Two-Step Targeting of Xenografted Colon Carcinoma Using a Bispecific Antibody and ^{188}Re-Labeled Bivalent Hapten: Biodistribution and Dosimetry Studies

Jean F. Gestin, Anthony Loussouarn, Manuel Bardiès, Emmanuel Gautherot, Anne Gruaz-Guyon, Catherine Saï-Maurel, Jacques Barbet, Chantal Curtet, Jean F. Chatal, and Alain Faivre-Chauvet

Institut de Biologie, Institut National de la Santé et de la Recherche Médicale, Nantes; Hopital St. Antoine, Institut National de la Santé et de la Recherche Médicale, Paris; and Immunotech, Marseille, France

Radioimmunotherapy (RIT) is currently being considered for the treatment of solid tumors. Although results have been encouraging for pretargeted ^{131}I RIT with the affinity enhancement system (AES), the radionuclide used is not optimal because of its long half-life, strong γ emission, poor specific activity, and low β particle energy. ^{188}Re, though unsuitable for direct antibody labeling, could be used with the AES two-step targeting technique. The purpose of this study was to compare the distribution and dosimetry of a bivalent hapten labeled with ^{188}Re or ^{125}I. For dosimetry calculations and biodistribution data, ^{125}I was substituted for ^{131}I. **Methods:** After preliminary injection of a bispecific anticarcinoembryonic antigen (CEA) or antihapten antibody (Bs-mAb F6-679), AG 8.1 or AG 8.0 hapten radiolabeled with ^{188}Re or ^{125}I was injected into a nude mouse model grafted subcutaneously with a human colon carcinoma cell line (LS-174-T) expressing CEA. A dosimetry study was performed for each animal from the concentration of radioactivity in tumor and different tissues. **Results:** Radiolabeling of AG 8.1 with ^{125}I afforded a 40% yield with a specific activity of 11.1 MBq/nmol after purification. Radiolabeling of AG 8.0 with ^{188}Re afforded a 72% yield with a specific activity of 31.82 MBq/nmol. In all experiments, the percentage of tumor uptake of ^{125}I-AG 8.1 was always significantly greater than that of ^{188}Re-AG 8.0. The corresponding tumor-to-tissue ratios reflected uptake values. The least favorable tumor-to-normal tissue ratios in the dosimetry study were 8.1 and 8.5 for ^{131}I (tumor-to-blood ratio and tumor-to-kidney ratio, respectively) and 2.3 for ^{188}Re (tumor-to-intestine ratio). **Conclusion:** This study indicates that ^{188}Re can be used for radiolabeling of hapten in two-step radioimmunotherapy protocols with the AES technique. ^{188}Re has a greater range than ^{131}I, which should allow the treatment of solid tumors around 1 cm in diameter. Although the method used for hapten radiolabeling did not provide optimal tumor uptake, the use of a bifunctional chelating agent associated with AG 8.1 should solve this problem.

Key Words: ^{188}Re; radioimmunotherapy; bispecific antibody; pretargeting

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Radioimmunotherapy (RIT) has proven to be efficient for the treatment of low-grade lymphomas, but results for solid (generally bulky) tumors have been disappointing (*1–4*) because of the low percentage of tumor uptake achieved (<0.01%) and the relatively high nonspecific uptake with the directly labeled monoclonal antibodies (in whole immunoglobulin G or fragment form) used in most studies. Two-step targeting techniques, which substantially reduce the uptake of activity in normal tissues, should help overcome these disadvantages (*5*). Several preclinical and clinical studies have shown the benefit of using the affinity enhancement system (AES), which associates a bispecific antibody with a bivalent hapten (Fig. 1). The haptens used have been radiolabeled first with ^{111}In for diagnostic studies and then with ^{131}I for RIT. The mean energy of β^- particles emitted by ^{131}I limits the efficacy of this radionuclide to small tumor targets. For larger targets, radionuclides emitting more energetic β^- particles seem to be preferable (*6*). Among these isotopes, ^{188}Re is a good candidate because of its physical characteristics (half-life, 16.98 h; β^-, 2.118 and 1.962 MeV) and production mode (^{188}W/^{188}Re generator) (*7–10*). However, the possibility of using this radionuclide with a directly radiolabeled antibody for RIT is limited by its short physical half-life and the specific activities obtained during direct radiolabeling of antibodies (70–80 MBq/nmol) (*7–9*) as well as slow tumor uptake (*11,12*). The AES two-step targeting technique provides rapid tumor uptake because of the small size and hydrophilic properties of the bivalent hapten and its long retention time at the tumor site attributed to specific binding to prelocalized antibodies (*5,13–15*).

The purpose of this study was to compare the distribution and dosimetry of a bivalent hapten labeled with ^{188}Re or ^{125}I after preliminary injection of a bispecific anticarcinoembryonic antigen (CEA) or antihapten antibody into a nude mouse model grafted subcutaneously with a human colon carcinoma cell line (LS-174-T) expressing CEA.

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For correspondence or reprints contact: Jean F. Gestin, PhD, Institut de Biologie, Unit 463, Institut National de la Santé et de la Recherche Médicale, 9 Quai Moncousu, 44093 Nantes cedex, France.

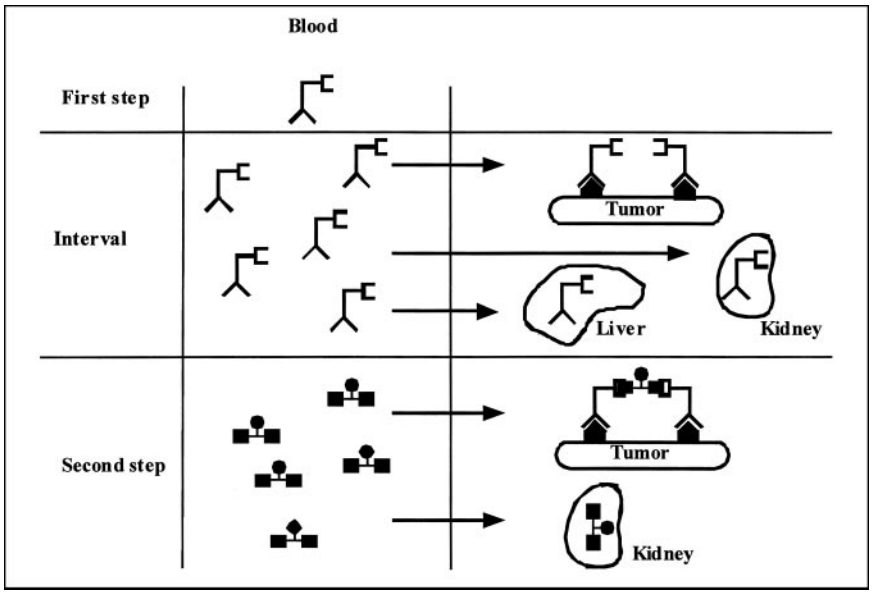

FIGURE 1. AES.

