Annals of Nuclear Medicine Vol. 15, No. 3, 199–202, 2001

Experimental radioimmunotherapy with ^{186}Re-MAG3-A7 anti-colorectal cancer monoclonal antibody: Comparison with ^{131}I-counterpart

Seigo KINUYA,* Kunihiko YOKOYAMA,* Katsutoshi KOBAYASHI,** Shoji MOTOISHI,** Katsuyuki ONOMA,** Naoto WATANABE,*** Noriyuki SHUKE,**** Hisashi BUNKO,***** Takatoshi MICHIGISHI* and Norihisa TONAMI*

*Department of Nuclear Medicine, Kanazawa University School of Medicine
**Production Division, Department of Research Reactor, Division of Radioisotopes, Japan Atomic Energy Research Institute
***Department of Radiology, Toyama Medical and Pharmaceutical University
****Department of Radiology, Asahikawa Medical College
*****Medical Informatics, Kanazawa University Hospital

A murine IgG_1 against a *Mr* 45 kD tumor-associated glycoprotein in human colorectal cancer, A7, was radiolabeled with ^{186}Re by a chelating method with a mercaptoacetyltriglycine (MAG3). Its specific activity was 119 MBq/mg, which would be high enough for a therapeutic purpose, and its immunoreactivity was preserved well as was ^{131}I-A7 labeled by the chloramine-T method. Growth of human colon cancer xenografts, 9.14 ± 0.44 mm in diameter, in nude mice was significantly suppressed by an intravenous dose of 4.48 MBq of ^{186}Re-A7. The therapeutic outcome with ^{186}Re-A7 was better than that with 4.63 MBq of ^{131}I-A7. Toxicity of treatments assessed by body weight change was similar with both conjugates. These results are likely caused by the tumor size and more favorable physical properties of ^{186}Re than those of ^{131}I.

Key words: radioimmunotherapy, ^{186}Re, colon cancer xenograft

INTRODUCTION

^{131}I is the radionuclide that has been most widely used to label monoclonal antibody (MAb) for radioimmunotherapy (RIT).[1,2] One of major disadvantages of ^{131}I is high energy γ emission, 364 keV, that is not ideal for gamma detection and exposes patients to unnecessary radiation. ^{186}Re appears to be a suitable radionuclide for RIT with its appropriate physical half-life of 3.7 days that is long enough for MAb to localize tumors and short enough to minimize toxicity in the whole body. Abundant intermediate energy β emission (71% of 1.07 MeV and 21% of 0.94 MeV) is comparable to ^{131}I, and γ emission of 137 keV (9%) that is suitable for external detection with gamma cameras, which may provide more accurate tissue absorbed radiation dose estimation than with ^{131}I, and produces a less 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),[3,4] 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,[5] so that indirect methods with ligands such as N_2S_2, N_2S_4 and N_3S compounds have been investigated.[6–9] Among these, a prechelating labeling method with *S*-benzoylmercaptoacetyltriglycine (MAG3), an N_3S ligand, appears to be a good choice because of its *in vivo* stability and possible high specific activity of labeled MAb.[8]

In this study of a mouse model xenografted with human colon cancer cells, we sought to determine the efficacy of RIT of ^{186}Re-MAG3-MAb. This study was performed as a part of the Working Group on Radioactive Rhenium supported by the Consultative Committee of Research on

Received November 20, 2000, revision accepted February 1, 2001.

For reprint contact: Seigo Kinuya, M.D., Department of Nuclear Medicine, Kanazawa University School of Medicine, 13–1 Takaramachi, Kanazawa, Ishikawa 920–8640, JAPAN.
E-mail: kinuya@med.kanazawa-u.ac.jp

Radioisotopes and the Subcommittee for Production and Radiolabeling in the Japan Atomic Energy Research Institute.

