

DEVELOPMENT AND EVALUATION OF COPPER-67 AND SAMARIUM-153 LABELED CONJUGATES FOR TUMOR RADIOIMMUNOTHERAPY

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INTRODUCTION

The potential of utilizing receptor-specific agents such as monoclonal antibodies (MAb), and MAb-derived smaller molecules, as carriers of radionuclides for the selective destruction of tumors has stimulated much research activity (Srivastava, 1988; Larson, 1994). The success of such applications depends on many factors, especially the tumor binding properties of the antibody reagent, the efficiency of labeling and in-vivo stability of the radioconjugate and, on the careful choice of the radionuclide best suited to treat the tumor under consideration. The radiolabeled antibody technique for radioimmunotherapy (RIT), however, has experienced many limitations, and its success has not matched the expectations that were raised more than a decade ago (Srivastava, 1988). The problems that have been identified include: (i) degradation of antibody immunoreactivity resulting from chemical manipulations required for labeling; (ii) lack of suitable radioisotopes and methods for stable attachment of the radiolabel; (iii) in-vivo instability of the radioimmunoconjugates; (iv) excessive accumulation of activity in non-target locations; and (v) lack of radioimmunoconjugate accessibility to cells internal to a tumor mass. A careful choice of the radionuclide(s) best suited to treat the tumor under consideration is one of the most important requirements for successful radioimmunotherapy.

Criteria for the selection of the optimum radionuclide(s) depend on the physical properties of the radionuclide, its production and chemistry, and its

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biological behavior (Mausner, 1993). Physical properties that are important to consider include the radionuclide half-life, the type, energy and branching ratio of particulate radiation and the gamma-ray energies and abundances. The physical half-life should be matched with the *in vivo* pharmacokinetics of the molecule under consideration. Time-dose fractionation is also an important criterion (Rao, 1993). For equal radioactivity concentrations in the target, radionuclides with long half lives will produce a lower absorbed dose rate than those with short half lives. The type of particulate emission also must be considered. Although Auger and low-energy conversion electrons can be potentially lethal (Kassis, 1982; Adelstein, 1987; Feinendegen, 1975), this effect can best be realized with intranuclear localization of the radionuclide, which does not generally occur with radiolabeled MAbs. Beta particles are less densely ionizing and have a range longer than α 's or the Auger and conversion electrons so that the distribution requirements are less restrictive for RIT of bulky disease or for macrometastases. The gamma-ray energies and abundances are also important physical properties, because the presence of gamma rays allows the possibility of external imaging.

As far as chemical variables are concerned, factors to be considered are the radionuclide specific activity, metal-ion contamination, the number of atoms that can be attached per MAb molecule without loss of immunological activity, and the stability of the radionuclide-protein attachment. The specific activity depends primarily on the method of production. Some of these issues have been reviewed for therapeutic radionuclides (Volkert, 1991). The convenience, efficiency, and gentleness of various radiolabeling procedures as well as the stability of the radionuclide attachment to the MAb reagent are very important factors and have received considerable attention (Srivastava, 1991). Finally, another practical aspect to be considered is that of radionuclide production - the routine availability at reasonable cost, and in sufficient quantities that will be required for widespread therapy. The above physical and chemical factors have to be assessed in light of available biological information on the distribution of the nuclide and/or the molecular conjugate. There is substantial variation in antibody uptake, macro- and micro-distribution, kinetics and processing (metabolism/catabolism) depending on the particular antibody, antibody dose, the variability of antigenic expression in the tumor, its size and stage, etc. Limitations due to normal tissue radiotoxicity are not entirely the function of radionuclide emissions but are largely governed by the pharmacokinetics of the labeled immunoconjugate. It is generally believed that one-half to three days is