MATERIALS AND METHODS

The bispecific antibody (Bs-mAb F6–679) used in this study was composed of an F(ab′) fragment of an antiCEA antibody (F6) coupled chemically to an F(ab′) fragment of an antiglycylsuccinimidylhistamine antibody (679). The bispecific antibody was supplied by Immunotech (Marseille, France) as a 3 mg/mL solution in 0.1 mol/L phosphate buffer and 0.05 mol/L EDTA, pH 7.

The hapten used, AG 8.1, was an octapeptide grafted with two glycylsuccinimidylhistamine residues in ε of lysines 1 and 3. Its semideveloped formula is shown in Figure 2. AG 8.1 may be readily radiolabeled with radioactive iodine.

For ^{188}Re labeling, the peptide was derivatized with an *S*-acetylthioacetyl residue at the N-terminal position. The new derivative thus obtained was designated as AG 8.0 (*16*). The two haptens (AG 8.1 and AG 8.0) were supplied by Immunotech as an aqueous solution at 1 mg/mL.

Hapten Radiolabeling

^{125}I-AG 8.1. AG 8.1 was labeled with ^{125}I using the chloramine-T technique (*17*). After labeling, the hapten was purified using a SepPak C_{18} cartridge (Millipore, Saint Quentin Yvelines, France) by successive injections of the labeling solution (5 mL 0.05% trifluoroacetic acid in water [Aldrich, Saint Quentin Fallavier, France] and then 5 mL of a 3:2 mixture of 0.1 mol/L phosphate buffer solution, pH 7, and ethanol). Under these conditions, free ^{125}I was eluted in the acid aqueous phase and the radiolabeled hapten was eluted in the first 2 mL of the ethanol phase. The radiochemical purity of the purified solution was measured by thin-layer silica gel chromatography (Kieselgel 60 F_{254}; Merck, Nogent sur Marne, France) using methanol as the migration solvent. The immunoreactive fraction was measured by determining the percentage of activity binding to tubes coated with the antihistamine antibody 679. The chromatograms were analyzed

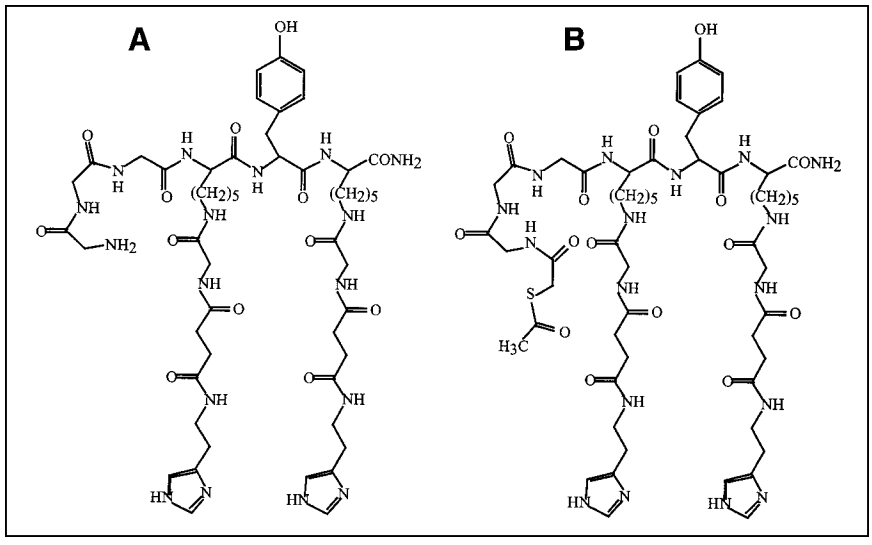

FIGURE 2. Plane semideveloped formula of AG 8.1 (A) and AG 8.0 (B).

after exposure on a phosphorus screen (Molecular Dynamics, Sunnyvale, CA) using IPLab-Gel software (Analytics Corp., Atlanta, GA).

^{188}Re-AG 8.0. AG 8.0 was labeled with ^{188}Re by ligand exchange according to a method derived from that of Joiris et al. (*18*). Briefly, ^{188}Re-glucoheptonate was obtained quantitatively by addition under a nitrogen atmosphere of 740 MBq (20 mCi; 3 mL) sodium perrhenate in a glass flask containing 150 mg sodium glucoheptonate (Aldrich), 8.4 mg sodium hydrogenocarbonate (Aldrich), and 1.25 mg stannous chloride (Aldrich). The solution was incubated for 1 h at room temperature and controlled by instant thin-layer chromatography on silica gel (ITLC-SG; Gelman-OSI, Elancourt, France) using 0.9% sodium chloride and acetone as migration solvents. During the incubation period, AG 8.0 was deacetylated by addition of excess sodium hydroxide (1N). A spot test with dithionitrobenzoic acid was performed on an aliquot of the deprotected solution to check for the presence of free thiol (*19*). Twenty-one nanomoles of deprotected AG 8.0 were added to the ^{188}Re-glucoheptonate solution, which was then incubated for 15 min at 100°C before purification, and the control solution under the same conditions as those during radiolabeling of AG 8.1 by ^{125}I.