MATERIALS AND METHODS

A7, an IgG_1 murine MAb recognizing *Mr* 45,000 tumor-associated glycoprotein of colorectal cancer, was used.[10] ^{186}Re-perrhenate ($^{186}ReO^{4-}$) was produced by ^{185}Re(n,γ) reaction (Japan Atomic Energy Research Institute, Tokaimura, Japan) at a specific activity of 19.0 TBq/g, chelated with *S*-benzoyl-mercaptoacetyltriglycine (MAG3) (a gift from Dr. Yasushi Arano) and conjugated to A7.[8] Briefly, the mixture of $^{186}ReO^{4-}$, $SnCl_2$ and *S*-benzoyl-MAG3 at the molar ratios of 2.3 : 1 for *S*-benzoyl-MAG3 : Re and 8.0 : 1 for Sn^{2+} : Re was heated under an N_2 stream, resulting in ^{186}Re-MAG3, which was conjugated to A7 after esterification with 2,3,5,6-tetrafluorophenol (TFP) (Nacalai Tesque, Kyoto). ^{186}Re-MAG3-A7 was then purified on a PD10 column (Pharmacia LKB Biotechnology, Uppsala, Sweden) with 5 mg/m*l* ascorbic acid as an eluant to prevent the radiolysis of the MAb. Immunoreactivity of ^{186}Re-MAG3-A7 was determined with 1.6×10^5 to 3×10^6 of LS180 human colon carcinoma cells (American Type Culture Collection, Rockville, MD, USA) as described by Lindmo et al.[11] The labeled MAb was sterilized by means of a filter (Millex-GV, 0.22 mm; Millipore, Bedford, MA, USA) prior to further experiments.

Animal studies were performed in compliance with the regulations of our institution. LS180 cells were grown in DMEM medium (Nissui Seiyaku, Tokyo), harvested with 0.1% trypsin, and then 5×10^6 of cells were subcutaneously xenografted into the thigh of Balb/c nu/nu mice (female, 20 g; NINOX Labo Supply Inc., Ishikawa). Tumor volume (mm^3) was calculated as length (mm) × width $(mm)^2 \times 0.5$, and expressed as the ratio of volume to the volume on day 0 (the day of starting the treatment). Tumor volume on day 0 was 376 ± 46 mm^3 and the diameter was 9.14 ± 0.44 mm. The tumoricidal activity of a dose of 4.48 MBq (121 μCi) of ^{186}Re-A7 was determined (n = 9). As a comparison, the therapeutic effect of 4.63 MBq (125 μCi) of ^{131}I-A7 labeled by the chloramine-T method was observed in the same model (n = 8). Tumor growth in non-treated mice was also observed as a reference (n = 5). Toxicity of the treatment was assessed by body weight loss of the animals.

Absorbed radiation dose in tissue with ^{186}Re-A7 was estimated under the assumption that ^{186}Re-A7 would show similar biodistribution to ^{131}I-A7: with the labeling condition yielding an appropriate conjugation ratio of ^{186}Re-MAG3 to MAb, ^{186}Re-MAbs was cleared from the circulation and accumulated into tumors similarly to ^{125}I-MAbs, and distribution of ^{186}Re-MAbs in normal tissue did not vary from that of ^{125}I-MAbs with some exceptions in gastric accumulation and their excretion routes.[12,13] These were demonstrated in both normal mice and tumor-bearing mice. In the estimation, we used the previous biodistribution data obtained with ^{125}I-A7,[14] and the physical half lives of ^{186}Re and ^{131}I were adapted to the data to obtain effective cumulative radioactivity within tissue for ^{186}Re and ^{131}I. Absorbed radiation dose was estimated by the formula: $D_\beta = \mu Ci \times h \times g^{-1} \times E$, where E of ^{131}I =