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usually required to reach maximum MAb tumor uptake (DeNardo, 1988; Larson, 1988; DeNardo, 1993). Optimum contrast with whole MAbs may take longer. Despite the presence of numerous antigen sites on cancer cells, evidence from various studies indicates a generally non-uniform MAb distribution (Wessels, 1985; Buchegger, 1988). This considerably reduces the attractiveness of very short-ranged electron emitters for radioimmunotherapy. The longer range of beta particles allows uniform tumor irradiation despite a marked heterogeneity of distribution of radioactivity within the tumor. Ionizing radiation with a range of one to several millimeters in tissue seems to be most desirable. Beta emitters also offer a much wider choice of candidates with a selection of particle ranges and chemical properties. The use of radionuclides with a gamma emitting component would allow diagnostic low-dose experiments to determine biodistribution before deciding to administer a therapeutic dose of the exact same preparation. In the clinical situation, it may indeed be a requirement to image each patient prior to therapy in order to assess antigenic status and to calculate radiation dose to tumor and sensitive tissue from the observed biodistribution. The energy of gamma emissions should be below 300 keV, and the γ abundance not too high but just sufficient for in vivo visualization.

Consideration of the above physical, chemical, biological, and practical factors has led us to conclude that ^{67}Cu and ^{153}Sm are two of the most attractive candidates for radioimmunotherapy of cancer. Both are intermediate-range beta emitters with suitable half lives and desirable imaging photons. Their properties are summarized in Table 1. Copper-67 has a 2.6 d half life, emits four beta particles with a weighted average energy of 141 keV (maximum 575 keV) and has an imageable gamma ray of 184.6 keV (49% abundance). The quantification of in-vivo radioactivity distribution in patients can be improved further using the positron emitter ^{64}Cu ($t_{1/2} = 12.7$ h) for imaging with PET. Copper-67 and ^{64}Cu are produced by our group at the Brookhaven Linac Isotope Producer (BLIP) using the $^{68}\text{Zn}(p,2p)$ and $^{66}\text{Zn}(p,2pn)$ reactions. Alternately, we have used the $^{67}\text{Zn}(n,p)^{67}\text{Cu}$ reaction at the High Flux Beam Reactor (HFBR) at BNL.

Samarium-153 also has attractive properties for RIT. Large amounts of ^{153}Sm can be easily produced in a nuclear reactor such as the HFBR using the $^{152}\text{Sm}(n,\gamma)^{153}\text{Sm}$ reaction. Although this route normally leads to a low specific activity product, the thermal neutron absorption cross section of ^{152}Sm is quite

Table 1. Physical and Nuclear Characteristics of Copper-67 and Samarium-153

Radionuclide	Copper-67	Samarium-153
Half life	2.58d	1.95d
Decay mode	β^-	β^-
Gamma emission	yes	yes
Energy (keV)	92,184	103
Abundance (%)	23,40	28
β^- particle emission	yes	yes
Energy (max), keV	577,484,395	640,710,810
Abundance (%)	20,23,56	30, 49, 20
Auger electron emission	negligible	some
Number of emissions	1	6
Energy range, keV	7.5	1.3-33.7
Total abundance per decay (%)	3	161

large ($\sigma=208$ b) and good specific activity is achievable even at a reactor of modest flux. The HFBR can theoretically provide specific activity of up to 11 mCi/ μ g. Although the therapeutic potential of this isotope is being utilized in the radiopharmaceutical ^{153}Sm -ethylenediaminetetramethylenephosphonic acid (EDTMP) for palliation of pain from metastatic bone cancer (Turner, 1989; Collins, 1993), little has been reported on the use of ^{153}Sm as an antibody label (Boniface, 1989; Izard, 1993).

Results thus far on the biodistribution of radioimmunoconjugates, especially in man, are not optimum for imaging, much less for RIT. For radiotherapy, a much higher absolute tumor uptake as well as better tumor to normal tissue ratios are required. A number of factors contribute to the specificity of biodistribution of radioimmunoconjugates (Srivastava, 1988, 1991).

One of the crucial issues is the in-vivo stability of the radiometal-MAb attachment, which is largely governed by the coordination chemistry of the radiometal and thermodynamic and kinetic stabilities of the radiometal chelate system. In parallel with other approaches to improve radioimmunoconjugate localization it is, therefore, necessary to develop better chelating systems for the radiometals of interest.