Animal Studies

For each radionuclide, 15 nude mice (Swiss/nu/nu; Iffa-Credo, L'Arbresle, France) were used (30 animals total). Each animal was xenografted subcutaneously in the right flank with 1 million cells of the LS-174-T colon cancer cell line (American Type Culture Collection, Rockville, MD), which strongly expresses CEA. Two weeks after grafting, the tumors measured 50–500 mm^3. Each mouse was injected intravenously in a tail vein with 50 μg (0.5 nmol/100 μL) Bs-mAb F6-679. On the basis of data obtained from other studies (*20–25*), a second injection was performed (24 h after this first injection) with either 0.25 nmol ^{125}I-AG 8.1 (11.1 MBq/nmol) or 0.25 nmol ^{188}Re-AG 8.0 (31.8 MBq/nmol).

Biodistribution Study

For each radionuclide, a group of 3 animals was killed at 5 min and at 1, 5, 24, and 48 h after injection of the radiolabeled hapten. For each time point, the main tissues and tumors were removed, dried, weighed, and counted in a γ scintillator (Compugamma; LKB-Pharmacia, Uppsala, Sweden) in parallel with a calibrated radioactive decay standard. The results are expressed as a percentage of the injected dose per gram (%ID/g) of tissue and as tumor-to-tissue ratios.

^{188}Re-AG 8.0 Stability

An analysis of the immunoreactive fraction of blood and urine was also performed using the same procedure as that used for the radiolabeling control. Statistical analysis of the results was done by ANOVA using Statview II software (Abacus Concepts, Berkeley, CA).

Measurements of immunoreactive fractions were performed at 5 min and at 1, 5, 24, and 48 h in blood, urine, and the ^{188}Re-labeled hapten solution after purification. This control solution was kept at room temperature throughout the experiment, with a starting activity of 185 MBq/mL (5 mCi/mL). Measurements were performed to study the instability of the radiolabeled hapten.

Dosimetry Study

For each mouse, the concentration of radioactivity (kBq/g) was calculated in different tissues or tumors. ^{131}I was considered for dosimetry instead of ^{125}I for several reasons: cost, radioprotection, and identical biodistributions. The counting and calculation of the percentage of the injected dose per animal tissue was determined simultaneously with that of a weighed standard aliquot of the injected dose. To minimize counting errors associated with radiation self-absorption by tissues, the same weight of each organ sample was measured. The mean value of this concentration at each time point allowed curves to be plotted according to a theoretic one-compartment extravascular model. The results (initial concentration, C_0) were used, after correction of the physical half-life, to calculate the mean dose delivered by a theoretic injection of 3.7 MBq ^{131}I or ^{188}Re according to a MIRD formula (*26*):

$$\bar{D}(\text{target} \leftarrow \text{source}) = \tilde{A}_{\text{source}} \times \frac{\Delta \times \phi(\text{target} \leftarrow \text{source})}{m_{\text{target}}},$$

where $\bar{D}$(target ← source) is the mean absorbed dose (in Gy) delivered to the target by the source, $\tilde{A}_{\text{source}}$ is the cumulated activity in the source (in Bq × s), Δ (in J/(Bq × s)) is the sum of energies emitted by desintegration, ϕ(target ← source) is the fraction of energy emitted by the source that is absorbed by the target, and m_{cible} is the mass of the target.

To simplify the calculations, it was considered in a first approximation that the sources and targets were identical, and the emissions were regarded as nonpenetrating. In this case,

$$\bar{D}(\text{source} \leftarrow \text{source}) = \tilde{A}_{\text{source}} \times \frac{\Delta \times \phi(\text{source} \leftarrow \text{source})}{m_{\text{source}}}$$

becomes:

$$\bar{D}_{\text{organ}} = \tilde{A}_{\text{organ}} \times \frac{\Delta}{m_{\text{organ}}} = \tilde{C}_{\text{organ}} \times \Delta,$$

which assumes that the emitted particles deposit their energy locally:

$$\phi(\text{source} \leftarrow \text{source}) = 1.$$

Calculations were performed with the tabulated Δ (*27*) for ^{131}I and ^{188}Re, which gave respectively:

$$\Delta = 1.24 \times 10^{-13}\ (\text{kg} \times \text{Gy})/(\text{Bq} \times \text{s}) \text{ for } ^{188}\text{Re}$$

and

$$\Delta = 3.04 \times 10^{-14}\ (\text{kg} \times \text{Gy})/(\text{Bq} \times \text{s}) \text{ for } ^{131}\text{I}.$$

RESULTS

Hapten Radiolabeling

^{125}I-AG 8.1. During radiolabeling of AG 8.1 with ^{125}I, a 40% yield was obtained after addition of 259 MBq ^{125}I to 11 nmol AG 8.1. The specific activity of ^{125}I-AG 8.1 after purification was 11.1 MBq/nmol, with a radiochemical purity of 100% by chromatography. The immunoreactive fraction was measured to 96.4%.

^{188}Re-AG 8.0. The radiochemical purity of the ^{188}Re-glucoheptonate solution used in the radiolabeling of AG 8.0 was 80% by chromatography. The impurities in the preparation represented 12% reduced and hydrolyzed rhenium and 8% perrhenate. Because these impurities could be re-

TABLE 1
Comparative F6–679/^{125}I-AG 8.1 Versus F6–679/^{188}Re-AG 8.0 Biodistribution After Injection in Nude Mouse Model Grafted Subcutaneously with Human Colon Carcinoma Cell Line (LS-174-T)

