125}I-A7 with a factor of cGy*g/μCi/h for ^{131}I of 0.40.[28]

Statistical analysis Statistical analysis was performed by use of the unpaired t test to compare the results. In the analysis, the level of significance was set at 5%.

RESULTS

Labeling efficiency of A7 and *in vitro* assessment of ^{186}Re-MAG3-A7 The incorporation of ^{186}Re into S-benzoyl-MAG3 was 87.2%, as assessed by TLC. The efficiency of ^{186}Re-MAG3-TFP production was 51.4–59.0%, and 56.5–59.5% of ^{186}Re-MAG3-TFP could be conjugated to A7 mAb. The recovery of radioactivity was 18.9–25.0%.

In 0.9% NaCl at 4°C, the radioactivity dissociated progressively from ^{186}Re-MAG3-A7, 12.7 MBq/mg (3.55 MBq/ml, Re-MAG3:mAb 0.76:1), as a small molecular weight moiety appearing at 13.5 min on HPLC analysis. During 7-day storage, 42.1% of ^{186}Re radioactivity was dissociated from the mAb (Fig. 1). The presence of 5 mg/ml ascorbic acid could prevent the radiolysis, and 90.3% of the radioactivity remained in the mAb on day 7. The effect of storage at −80°C with or without ascorbic acid was similar. Radiolysis of ^{186}Re-MAG3-A7 labeled at lower specific activity, 2.48 MBq/mg (2.78 MBq/ml, Re-MAG3:mAb 0.57:1), was negligible even at 4°C without ascorbic acid, as shown in Fig. 2. In human plasma at a concentration of 13.2 μg/0.17 MBq/ml at 37°C, 13.3% of the ^{186}Re radioactivity dissociated as a small moiety during 7 days (Fig. 1).

Immunoreactivity of ^{186}Re-MAG3-A7 was well preserved, being 68.2% at infinite antigen excess immediately after the PD-10 purification. This was comparable to that of ^{125}I-A7, 57.8%, and that of ^{111}In-A7, 62.9%, labeled by the cyclic diethylenetriaminepentaacetic acid, DTPA, method using the same batch of A7 at a 2:1 molar ratio of DTPA:mAb, conditions which would not affect the immunoreactivity of the mAb. The protective effect of ascorbic acid was monitored for 8 days at the specific activity of 6.70 MBq/mg (6.66 MBq/ml, Re-MAG3:mAb 0.43:1). As shown in Fig. 3, the rate of radiolysis of this preparation was comparable to that of the preparation of 12.7 MBq/mg (3.55 MBq/ml, Re-MAG3:mAb 0.86:1) (Fig. 1). The reduction of immunoreactivity almost paralleled the dissociation of ^{186}Re radioactivity from the mAb.

Biodistribution of ^{186}Re-MAG3-A7 and calculation of radiation dose The results of the biodistribution study in

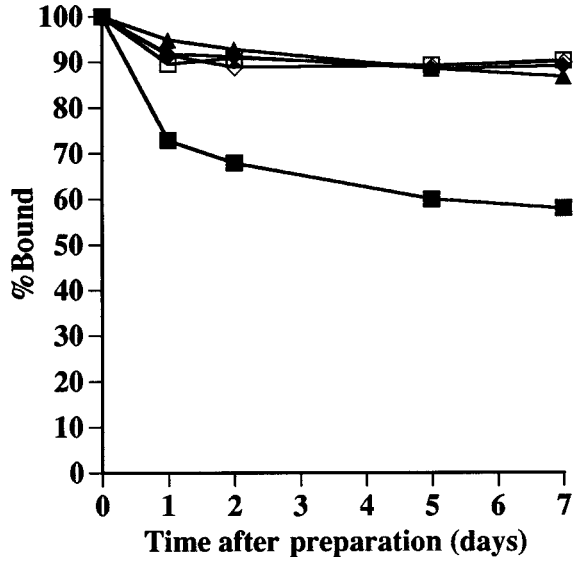

Fig. 1. Stability of ^{186}Re-MAG3-A7 with the specific activity of 12.7 MBq/mg (3.55 MBq/ml, Re-MAG3:mAb 0.76:1). ■, stored at 4°C without ascorbic acid; □, at 4°C with 5 mg/ml ascorbic acid; ◆, at −80°C; ◇, at −80°C with 5 mg/ml ascorbic acid; ▲, in plasma at 37°C.