RADIONUCLIDE PRODUCTION

Our group published the first definitive work on the production of ^{67}Cu in 1986 (Mirzadeh, 1986). We produce ^{67}Cu at the BLIP by $^{68}\text{Zn}(p,2p)$ and $^{70}\text{Zn}(p,\alpha)$ reactions with 193 MeV protons and at the HFBR using the $^{67}\text{Zn}(n,p)$ reaction. The effective cross sections of $^{64,67}\text{Zn}(p,2pxn)$ $^{64,67}\text{Cu}$ reactions were measured at 193 MeV proton energy and compared to predicted values obtained with the semiempirical method of Silberberg and Tsao (Silberberg, 1973). A remote method was devised to separate the no-carrier-added copper from the zinc targets (DasGupta, 1991). The target is dissolved in concentrated HCl. Major species in the target solution are ^{65}Zn , $^{64,67}\text{Cu}$, ^{67}Ga , along with traces of ^{51}Cr , $^{56,57,58}\text{Co}$, and ^{54}Mn . Chlorine gas is bubbled through the solution to ensure that the dissolved copper remains as Cu (II). This solution is evaporated to dryness, redissolved in 0.5 N HCl, and treated with 0.01% dithizone in CCl_4 . On average, 96% of the Cu extracts into the organic phase with 0.1% Zn and 2% Ga contamination. After washing the dithizone layer with 0.02 N HCl, the Cu is back-extracted into 7.2 N HCl (with a few drops of H_2O_2). Residual ^{67}Ga is removed by extraction with isopropyl ether saturated with 7.2 N HCl. The aqueous phase is warmed to remove dissolved ether and is then passed through a Dowex-1 anion exchange column equilibrated with 6 N HCl. Manganese-54 is not retained by the column under these conditions. Last traces of Cr and Co isotopes are removed from the column with 4.5 N HCl and the Cu is eluted with 1 N HCl. The overall ^{67}Cu recovery is 90% under remote conditions with an average specific activity of $2 \mu\text{Ci}/\mu\text{g}$. The Zn separation factor is about 1×10^5 . The chemical procedure requires less than 6 hours.

The production of ^{67}Cu was recently scaled up to therapeutic levels of 400 mCi per batch. The difficult problems of maintaining high specific activity, and very low metal impurity levels have largely been solved for therapy batches. To achieve this performance, a new radiochemical procedure was developed to separate Cu from the Zn targets based on a one-step gross Cu/Zn separation

using a Chelex-100 column. This column method is faster and easier (less manipulations and fewer reagents) compared to the solvent extraction procedure previously employed and also improves specific activity and reliability. The Chelex method is also more easily scaled up to the large Zn target mass required to reach ^{67}Cu production levels needed for RIT. The method involves evaporating the Zn target solution (conc. HCl) to dryness and redissolving in 0.5 M acetate buffer. After adjusting the pH to 3 with NaOH the solution is passed through the Chelex 100 column by gravity. After washing the column with 100 ml of 0.001 N HCl, and 100 ml of 0.01 N HCl, the ^{67}Cu is eluted in 30 ml of 3 N HCl. To remove traces of ^{65}Zn , ^{56}Co , ^{67}Ga , and ^{54}Mn , a clean-up anion exchange column is used. This column is loaded at 7.2 N HCl, then washed with 4.5 N HCl. The Cu fraction is then eluted at 3 N HCl. Assay for Cu is performed by a spectrophotometric method using the complex salicylaldehyde. Traces of stable Fe in the product are measured by a spectrophotometric method based on the Fe complex with diphenylphenanthroline. A polarographic technique is used to assay for Pb, Zn, Cd and Bi.