	Time									
	5 min		1 h		5 h		24 h		48 h	
%ID/g	^{125}I-AG 8.1	^{188}Re-AG 8.0	^{125}I-AG 8.1	^{188}Re-AG 8.0	^{125}I-AG 8.1	^{188}Re-AG 8.0	^{125}I-AG 8.1	^{188}Re-AG 8.0	^{125}I-AG 8.1	^{188}Re-AG 8.0
Tumor	4.80 ± 1.21	2.44 ± 0.93	6.88 ± 1.09	4.01 ± 0.41	10.4 ± 1.6	5.42 ± 0.58	7.92 ± 2.78	4.04 ± 1.42	5.92 ± 0.61	2.12 ± 0.76
Blood	6.49 ± 1.15	5.02 ± 0.94	3.58 ± 0.63	2.59 ± 0.53	3.03 ± 0.56	1.58 ± 0.37	1.32 ± 0.29	0.36 ± 0.21	0.35 ± 0.12	0.39 ± 0.02
Liver	2.20 ± 0.50	8.12 ± 2.36	0.93 ± 0.03	0.97 ± 0.30	0.79 ± 0.09	0.50 ± 0.05	0.74 ± 0.27	0.25 ± 0.10	0.46 ± 0.04	0.15 ± 0.05
Kidney	8.12 ± 3.20	4.24 ± 0.88	3.11 ± 0.15	1.29 ± 0.35	2.06 ± 0.17	0.75 ± 0.05	1.47 ± 0.33	0.29 ± 0.08	1.03 ± 0.17	0.20 ± 0.04
Intestine	1.32 ± 0.37	3.34 ± 2.55	1.43 ± 0.67	16.7 ± 13.4	0.38 ± 0.09	0.27 ± 0.04	0.17 ± 0.05	0.12 ± 0.03	0.07 ± 0.01	0.10 ± 0.03
Spleen	1.30 ± 0.24	1.04 ± 0.24	0.62 ± 0.07	0.43 ± 0.09	0.55 ± 0.09	0.34 ± 0.10	0.48 ± 0.17	0.14 ± 0.02	0.24 ± 0.02	0.19 ± 0.02
Bone	1.32 ± 0.31	1.31 ± 0.27	0.62 ± 0.13	0.54 ± 0.17	0.38 ± 0.05	0.32 ± 0.09	0.28 ± 0.09	0.23 ± 0.05	0.09 ± 0.02	0.29 ± 0.06

Data are expressed as mean ± SD.

moved easily during the purification step for the radiolabeled hapten, the solution was used in this state. Under these conditions, the uptake yield after addition of 1 GBq ^{188}Re-glucoheptonate to 21 nmol AG 8.0 was 72%. The specific activity of ^{188}Re-AG 8.0 after purification was estimated at 90% by chromatography. The immunoreactive fraction was measured to 91%. The major impurities detected by chromatography could not be identified. The purification step allowed elimination of perrhenate and ^{188}Re hydrolysates.

Animal Study

Biodistribution Study. The results obtained (Table 1) show that the maximum injected dose in all tissues appeared at the same time point for both radionuclide-associated haptens: 5 min for blood, liver, kidney, spleen, and bone; 1 h for intestine; and 5 h for tumor. The percentage of tumor uptake of ^{125}I-AG 8.1 was always statistically greater than that of ^{188}Re-AG 8.0 ($P < 0.01$ for all time points). The distribution of radioactivity was comparable for blood, spleen, and bone up to 1 h after injection and then became statistically greater for ^{125}I-AG 8.1 than for ^{188}Re-AG 8.0—except for bone, in which uptake of ^{188}Re-AG 8.0 was greater than that of ^{125}I-AG 8.1 at 48 h ($P < 0.01$).

The corresponding tumor-to-tissue ratios (Fig. 3) reflected these uptake values. They were higher with ^{125}I-AG 8.1 (Fig. 3A) at 5 min for blood, spleen, and bone ($P < 0.05$) and were comparable with the ratios of ^{188}Re-AG 8.0 (Fig. 3B) for these same tissues at 1, 5, and 24 h. At late times (48 h), ^{188}Re-AG 8.0 showed ratios that were lower than those for ^{125}I-AG 8.1, except in liver and kidney ($P < 10^{-4}$), which were indicative of higher tumor uptake and lower bone uptake for ^{125}I-AG 8.1. At 5 min, liver uptake of ^{188}Re-AG 8.0 was higher than that of ^{125}I-AG 8.1, resulting in very low tumor-to-liver ratios ($P < 0.01$), whereas at 5, 24, and 48 h uptake was greater for ^{125}I-AG 8.1 ($P < 0.05$), with comparable tumor-to-liver ratios for both substances ($P > 0.05$). In the intestine, uptake of ^{125}I-AG 8.1 and ^{188}Re-AG 8.0 was not significantly different at any time point, except at 1 h when uptake was markedly greater for ^{188}Re-AG 8.0 ($P < 0.01$), with a very large SD indicative of considerable interindividual variation. In kidney, regardless of analysis time, uptake percentages for ^{125}I-AG 8.1 were greater than those for ^{188}Re-AG 8.0 ($P < 0.05$). Despite high tumor uptake of ^{125}I-AG 8.1, except at 5 min, the tumor-to-kidney ratios obtained with ^{188}Re-AG 8.0 were greater than those for ^{125}I-AG 8.1 ($P < 0.05$).

In summary, tumor-to-tissue ratios were highest for ^{188}Re-AG 8.0 at 24 h, except for the tumor-to-bone ratio, which was highest at 5 h. For ^{125}I-AG 8.1, the tumor-to-blood ratio was highest at 48 h, whereas the other ratios were highest between 5 and 48 h.

^{188}Re-AG 8.0 Stability. The results for immunoreactive measurements are shown in Figure 4. Analysis of the stability of the purified ^{188}Re-AG 8.0 solution over time showed that the ^{188}Re-labeled peptide, used as a control, lost a large part of its immunoreactivity at early times. It decreased from 90.8% at 5 min to 17.7% at 5 h but remained quite constant after that time point: 14.2% at 24 h and 8% at 48 h.

The immunoreactive fraction in urine was around 38% at 5 min and then became very low (7.1%) between 1 and 5 h before rising to 44% at 24 h and dropping slightly to 36% at 48 h. Most of the radioactivity was eliminated rapidly. The immunoreactive fractions were quite stable in blood, ranging from 84% at 5 min to 26.7% at 48 h. This phenomenon could have been caused by radiolysis resulting from the high activity used or by instability of the radiolabeled hapten (or both).