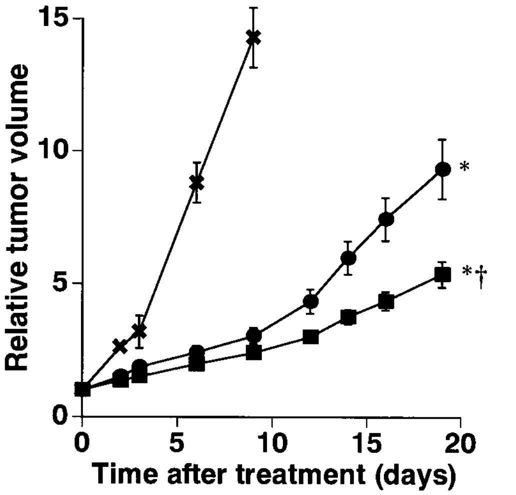

Fig. 1 Growth of LS180 human colon cancer xenografts in mice, expressed as a ratio of volumes to the volume obtained on day 0 (mean ± SEM). (✖), control; (●), ^{131}I-A7 4.63 MBq; (■), ^{186}Re-A7 4.48 MBq. *, $p < 0.0001$ vs. control; †, $p < 0.05$ vs. ^{131}I-A7.

Table 1 Experimental groups and therapeutic results

	Relative tumor volume On day 19	Maximum body weight loss (%)
Control	(8.79 ± 1.13 on day 6)	—
^{131}I-A7 4.63 MBq	9.33 ± 1.27	14.8 ± 2.01
^{186}Re-A7 4.48 MBq	5.35 ± 0.77*	15.4 ± 2.11

*$p < 0.02$ versus other RIT groups. n = 5–9.

Table 2 Tissue absorbed radiation doses (Gy)

	^{131}I-A7 4.63 MBq	^{186}Re-A7 4.48 MBq
Tumor	11.00	16.34
Blood	6.79	10.24
Liver	1.30	1.89
Spleen	1.39	2.02
Kidney	1.25	1.83
Bone	0.57	0.82
Muscle	0.40	0.56
Intestine	0.42	0.63
Whole body	1.52	2.06

Estimated using the data published in reference 14, assuming the same biodistribution with ^{131}I-A7 and ^{186}Re-A7.

0.3985 and E of ^{186}Re = 0.73.[13] The contribution of γ emission was neglected in the calculation.

RESULTS

The efficiency of ^{186}Re-MAG3-TFP production was 74%, and 60% of ^{186}Re-MAG3-TFP was conjugated to A7. The specific activity of ^{186}Re-A7 was 119 MBq/mg, and its immunoreactivity at infinite antigen excess was 72%. Those of ^{131}I-A7 were 140 MBq/mg and 71%.

RIT with ^{186}Re-A7 significantly suppressed the growth of xenografts as compared to no treatment (Fig. 1 and Table 1). A dose of 4.48 MBq of ^{186}Re-A7 showed better tumor suppression than did a dose of 4.63 MBq of ^{131}I-A7. Maximum body weight loss was similar with both conjugates at this dose level (Table 1), but the loss with ^{131}I-A7 tended to appear later and persist longer than that with ^{186}Re-A7: a nadir on day 6 with ^{186}Re-A7 and on day 12 with ^{131}I-A7. No mouse died from the treatment during the observation period.

Estimated tissue absorbed radiation doses are shown in Table 2. The absorbed radiation dose caused by β emissions to the tumor with a dose of 4.48 MBq of ^{186}Re-A7 was 1.67-fold greater than that with 4.63 MBq of ^{131}I-A7. Doses absorbed by normal tissue from β emissions were approximately 1.5-fold greater with ^{186}Re-A7.

DISCUSSION

A7 MAb was able to be labeled with ^{186}Re-MAG3 at sufficiently high specific activity for a therapeutic purpose, and its immunoreactivity was well preserved. We found significant tumoricidal effect of ^{186}Re-A7 *in vivo*, and ^{186}Re-A7 produced better tumor response than did ^{131}I-A7 at the similar dose level. Estimation of the tissue absorbed radiation dose indicates that ^{186}Re-A7 produced a much greater tumor dose than ^{131}I-A7, which would be the major reason for the better outcome with ^{186}Re-A7.