Samarium-153 was produced by the thermal neutron reaction ^{152}Sm (n,γ) ^{153}Sm ($\sigma_n = 208$ b) as mentioned above. There also exists a significant fast neutron cross section for this reaction ($\sigma_n = 3000$ b). Microgram samples of enriched $^{152}\text{SmO}_3$ (98.29% enrichment) have been irradiated in the "core-edge" position of the HFBR at BNL. The thermal neutron flux at this position is 4.2×10^{14} n/cm²-s and the fast neutron flux is 4.5×10^{13} n/cm²-s for $E_n > 1$ MeV. Two-day irradiations produced a specific activity of 7.05 mCi/ μg at end-of-bombardment (EOB) in good agreement with theoretical calculation. After irradiations, the oxide targets were dissolved in 0.1 N HCl with no further purifications required, due to the low level of impurities.

LIGAND DEVELOPMENT

For chelating ligands, our initial approach (Mease, 1988; Srivastava, 1990) was to combine the rigidity of the ligand, which is important in cyclams and porphyrins, with the general utility of polyaminocarboxylates such as EDTA and DTPA (Figure 1). The starting point in these studies was the investigation of the semi-rigid chelate, cyclohexyl EDTA (CDTA). CDTA exists in the trans-diequatorial form and its conversion to the trans-diaxial form is greatly disfavored. CDTA can be viewed as having the ethylenediamine portion of EDTA incorporated into a cyclohexane ring. This ring locks the nitrogens into

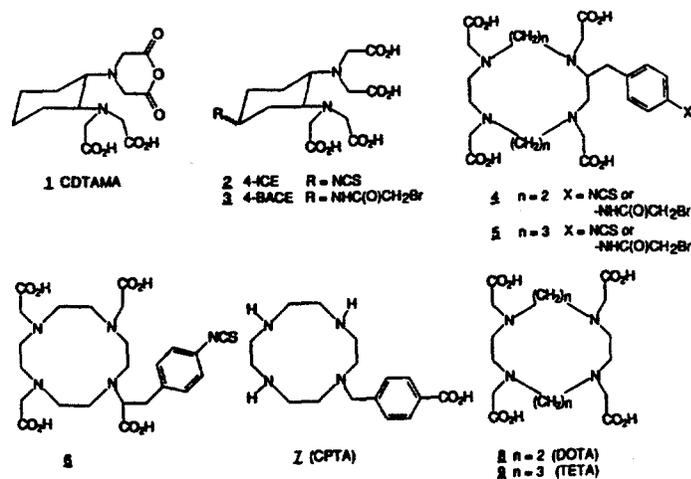


Figure 1. Ligands under study at BNL for preparing stable radiometal immunoconjugates

place, providing preorientation of a portion of the ligand for chelating the metal as well as some steric blockage of competing ligands. Extensive investigations (Mease, 1992a; Srivastava, 1994) have shown that a number of these rigid polyaminocarboxylates, especially 4-isothiocyanato cyclohexyl EDTA (4-ICE) produced very stable immunoconjugates with ¹¹¹In, ⁵⁷Co, and ⁴⁷Sc, metals that all have low ionic radii.

However, 4-ICE (2, Figure 1) did not produce stable copper immunoconjugates. These preparations all yielded high liver uptake. This failure confirmed the observation by others that copper requires a cyclic tetraaza structure (Meares, 1990). Compounds 4, 5, 6 and 7 (Figure 1) have been prepared by others and contain functionality which allows them to be conjugated to MAbs. Compounds 4, 5 and 7 have been used to produce stable copper conjugates. Because the one-step synthesis and in-situ functionalization of 7 is simpler than the multi-step synthesis of 4 and 5, we chose to investigate compound 7 (CPTA; Smith-Jones, 1991), as well as just the unfunctionalized parent structures of compounds 4 and 5 (compounds 8 and 9, DOTA and TETA respectively, Figure 1). The one-step synthesis of TETA by alkylation of commercial 1,4,8,11-tetraazacyclotetradecane with bromoacetic acid in strong

base required purification by ion-exchange chromatography. This synthesis produced an intractable sticky, hygroscopic solid. Instead, DOTA and TETA were each prepared by a simple high yield two-step method, which required no chromatography (Figure 2; Mease, 1992). Specifically, commercial 1,4,7,10-tetraazacyclododecane (10) and 1,4,8,11-tetraazacyclotetradecane (11) were cyanomethylated using formaldehyde and potassium cyanide in acid media to