The study of immunoreactive fractions relating to ^{188}Re-labeled hapten showed that urinary radioactivity was eliminated mainly in the form of nonimmunoreactive metabolites, whereas circulating forms in the blood were composed mainly of immunoreactive hapten until 5 h after injection and were then considerably reduced at late times.

Dosimetry Study. The results of the dosimetric study are shown in Table 2. For each tissue and tumor, the cumulative concentration is indicated in kBq/s/kg, as extrapolated by exponential adjustment of kinetic values and the constant of the dose corresponding to each radionuclide studied. Cal-

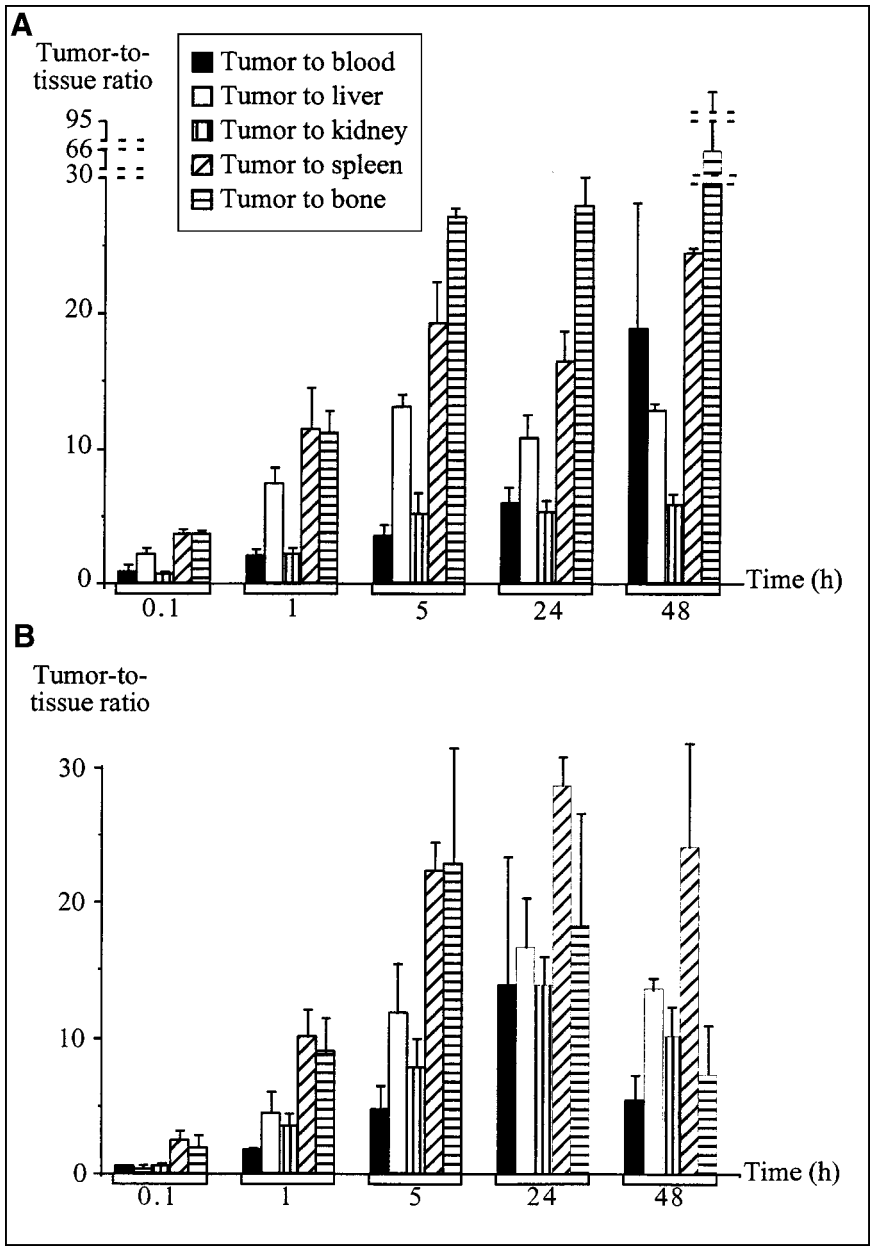

FIGURE 3. Comparison of tumor-to-tissue ratios obtained with ^{125}I-AG 8.1 (A) and ^{188}Re-AG 8.0 (B) after injection in nude mouse model grafted subcutaneously with human colon carcinoma cell line (LS-174-T). Data are expressed as mean ± SD.

culations for ^{131}I were performed on the basis of results for ^{125}I, assuming that the kinetics of AG 8.1 kinetics was identical for the two iodine radioisotopes.

With ^{188}Re, the highest doses were obtained for tumor, intestine, blood, and kidney, whereas with ^{125}I the most irradiated tissues were tumor, kidney, blood, and liver. The least favorable tumor-to-normal tissue ratios were 8.1 and 8.5 for ^{131}I (tumor-to-blood ratio and tumor-to-kidney ratio, respectively) and 2.3 for ^{188}Re (tumor-to-intestine ratio).

DISCUSSION

The use of mercaptoacetyltriglycine (MAG3) derivatives for the radiolabeling of proteins with ^{99m}Tc or ^{188}Re is one of the most common techniques (*28–32*). With ^{188}Re, the usual approach is to radiolabel MAG3 first and then purify the ^{188}Re-MAG3 thus obtained before activating an acid residue (generally butyric acid). The radiolabeling of proteins is then achieved by incubating the activated, radiolabeled MAG3 with a slightly alkaline protein solution (*28,29*). Although this procedure provides radiolabeling with high radiochemical purity, it is time-consuming and thus unsuitable in terms of the short physical half-life of ^{188}Re. Radiolabeling of antibodies with ^{188}Re can be performed directly (*7–9,33*), but the radioantibodies obtained generally lack stability in vivo (*8,29*). To avoid these drawbacks, the modified derivative of AG 8.1 used in this study had a structure derived from MAG3 but could be radiolabeled directly with ^{188}Re. Moreover, to facilitate the radio-