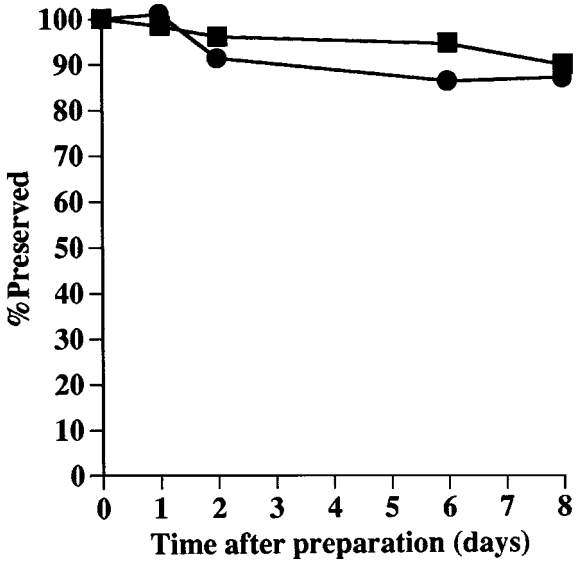

Fig. 3. Stability and immunoreactivity of ^{186}Re-MAG3-A7 with the specific activity of 6.70 MBq/mg (6.66 MBq/ml, Re-MAG3:mAb 0.43:1) stored at 4°C with 5 mg/ml ascorbic acid. ■, radioactivity bound to protein; ●, immunoreactivity at infinite antigen excess relative to that observed immediately after the purification.

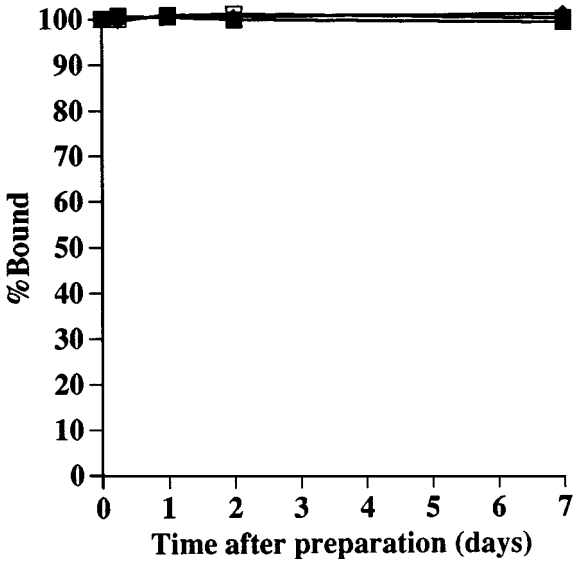

Fig. 2. Stability of ^{186}Re-MAG3-A7 with the specific activity of 2.48 MBq/mg (2.78 MBq/ml, Re-MAG3:mAb 0.57:1). ■, stored at 4°C without ascorbic acid; □, at 4°C with 5 mg/ml ascorbic acid; ◆, at −80°C without ascorbic acid.

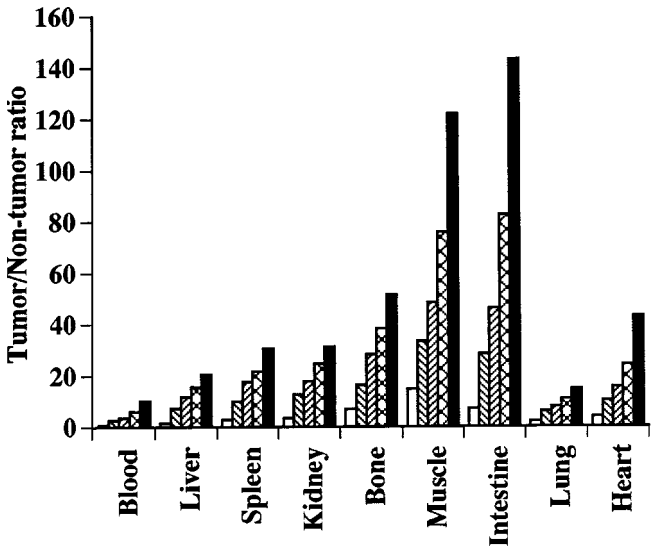

Fig. 4. Tumor-to-nontumor uptake ratios of ^{186}Re-MAG3-A7 in colon cancer xenografted mice. □, 6 h; ▨, 1 day; ▨, 2 days; ▨, 5 days; ■, 8 days.