The size of tumors may be another factor in the more pronounced tumor suppression with ^{186}Re-A7 than ^{131}I-A7. The efficacy of RIT is affected by the properties of the radionuclide labeled to MAbs, and a mathematical model assuming uniform radionuclide distribution in tumors indicates that the optimal cure tumor size for β-particles of ^{186}Re (71% of 1.07 MeV and 21% of 0.94 MeV) is 7.0–12.0 mm in diameter in contrast to 2.6–5.0 mm for ^{131}I (86% of 0.606 MeV and 13% of 0.336 MeV).[15] Kievit et al.[13] reported the slight superiority of ^{131}I-MAb to ^{186}Re-MAb in 5.0–7.0 mm ovarian cancer xenografts delivered with the equal tumor absorbed dose by two conjugates, concluding that the tumor size contributed to producing these findings. In contrast, the diameter of tumors used in this study was 9.14 ± 0.44 mm, being within the optimal cure range for ^{186}Re. In current clinical settings, patients with recurrent lesions and metastatic lesions are candidates for RIT. In general, the minimal size of a tumor that is detectable with imaging methods is around 1 cm, which is within the suitable range for the β-particles of ^{186}Re. In addition, to treating larger tumors, the so-called cross-fire effect from radiolabeled MAbs heterogeneously distributed within tumors may be more significant with β-particles of ^{186}Re than those of ^{131}I. These several factors suggest the superiority of ^{186}Re-A7 to ^{131}I-A7 as an RIT compound.

Body weight was monitored to assess the toxicity of treatments, indicating that maximum body weight loss in the group treated with a dose of 4.48 MBq of ^{186}Re-A7 was similar to that with 4.63 MBq of ^{131}I-A7. In contrast, absorbed radiation doses within normal tissues including whole body doses were approximately 1.5-fold greater with ^{186}Re-A7 than with ^{131}I-A7 at these doses. We neglected the contribution of γ emissions in the estimation of tissue radiation dosimetry, and abundant high energy γ emission of 364 keV of ^{131}I may have produced a considerable whole body radiation dose, as compared with the lower energy γ emission of ^{186}Re, so that the actual whole body radiation dose with ^{131}I-A7 is likely to be closer to that with ^{186}Re-A7 than shown in Table 2, which made the toxicity similar with both conjugates. γ emissions would contribute to the whole body dose in human subjects more significantly than in small animals, suggesting that the advantage of ^{186}Re-A7 over ^{131}I-A7 would be greater in human subjects than in animals with regard to toxicity. The different profile in terms of the duration of body weight loss with two conjugates may depend on the difference between the physical half-lives of these radionuclides.

In conclusion, RIT with ^{186}Re-A7 suppressed the growth of colon cancer xenografts more effectively than that with ^{131}I-A7 at a similar dose level. They were equally toxic when assessed by body weight change. These results are likely to be caused by the tumor size treated in this study and the more favorable physical properties of ^{186}Re than those of ^{131}I.

ACKNOWLEDGMENTS

We thank former Professor Toshio Takahashi and Dr. Toshiharu Yamaguchi, First Department of Surgery, Kyoto Prefectural University of Medicine, for providing A7 MAb, and Dr. Yasushi Arano, Faculty of Pharmaceutical Sciences, Kyoto University (currently Professor of Faculty of Pharmaceutical Sciences, Chiba University), for providing *S*-benzoyl-MAG3.