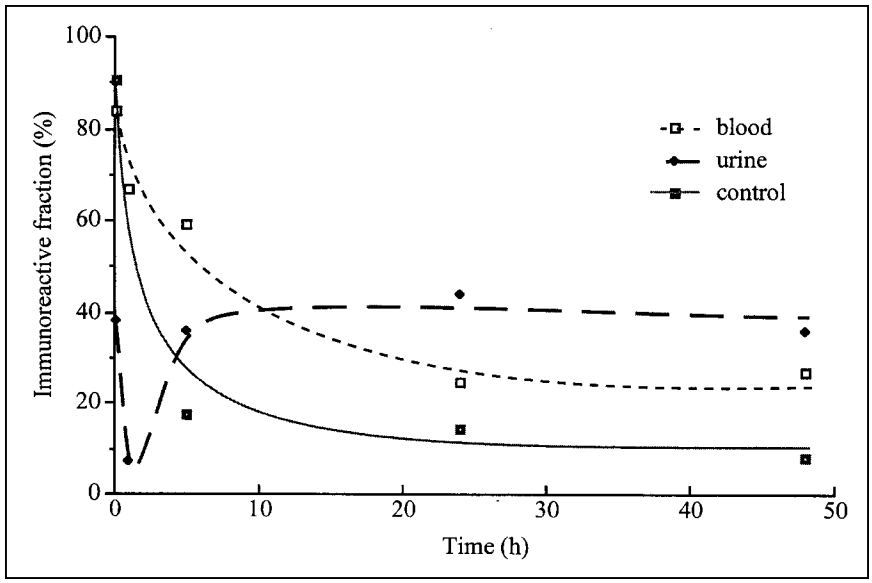

FIGURE 4. Analysis of stability of ^{188}Re-AG 8.0 by measurement of serum and urinary immunoreactive fractions after injection in nude mouse model grafted subcutaneously with human colon carcinoma cell line (LS-174-T).

labeling of this AG 8.0 peptide, a ligand-exchange technique was developed, which allowed ^{188}Re-AG 8.0 to be obtained in 15 min with good yields. As a reference, we used another peptide closely related to AG 8.0, which was labeled with a low specific activity of ^{125}I to minimize radiolysis (11.1 MBq/nmol). AG 8.0 was labeled with a higher specific activity of ^{188}Re (31.82 MBq/nmol), which should make ^{188}Re-AG 8.0 suitable for future RIT protocols. The same purification technique used for labeling both radionuclides afforded excellent results for ^{125}I-AG 8.1 but was less efficient in removing impurities from the ^{188}Re-AG 8.0 solution. However, the latter was used because radiochemical purity was better than or equal to that reported for direct radiolabeling of antibodies with ^{188}Re (60%–80% as measured by the immunoreactive fraction) (*7–9*).

The results obtained with ^{125}I-AG 8.1 were comparable with those reported in the same animal model (*34*) for a radiolabeled peptide that was only slightly different from the one used in this study. The results for tumor uptake of ^{188}Re-AG 8.0 were poorer than those with ^{125}I labeling but were comparable with values in the literature for antibodies radiolabeled directly with ^{188}Re (*16,29*). Kinetic analysis of biodistribution showed that the blood clearance of ^{125}I-AG 8.1 was lower than that of ^{188}Re-AG 8.0 (1.23 mL/h vs. 2.1 mL/h). The higher blood clearance of AG 8.0 was apparently one of the reasons for lower tumor uptake with this peptide than with AG 8.1. However, the radiochemical purity of ^{188}Re-AG 8.0 decreased very rapidly over time when it was concentrated in the purification medium, which suggests that impurities formed during the period required for preparation and injection of ^{188}Re-AG 8.0 into the animal. These impurities associated with radiolabeling complexation instability or radiolysis (or both) were apparently responsible for part of the increase in the renal clearance of radioactivity and for reduced tumor uptake. This was confirmed by the study of urinary immunoreactive fractions, which showed that only 8% of the radioactivity consisted of hapten that was recognized by antibody 679 at 1 h, whereas this fraction was >30% at all other time points. This difference may be attributed to rapid urinary elimination of

TABLE 2
Dosimetry Estimations Obtained from Biodistribution Results for ^{125}I-AG 8.1 and ^{188}Re-AG 8.0 for Theoretic Injection of 3.7 MBq ^{131}I or ^{188}Re in Nude Mouse Model Grafted Subcutaneously with Human Colon Carcinoma Cell Line (LS-174-T)

	^{131}I			^{188}Re		
Tissue	$C_{cumulative}$ (kBq/s/kg)	D (cGy)	Tumor/nontumor dosimetry ratio	$C_{cumulative}$ (kBq/s/kg)	D (cGy)	Tumor/nontumor dosimetry ratio
Tumor	6.59	259.79		1.07	172.63	
Blood	0.82	32.16	8.1	0.28	45.39	3.8
Liver	0.41	16.06	16.2	0.14	22.76	7.6
Kidney	0.78	30.65	8.5	0.15	25.04	6.9
Intestine	0.13	5.21	49.9	0.47	75.34	2.3
Spleen	0.26	10.37	25	0.06	10.42	16.6
Bone	0.13	5.29	49.1	0.07	12.24	14.1

impurities caused by the release of noncomplexed ^{188}Re from ^{188}Re-AG 8.0 before administration. At later time points, the metabolism of this radiolabeled peptide was a contributing factor, although a highly significant fraction of peptide circulating in the blood remained immunoreactive over time. Moreover, the results of the biodistribution study show that ^{188}Re-AG 8.0 had very high hepatic and intestinal uptake at early time points compared with that of ^{125}I-AG 8.1, apparently because of biliary elimination of ^{188}Re-AG 8.0 radioactivity. This mode of elimination could have caused increased clearance of the radiolabeled peptide and thus decreased tumor uptake at later times. It appears that ^{125}I-AG 8.1 was eliminated preferentially by the renal route. In fact, the percentages of renal uptake were always greater for ^{125}I-AG 8.1 than for ^{188}Re-AG 8.0 at all time points. Finally, bone uptake was greater at 48 h with ^{188}Re-AG 8.0 than with ^{125}I-AG 8.1, possibly because ^{188}Re-AG 8.0 lacked labeling stability in vivo. This could have caused local uptake by exchange between the ^{188}Re-MAG3 complex of the peptide and the phosphocalcic structures of the bone network.