tumor-bearing mice are summarized in Table I and Fig. 4. Tumor uptake of ^{186}Re-MAG3-A7 was considerable, amounting to 15.65%ID/g at 6 h after the injection, and peaked on day 1 at 31.00%ID/g. The distribution in normal tissues was most prominent at 6 h and rapidly cleared with time. Although radioactivity was gradually washed

Table I. Biodistribution of ^{186}Re-MAG3-A7 in Tumor-bearing Nude Mice

Tissue	6 h[a)]	1 day	2 days	5 days	8 days
Blood	17.38 (1.71)[b, c)]	11.70 (1.36)[c)]	7.87 (1.04)[c)]	2.22 (1.31)	0.59 (0.37)
Liver	9.43 (1.41)[c)]	4.22 (0.60)	2.30 (0.12)	0.80 (0.25)	0.24 (0.07)
Spleen	5.34 (0.52)	3.17 (0.56)	1.57 (0.34)[c)]	0.60 (0.26)	0.17 (0.06)
Kidney	4.71 (0.41)	2.48 (0.44)[c)]	1.59 (0.25)	0.53 (0.26)	0.16 (0.06)
Bone	2.26 (0.26)	1.92 (0.32)	1.01 (0.13)	0.34 (0.14)	0.10 (0.05)
Muscle	1.16 (0.40)	0.93 (0.06)	0.62 (0.13)	0.17 (0.09)	0.05 (0.02)
Intestine	2.24 (0.50)	1.13 (0.28)	0.63 (0.10)	0.16 (0.07)	0.04 (0.02)
Lung	7.05 (0.89)[c)]	5.13 (0.84)[c)]	3.55 (0.30)[c)]	1.21 (0.58)	0.57 (0.56)
Brain	0.40 (0.06)	0.25 (0.03)[c)]	0.18 (0.02)[c)]	0.05 (0.03)	0.01 (0.01)
Heart	4.12 (0.60)[c)]	3.13 (0.56)	1.89 (0.32)[c)]	0.53 (0.25)	0.14 (0.08)
Tumor	15.65 (2.25)	31.00 (2.09)[c)]	27.21 (3.22)[c)]	12.73 (5.13)	5.05 (1.48)

a) Time after IV injection.
b) Expressed as % injected dose/gram tissue. Mean (SD) of 4–5 mice.
c) Significant by unpaired *t* test vs. ^{125}I-A7 shown in Table III ($P<0.05$). Comparison was performed only for the time-matched data, at 6 h, 1 day and 2 days.

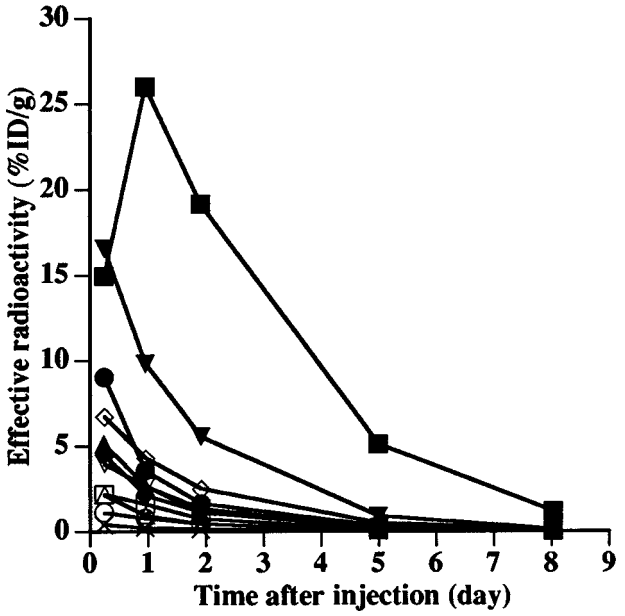

Fig. 5. Effective radioactivity of ^{186}Re-MAG3-A7 in tissues expressed after taking into account physical decay. ▼, blood; ●, liver; ▲, spleen; ◆, kidney; □, bone; ○, muscle; △, intestine; ◇, lung; ×, brain; ▽, heart; ■, tumor.