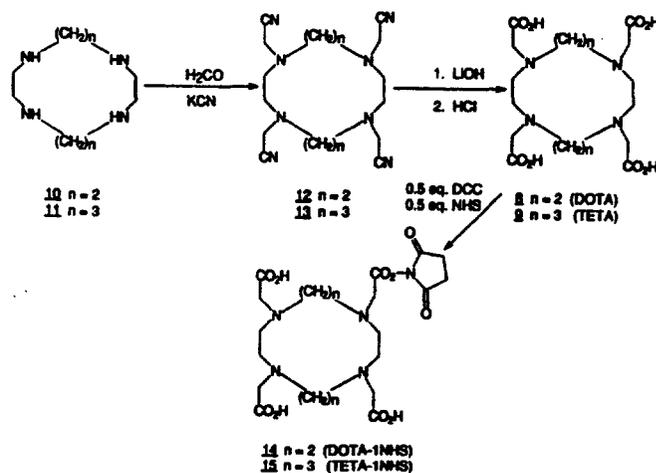


Figure 2. Synthesis of DOTA and TETA and their N-hydroxysuccinimide (NHS) esters

give tetranitriles 12 and 13, respectively. The product simply precipitated from the reaction upon the addition of base. Lithium hydroxide hydrolysis of 12 and 13 followed by acidification, evaporation to dryness, reconstitution in methanol, and precipitation with acetone gave DOTA (8) and TETA (9). N-hydroxysuccinimide esters 14 and 15 were prepared using a modification of the DTPA esterification procedure (Buckley, 1984). One molar equivalent of DOTA/TETA was reacted with 0.5 molar equivalents of dicyclohexylcarbodiimide and N-hydroxysuccinimide in DMSO overnight. After filtration of the resulting dicyclohexyl urea, the DMSO filtrate containing the activated ester was stored at 0°C until needed. These activated esters are stable at 0°C for several months. The major advantage of using ligands 14 and 15 again is that they can be prepared by a simple, high yield, three-step method which requires no chromatography.

LABELING AND IN-VIVO STUDIES

Whole IgG or F(ab')₂ fragments of anticolon carcinoma monoclonal antibody 17-1A (used as model systems) were conjugated with macrocycles DOTA, TETA or CPTA, 4-ICE, or with other bifunctional chelating agents using published procedures (Srivastava, 1991). The number of chelating groups bound to antibody (usually 1-3) was determined using the ⁵⁷Co TLC method (Meares, 1984). Molecular weight cut-off membrane filters (Centricon C-30) were used for purification of the conjugates. Radiolabeling was carried out by incubating the ⁶⁷Cu or ⁶⁴Cu chloride solution in 0.05 N HCl with the conjugates in 0.1 M sodium bicarbonate, pH 8.4 buffer, with 0.1 M acetate as the weakly chelating buffer system. Purification was carried out by HPLC using a Zorbax-GF250 sizing column and 0.1 M, pH 7.0 phosphate buffer as the eluent. EDTA (10 µl of a 0.05 M solution) was used to remove unbound and/or non-specifically bound copper. Labeling efficiencies were 80-90% for all conjugates except the CPTA conjugate which was only 10-20%. Immunoreactivity retention ranged between 60-90%, as determined from live cell-binding assays using SW-948 human colon carcinoma cells.

Serum incubation studies were performed to determine the relative stability of the various ligands for chelating copper. The labeled conjugates were incubated in mouse serum at 37°C for up to 5 d. Aliquots were removed and injected on to a Zorbax GF-250 HPLC column and eluted with 0.1 M phosphate buffer, pH 7.0, at a flow rate of 1 ml/min. Fractions were collected and counted for radioactivity using a gamma counter. The percent of injected activity that eluted with the antibody was determined. DOTA and TETA conjugates showed 90-95% stability after 96 h whereas CPTA and 4-ICE conjugates retained only 40% and 39% MAb-bound activity, respectively, after 24 hours. All conjugates retained 95-99% of the copper as MAb-bound activity when incubated with saline only, for up to 96 hours.