For all normal tissues studied, the %ID/g with ^{125}I-AG 8.1 was lower than that for antibody radiolabeled directly with ^{125}I (*5*,*16*,*35*,*36*). This phenomenon, which has been reported for other bispecific antibody or hapten pairs (*14*,*15*), resulted in tumor-to-tissue ratios that were greater than those observed with antibodies that were radiolabeled directly (*16*,*28*,*37*). Comparison of the biodistribution obtained with AG 8.1 labeled with ^{125}I and ^{188}Re showed differences in the in vivo distribution of radioactivity depending on the radioelement used. In fact, this phenomenon could have been attributed to the structural difference between the two haptens. The elimination kinetics of ^{188}Re-AG 8.0 appeared to be faster than that of ^{125}I-AG 8.1, partly because ^{188}Re-AG 8.0 has significant hepatobiliary clearance.

Two assumptions were made in this study relative to dosimetry calculations. First, the effect of γ emissions was disregarded at the doses delivered because of the low proportion of photon emissions by ^{188}Re (9.18×10^{-15} kg $\times$ Gy/Bq $\times$ s) compared with particle emissions (1.24×10^{-13} kg $\times$ Gy/Bq $\times$ s) and the fact that this energy was deposited at greater distances than with β^- emissions. Second, particle emissions were considered nonpenetrating in the geometry of our experimental conditions. This assumption, though frequently made for dosimetry in vivo in humans, cannot be verified in mice. Thus, our results need to be interpreted with caution, given the size of murine organs and tissues, which are generally smaller than the maximal range of β^- particles of ^{188}Re (*6*). Nevertheless, it is necessary to make these calculations to compare the results obtained with both radionuclides with those published using the same animal model (*21–24*).

On the whole, our results are encouraging for the development of RIT protocols with this technique, even if improvement of ^{188}Re-AG 8.0 stability is still necessary. Under these conditions, the injection of quantities of radioactivity three to four times higher than the quantities we used may allow the delivery of doses approximating those reported in other studies relating to this method of human tumor treatment (*33*,*38*,*39*).

CONCLUSION

This study indicates that ^{188}Re can be used for the radiolabeling of hapten in two-step RIT protocols with the AES. The main advantage of this radionuclide over ^{131}I is its range, which should allow the treatment of solid tumors around 1 cm in diameter. However, the method used for hapten radiolabeling did not provide optimal tumor uptake. Other rhenium-chelating agents easily substituted for the N-terminal end of AG 8.1 could offer improved labeling efficiency and in vivo stability.

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REFERENCES

1. Behr TM, Sharkey RM, Juweid ME, et al. Phase I/II clinical radioimmunotherapy with an iodine-131-labeled anti-carcinoembryonic antigen murine monoclonal antibody IgG. *J Nucl Med.* 1997;38:858–870.
2. Alvarez RD, Partridge EE, Khazaeli MB, et al. Intraperitoneal radioimmunotherapy of ovarian cancer with Lu-177-CC49: a phase I/II study. *Gynecol Oncol.* 1997;65:94–101.
3. Juweid M, Sharkey RM, Alavi A, et al. Regression of advanced refractory ovarian cancer treated with iodine-131-labeled anti-CEA monoclonal antibody. *J Nucl Med.* 1997;38:257–260.
4. Juweid ME, Sharkey RM, Behr T, et al. Radioimmunotherapy of patients with small-volume tumors using iodine-131-labeled anti-CEA monoclonal antibody np-4 F(ab′)$_2$. *J Nucl Med.* 1996;37:1504–1510.
5. Rosebrough SF. Two-step immunological approaches for imaging and therapy. *Q J Nucl Med.* 1996;40:234–251.
6. Bardies M, Chatal JF. Absorbed doses for internal radiotherapy from 22 beta-emitting radionuclides: beta dosimetry of small spheres. *Phys Med Biol.* 1994; 39:961–981.
7. Griffiths GL, Goldenberg DM, Knapp FF, Callahan AP, Chang CH, Hansen HJ. Direct radiolabeling of monoclonal antibodies with generator-produced rhenium-188 for radioimmunotherapy: labeling and animal biodistribution studies. *Cancer Res.* 1991;51:4594–4602.
8. Rhodes BA, Lambert CR, Marek MJ, Knapp FF, Harvey EB. Re-188 labelled antibodies. *Appl Radiat Isot.* 1996;47:7–14.
9. Winnard P, Virzi E, Fogarasi M, Rusckowski M, Hnatowich DJ. Investigations of directly labeling antibodies with rhenium-188. *Q J Nucl Med.* 1996;40:151–160.
10. Eary JF, Durack L, Williams D, Vanderheyden JL. Considerations for imaging Re-188 and Re-186 isotopes. *Clin Nucl Med.* 1990;15:911–916.
11. Goldrosen MH, Biddle WC, Pancook J, 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.
12. Yokota T, Milenic DE, Whitlow M, Schlom J. Rapid tumor penetration of single-chain Fv and comparison with other immunoglobulin forms. *Cancer Res.* 1992;52:3402–3408.
13. Le Doussal JM, Barbet J, Delaage M. Bispecific-antibody-mediated targeting of radiolabeled bivalent haptens: theoretical, experimental and clinical results. *Int J Cancer Suppl.* 1992;7:58–62.
14. Bardies M, Bardet S, Faivre-Chauvet A, et al. Bispecific antibody and iodine-