Table II. Dosimetry of ^{186}Re-MAG3-A7 in Tumor-bearing Mice

Tissue	μCi*h/g[a)]	cGy/μCi[b)]	Ratio[c)]
Blood	9.13	6.67	2.23
Liver	2.78	2.03	7.32
Spleen	1.84	1.35	11.04
Kidney	1.65	1.20	12.37
Bone	1.02	0.75	19.88
Muscle	0.55	0.40	37.04
Intestine	0.70	0.51	29.07
Lung	3.25	2.37	6.27
Brain	0.16	0.12	125.08
Heart	1.79	1.31	11.37
Whole body	1.65	1.21	12.31
Tumor	20.36	14.86	—

a) Calculated for 1 μCi injection by the trapezoid integration method using the biodistribution data up to 8 days.
b) Obtained by multiplying by a factor, g*cGy/(μCi*h), of 0.73.
c) Ratios of tumor dose to normal tissue dose.

out from the tumor to 5.05%ID/g on day 8, the clearance rate was slower than those of normal tissues, so the tumor-to-nontumor uptake ratios increased with time (Fig. 4).

Dosimetry calculation was performed by the trapezoid integration method using the biodistribution data (Table II). The calculation was performed up to 8 days, and the later radiation contribution was neglected. After day 8, the doses to normal tissues were negligible and the dose to the tumor would have little effect (Fig. 5). The ratios of tumor dose to blood and liver doses were 2.23 and 7.32, respectively. For other tissues except for lung, the ratios were greater than 10. Tumor dose was greater than the whole-body dose by a factor of 12.31.

These results were compared with the biodistribution of ^{125}I-A7 and estimated dosimetry for ^{131}I-A7 (Tables III and IV). Blood clearance of ^{186}Re-MAG3-A7 was significantly faster than that of ^{125}I-A7 and the distribution to other normal tissues tended to be less with ^{186}Re-MAG3-A7, except for the initial hepatic uptake. Tumor uptake of ^{186}Re-MAG3-A7 was greater until 2 days after the injec-

Table III. Biodistribution of ^{125}I-A7 in Tumor-bearing Nude Mice

Tissue	6 h[a)]	1 day	2 days	4 days	7 days
Blood	24.53 (2.29)[b)]	15.61 (1.97)	11.28 (0.76)	5.20 (0.63)	2.21 (1.68)
Liver	5.44 (0.98)	3.55 (0.51)	2.23 (0.25)	1.23 (0.09)	0.54 (0.46)
Spleen	5.58 (1.06)	3.81 (0.45)	2.39 (0.37)	1.27 (0.26)	0.61 (0.43)
Kidney	4.86 (0.29)	3.51 (0.41)	2.20 (0.73)	1.21 (0.16)	0.41 (0.27)
Bone	1.94 (0.23)	1.62 (0.09)	1.02 (0.13)	0.55 (0.10)	0.24 (0.16)
Muscle	0.89 (0.29)	1.07 (0.12)	0.89 (0.09)	0.45 (0.03)	0.16 (0.12)
Intestine	1.79 (0.10)	1.21 (0.14)	0.76 (0.07)	0.38 (0.01)	0.13 (0.08)
Lung	10.31 (1.46)	7.32 (0.56)	4.82 (0.38)	2.46 (0.20)	1.11 (0.90)
Brain	0.48 (0.03)	0.32 (0.02)	0.24 (0.01)	0.12 (0.02)	0.05 (0.04)
Heart	5.97 (0.56)	3.88 (0.39)	2.98 (0.46)	1.34 (0.14)	0.63 (0.52)
Tumor	11.96 (3.66)	17.70 (4.25)	19.64 (2.23)	14.22 (2.48)	10.61 (1.96)

a) Time after IV injection.
b) Expressed as % injected dose/gram tissue. Mean (SD) of 4 mice.

Table IV. Dosimetry of ^{131}I-A7

Tissue	μCi*h/g[a)]	cGy/μCi[b)]	Ratio[c)]
Blood	13.63	5.52	1.56
Liver	2.60	1.05	8.17
Spleen	2.79	1.13	7.61
Kidney	2.51	1.01	8.48
Bone	1.15	0.46	18.53
Muscle	0.79	0.32	26.81
Intestine	0.85	0.34	24.94
Lung	5.32	2.15	4.00
Brain	0.25	0.10	86.75
Heart	2.99	1.21	6.39
Whole body	2.90	1.17	7.34
Tumor	21.25	8.60	—

a) Calculated for 1 μCi injection by the trapezoid integration method using the biodistribution data of ^{125}I-A7 up to 10 days.
b) Obtained by multiplying by a factor, g*cGy/(μCi*h), of 0.40.
c) Ratios of tumor dose to normal tissue dose.

tion and showed faster washout thereafter as compared with ^{125}I-A7. The tumor dose, cGy/μCi, was 1.73-fold greater with ^{186}Re-MAG3-A7, and therapeutic ratios of ^{186}Re-MAG3-A7 were better than ^{125}I-A7 for all tissues except liver. In particular, the whole-body therapeutic ratio of ^{186}Re-MAG3-A7 was improved by a factor of 1.67 as compared with ^{131}I-A7.