REFERENCES

1. Kaminski MS, Zasadny KR, Francis IR, Milik AW, Ross CW, Moon SD, et al. Radioimmunotherapy of B-cell lymphoma with [^{131}I]anti-B1 (anti-CD20) antibody. *N Engl J Med* 1993; 329: 459–465.
2. Press OW, Eary JF, Appelbaum FR, Martin PJ, Badger CC, Nelp WB, et al. Radiolabeled-antibody therapy of B-cell lymphoma with autologous bone marrow support. *N Engl J*

Med 1993; 329: 1219–1224.
3. Behr TM, Becker WS, Bair H-J, Klein MW, Stühler CM, Cidlinsky KP, et al. Comparison of complete versus fragmented technetium-99m-labeled anti-CEA monoclonal antibodies for immunoscintigraphy in colorectal cancer. *J Nucl Med* 1995; 36: 430–441.
4. Oriuchi N, Endo K, Watanabe N, Sugiyama S, Asao T, Takenoshita S, et al. Semiquantitative SPECT tumor uptake of technetium-99m-labeled anti-CEA monoclonal antibody in colorectal tumor. *J Nucl Med* 1995; 36: 679–683.
5. Griffiths GL, Goldenberg DM, Knapp FF Jr, Callahan AP, Chang C-H. Direct radiolabeling of monoclonal antibodies with generator-produced rhenium-188 for radioimmunotherapy: labeling and animal biodistribution studies. *Cancer Res* 1991; 51: 4594–4602.
6. Fritzberg AR. Advances in ^{99m}Tc-labeling of antibodies. *Nucl Med* 1987; 26: 7–12.
7. Najafi A, Alauddin MM, Sosa A, Ma GQ, Chen DCP, Epstein AL, et al. The evaluation of ^{186}Re-labeled antibodies using N_2S_4 chelate *in vitro* and *in vivo* using tumor-bearing nude mice. *Nucl Med Biol* 1992; 19: 205–212.
8. Visser GMW, Gerretsen M, Herscheid JDM, Snow GB, van Dongen GAMS. Labeling of monoclonal antibodies with rhenium-186 using MAG3 chelate for radioimmunotherapy of cancer: a technical protocol. *J Nucl Med* 1993; 34: 1953–1963.
9. Goldrosen MH, Biddle WC, Pancook J, Bakshi S, Vanderheyden J-L, Fritzberg AR, et al. Biodistribution, pharmacokinetic, and imaging studies with ^{186}Re-labeled NR-LU-10 whole antibody in LS174T colonic tumor-bearing mice. *Cancer Res* 1990; 50: 7973–7978,
10. Kotanagi H, Takahashi T, Masuko T, Hashimoto Y, Koyama K. A monoclonal antibody against human colon cancers. *Tohoku J Exp Med* 1986; 148: 353–360.
11. Lindmo T, Boven E, Cuttitta C, Fedorko J, Bunn PA Jr. Determination of the immunoreactive fraction of radiolabeled monoclonal antibodies by linear extrapolation to binding at infinite antigen excess. *J Immunol Methods* 1984; 72: 77–89.
12. van Gog FB, Visser GWM, Klok R, van der Schos R, Snow GB, van Dongen GAMS. Monoclonal antibodies labeled with rhenium-186 using MAG3 chelate: relationship between the number of chelated groups and biodistribution characteristics. *J Nucl Med* 1996; 37: 352–362.
13. Kievit E, van Gog FB, Schluper HMM, van Dongen GAMS, Pinedo HM, Boven E. Comparison of the biodistribution and the efficacy of monoclonal antibody 323/A3 labeled with either ^{131}I or ^{186}Re in human ovarian cancer xenografts. *Int J Radiat Oncol Biol Phys* 1997; 38: 813–823.
14. Kinuya S, Yokoyama K, Kawashima A, Hiramatsu T, Konishi S, Shuke N, et al. Pharmacologic intervention with angiotensin II and kininase inhibitor enhanced efficacy of radioimmunotherapy in human colon cancer xenografts. *J Nucl Med* 2000; 41: 1244–1249.
15. O'Donoghue JA, Bardies M, Wheldon TE. Relationships between tumor size and curability for uniformly targeted therapy with beta-emitting radionuclides. *J Nucl Med* 1995; 36: 1902–1909.