131-labeled bivalent hapten dosimetry in patients with medullary thyroid or small-cell lung cancer. *J Nucl Med.* 1996;37:1853–1859.
15. Devys A, Thédrez P, Gautherot E, et al. Comparative targeting of human colon-carcinoma multicell spheroids using one- and two-step (bispecific antibody) techniques. *Int J Cancer*. 1996;67:883–891.
16. Gruaz-Guyon A, Le Doussal JM, Delaage M, Barbet J, inventors; Immunotech Partners, assignee. Bihaptenic derivatives for binding of technetium or rhenium, process for their preparation, application to diagnosis and therapy, kits and immunological reagents comprising them. European patent application 595743. October 26; 1993.
17. Greenwood F, Hunter W. The preparation of I-131-labeled human growth hormone of high specific radioactivity. *Biochem J.* 1963;89:114–123.
18. Joiris E, Bastin B, Thornback JR. A new method for labelling of monoclonal antibodies and their fragments with technetium-99m. *Nucl Med Biol.* 1991;18: 353–356.
19. Aitken A. Quantification and location of disulfide bonds in proteins. *Methods Mol Biol.* 1997;64:317–328.
20. Gautherot E, LeDoussal JM, Bouhou J, et al. Delivery of therapeutic doses of radio-iodine using bispecific antibody-targeted bivalent haptens: a comparative pharmacokinetic and dosimetry study. *J Nucl Med.* 1998;39:1937–1943.
21. Kraeber-Bodéré F, Faivre-Chauvet A, Saï Maurel C, et al. Bispecific antibody and bivalent hapten radioimmunotherapy in CEA-producing medullary thyroid cancer xenograft. *J Nucl Med.* 1999;40:198–204.
22. Hosono M, Hosono MN, Kraeber-Bodéré F, et al. Two-step targeting and dosimetry for small cell lung cancer xenograft with anti-NCAM/antihistamine bispecific antibody and radioiodinated bivalent hapten. *J Nucl Med.* 1999;40: 1216–1221.
23. Gautherot E, Kraeber-Bodéré F, Daniel L, et al. Immunohistology of carcinoembryonic antigen (CEA)-expressing tumors grafted in nude mice after radioimmunotherapy with ^{131}I-labeled bivalent hapten and anti-CEA × anti-hapten bispecific antibody. *Clin Cancer Res.* 1999;5:3177–3182.
24. Kraeber-Bodéré F, Faivre-Chauvet A, Saï Maurel C, et al. Toxicity and efficacy of radioimmunotherapy in carcinoembryonic antigen-producing medullary thyroid cancer xenograft: comparison of iodine 131-labeled F(ab′)$_2$ and pretargeted bivalent hapten and evaluation of repeated injections. *Clin Cancer Res.* 1999;5: 3183–3189.
25. Gautherot E, Rouvier E, Daniel L, et al. Pretargeted radioimmunotherapy of human colorectal xenografts with bispecific antibody and ^{131}I-labeled bivalent haptens. *J Nucl Med.* 2000;41:480–487.
26. Loevinger R, Budinger TF, Watson EE. *MIRD Primer for Absorbed Dose Calculations.* New York, NY: Society of Nuclear Medicine; 1988.
27. Weber DA, Eckerman KF, Dillman LT, Ryman JC. *MIRD Radionuclide Data and Decay Schemes*. New York, NY: Society of Nuclear Medicine; 1989.
28. Visser GW, Gerretsen M, Herscheid JD, Snow GB, Van Dongen G. Labeling of monoclonal antibodies with rhenium-186 using the MAG3 chelate for radioimmunotherapy of cancer: a technical protocol. *J Nucl Med.* 1993;34:1953–1963.
29. Van Gog FB, Visser GW, Klok R, Van der Schors R, Snow GB, Van Dongen G. Monoclonal antibodies labeled with rhenium-186 using the MAG3 chelate: relationship between the number of chelated groups and biodistribution characteristics. *J Nucl Med.* 1996;37:352–362.
30. Rajagopalan R, Grummon GD, Bugaj J, et al. Preparation, characterization, and biological evaluation of technetium (V) and rhenium (V) complexes of novel heterocyclic tetradentate N_3S ligands. *Bioconjug Chem.* 1997;8:407–415.
31. Yoo TM, Chang HK, Choi CW, et al. Technetium-99m labeling and biodistribution of anti-TAC disulfide-stabilized Fv fragment. *J Med Nucl.* 1997;38:294–300.
32. Lei K, Rusckowski M, Chang F, Qu T, Mardirossian G, Hnatowich DJ. Technetium-99m antibodies labeled with MAG3 and SHNH: an *in vitro* and animal *in vivo* comparison. *Nucl Med Biol.* 1996;23:917–922.
33. Gerretsen M, Visser GW, van Walsum M, Meijer CJ, Snow GB, van Dongen GA. ^{186}Re-labeled monoclonal antibody E48 immunoglobulin G-mediated therapy of human head and neck squamous cell carcinoma xenografts. *Cancer Res.* 1993; 53:3524–3529.
34. Janevik-Ivanovska E, Gautherot E, Hillairet de Boisferon M, et al. Bivalent hapten-bearing peptides designed for iodine-131 pretargeted radioimmunotherapy. *Bioconjug Chem.* 1997;8:526–533.
35. Hosono MN, Hosono M, Endo K, Ueda R, Onoyama Y. Effect of hyperthermia on tumor uptake of radiolabeled anti-neural cell adhesion molecule antibody in small-cell lung cancer xenograft. *J Nucl Med.* 1994;35:504–509.
36. Kwa HB, Verhoeven AH, Storm J, Van Zandwijk N, Mooi WJ, Hilkens J. Radioimmunotherapy of small-cell lung cancer xenografts using ^{131}I-labelled anti-NCAM monoclonal antibody 123C3. *Cancer Immunol Immunother*. 1995; 41:169–174.
37. Griffiths GL, Goldenberg DM, Jones AL, Hansen HJ. Radiolabeling of monoclonal antibodies and fragments with technetium and rhenium. *Bioconjug Chem.* 1992;3:91–99.
38. Breitz HB, Durham JS, Fisher DR, Weiden PL. Radiation-absorbed dose estimates to normal organs following intraperitoneal ^{186}Re-labeled monoclonal antibody: methods and results. *Cancer Res.* 1995;55:5817–5822.
39. Breitz HB, Fisher DR, Weiden PL, et al. Dosimetry of rhenium-186-labeled monoclonal antibodies: methods, prediction from technetium-99m-labeled antibodies and results of phase I trials. *J Nucl Med.* 1993;34:908–917.