DISCUSSION

A major drawback to the usage of ^{186}Re as a radiolabel of mAb is its low specific activity, because of carrier contamination when ^{186}Re is produced via the ^{185}Re(n, γ) reaction.[29)] To obtain suitable radioimmunoconjugates of high specific activity for RIT, several ^{186}Re-MAG3 groups should be coupled to a single mAb molecule.[15, 29, 30)] We could label A7 mAb with ^{186}Re-MAG3 without destroying its immunoreactivity. However, the specific activity of ^{186}Re-MAG3-A7 was only 12.7 MBq/mg (344 μCi/mg) at best, with a coupling ratio of Re-MAG3:mAb of 0.76:1. In the published results by other investigators, ^{186}Re-mAb of higher specific activity could be obtained by the conjugation of up to 20 Re-MAG3 groups per mAb, though the immunoreactivity deteriorated with Re-MAG3:mAb higher than 12.[30)] In the present study, ^{186}Re incorporation into MAG3 was performed at Re:MAG3 and Re:Sn^{2+} molar ratios of approximately 1 and 6, respectively. Although the incorporation rate, 87.2%, was close to the results of Visser *et al*., >90%,[15)] it appeared that, to obtain a mAb of high specific activity, the Re:MAG3 and Re:Sn^{2+} molar ratios should have been increased, as their reaction conditions were Re:MAG3 1:2.3 and Re:Sn^{2+} 1:8, and that the reaction volume for the conjugation of mAb should have been reduced or the ^{186}Re-MAG3:mAb ratio should have been increased. Under such labeling conditions, ^{186}Re-MAG3-A7 of high specific activity should be obtained.

Although high specific activity would be essential for clinical RIT, difficulties would arise if the labeling of mAb were performed at an extreme molar ratio of Re-MAG3:mAb.[30, 31)] It has been reported that coupling of too many Re-MAG3 groups results in an alteration of *in vivo* distribution and a reduction of immunoreactivity because of the change in the net charge of the mAb and steric hindrance at the mAb binding site. A further drawback to mAbs of high specific activity would be an increase in the susceptibility to autoradiolysis.[19, 20)] We observed time-dependent dissociation of ^{186}Re radioactivity during storage even at 12.7 MBq (344 μCi)/mg, 3.55 MBq/ml. Although labeling at lower specific activity would reduce the risk of radiolysis, as shown in Fig. 2, such low spe-

cific activity would not be ideal for clinical RIT because of the need to inject a large amount of mAb to provide sufficient radioactivity. In clinical RIT, 120 mCi/m^2 of ^{186}Re-mAb could be injected as a maximum tolerated dose in heavily pretreated patients.[32] In this case, 180–259 mCi of ^{186}Re-NR-LU-10 was injected with 30 ml of 0.9% NaCl at the concentration of 222–318 MBq (6.0–8.6 mCi)/ml, 45–260 mCi/33–47 mg.[32] At this dose level, measures to prevent autoradiolysis of labeled mAbs are essential.[15]

Addition of 5 mg/ml ascorbic acid as a radioprotectant to the mAb solution could effectively prevent radiolysis of the mAb. Storage at −80°C, cryopreservation, was equally effective. Since the reduction of immunoreactivity is not completely accounted for by the breakdown products,[20] immunoreactivity should be monitored as well in the assessment of radiolysis. As shown in Fig. 3, 5 mg/ml ascorbic acid could protect A7 from loss of immunoreactivity, as well as breakdown. Because the susceptibility to radiation may vary among mAbs, assessment of individual mAbs may be necessary. Although long-term storage of ^{186}Re-mAb would not be needed in the clinical setting, radiolysis can occur in a very short period. It was reported that 6% of radioactivity was lost from ^{186}Re-MAG3 labeled anti-squamous cell carcinoma mAb within 20 min.[15] Therefore, we have to recognize that, without protection of the mAb from possible radiolysis and loss of immunoreactivity, the results of RIT could be affected even by brief storage.

In human plasma, 86.7% of ^{186}Re radioactivity remained on A7 mAb after a 7-day incubation. Similar stability in human plasma was observed with ^{186}Re-MAG3-ZCE025, an anti-CEA mAb: 92.8% and 87.5% of ^{186}Re remained bound on day 7 and day 12, respectively (data not shown). In contrast, only 58–64% of ^{186}Re was found on a directly labeled ^{186}Re-Mu-9 in human serum on day 7, or 85% of ^{188}Re-Mu-9 on day 2.[12] These findings indicated that mAb labeled with a MAG3 ligand would be more stable than directly labeled mAbs in terms of dissociation of radioactivity, although comparison is difficult since Griffiths *et al*. did not give the concentration of mAb in their test solution. Furthermore, although they mentioned the *in vivo* stability, showing that all of the radioactivity was bound to Mu-9 at 24 h postinjection in serum from mice injected with ^{188}Re-Mu-9,[12] this finding does not confirm the stability of their Re-mAbs because the mechanism of instability of directly labeled Re-mAbs would be reoxidation of conjugated Re to produce perrhenate that is rapidly cleared from the circulation. The precise form of the breakdown products found in the present study was not clear. They were eluted at 13.5 min on the size exclusion HPLC column used in this study, but both ^{186}Re-MAG3 and ^{186}Re-perrhenate would emerge at the same retention time. Further analyses by TLC or reverse-phase HPLC would cast light on this issue.

Tumor uptake of ^{186}Re-MAG3-A7 was very high, peaking on day 1 with slower washout than from normal tissues, so that the tumor-to-nontumor ratios increased with time. Tumor accumulation was greater than that of ^{125}I-A7. ^{186}Re radioactivity was cleared from tumors faster than ^{125}I after the peak uptake. However, this would not be a serious problem for RIT because of the shorter physical half life, 3.7 days, than that of ^{131}I, 8.0 days. Beaumier *et al*. reported that $LD_{50/30}$ was 600 μCi, corresponding to 880 cGy of whole-body dose, in an experimental RIT study with ^{186}Re-NR-LU-10 in a mouse model bearing small cell lung cancer.[33] In our model, a value of 726 cGy of whole-body dose was estimated, suggesting that the toxicity of ^{186}Re-MAG3-A7 is comparable to that of ^{186}Re-NR-LU-10. The tumor-to-whole body radiation dose in the present study was 12.34, which is 5-fold larger than their result. In their study, 19% remission was obtained with a total of 500–600 μCi injection (2012–2671 cGy to the tumor). Gerretsen *et al*. reported another experimental RIT in a head and neck squamous cell cancer model using ^{186}Re-MAG3-E48, obtaining 33% remission by 400–600 μCi injection, with 3432 cGy to the tumor at 600 μCi.[27] In our model, we estimated a tumor dose of 8916 cGy with 600 μCi of ^{186}Re-MAG3-A7. Although radiation sensitivity might vary among these cell types and we could not conduct an experimental RIT with ^{186}Re-MAG3-A7 because of the limited amount of ^{186}Re permitted to be used under the regulations of our institution, the dosimetry results in the present study indicate the feasibility of RIT with ^{186}Re-MAG3-A7. Our previous experimental RIT study with 9.25 MBq (250 μCi) of ^{131}I-A7 in the same animal model as used in this study showed a significant suppression of tumor growth.[34] This dosage could produce a tumor burden of 2150 cGy, which is far less than the expected dose with ^{186}Re-MAG3-A7 mentioned above. Because of the higher therapeutic ratios of ^{186}Re-MAG3-A7, ^{186}Re-MAG3-A7 would be more favorable than ^{131}I-A7 in terms of toxicity as well. Therefore, it is clear that a greater tumor radiation dose could be delivered by ^{186}Re-MAG3-A7 with the same toxicity to normal tissues as in the case of ^{131}I-A7, and better results of RIT should be achieved with ^{186}Re-MAG3-A7.

In conclusion, it is crucial to protect ^{186}Re-mAb against autoradiolysis. The *in vivo* characteristics of ^{186}Re-MAG3-A7 were better than those of conventional ^{131}I-A7 in terms of both tumor dosimetry and normal tissue irradiation doses, indicating that ^{186}Re-MAG3-A7 should be superior to ^{131}I-A7 as a candidate for RIT of colorectal cancer. Although some improvement in the labeling process would be needed to get ^{186}Re-MAG3-A7 of higher specific activity, RIT with ^{186}Re-MAG3-A7 seems to be a promising modality.

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