Biodistribution of ⁶⁷Cu- and ⁶⁴Cu-labeled conjugates of 17-1A (IgG and F(ab')₂) was determined in normal (Balb/c) mice as well as human colon carcinoma (SW 948 cells) xenografted nude mice. Representative data are summarized in Tables 2 and 3. The animals were sacrificed following the i.v. administration of the various immunoconjugates. In-vivo stability in normal mice (Table 2) was in the order DOTA ≈ CPTA ≥ TETA >> 4-ICE. CPTA conjugate, despite its low serum stability, showed biodistribution comparable to

Table 2. Biodistribution^a of ⁶⁷Cu Labeled Anticolon Carcinoma Monoclonal Antibody 17-1A in Normal Mice

Conjugate	Time(h)	Blood	Kidney	Liver	Whole body retention ^b
CuCl ₂ (control)	24	1.7	5.7	9.1	59
	96	0.8	4.5	6.1	38
4-ICE-IgG	24	2.7±0.4	9.2±0.7	17.9±0.4	80±3
	96	1.2±0.1	7.1±0.6	10.3±0.8	50±3
CPTA-IgG	24	16.6±0.2	5.2±0.4	8.5±0.7	98±2
	72	14.±0.63	5.6±0.3	8.5±0.9	87±3
DOTA-IgG	24	18.5±1.9	4.8±0.3	8.7±0.4	83±6
	96	12.9±0.5	4.2±0.2	7.0±0.9	68±3
TETA-IgG	24	15.4±0.3	5.4±0.2	7.7±0.4	88±4
	72	11.1±1.1	5.0±0.4	6.3±0.5	73±4

^a% Injected dose/g normalized for 25 g body weight; n=5.

^b% Injected dose remaining.

the DOTA and TETA conjugates. As expected, F(ab')₂ conjugates cleared faster from blood than whole IgG conjugates. DOTA conjugates yielded higher tumor uptakes (Table 3) than the TETA conjugates. The ⁶⁷Cu-IgG-DOTA conjugate yielded the highest tumor uptake at 96 h and thus the tumor to organ ratios were higher than with other conjugates. F(ab')₂ conjugates, as expected, cleared faster than the whole IgG conjugates. Results with ⁶⁴Cu for all the conjugates were generally similar to those with ⁶⁷Cu. Although further improvement is desirable, these biodistribution results are quite favorable for imaging and therapy with MAbs labeled with copper radionuclides.

Table 3. Biodistribution* of ⁶⁷Cu Labeled Anticolon Carcinoma Monoclonal Antibody 17-1A in Human Tumor (SW 948 cells) Xenografted Nude Mice

Conjugate	Time(h)	Tumor	Blood	Kidney	Liver	Whole body retention ^b
DOTA-IgG	24	11.2±1.3	15.5±1.2 (0.7)	4.7±0.3 (2.4)	9.4±0.9 (1.2)	112±7
	96	13.5±1.5	10.6±1.6 (1.3)	4.9±0.2 (3.0)	7.0±0.8 (2.1)	84±6
DOTA-F(ab') ₂	24	10.3±2.2	9.4±0.6 (1.1)	14.7±0.7 (0.7)	15.4±0.7 (0.7)	122±2
	96	5.2±0.4	1.3±0.2 (4.0)	7.5±0.6 (0.7)	7.5±0.6 (0.7)	57±3
TETA-IgG	24	10.3±0.7	16.8±1.0 (0.6)	4.9±0.3 (2.1)	8.6±0.7 (1.2)	103±4
	96	7.5±1.2	9.4±0.7 (0.8)	4.6±0.2 (1.6)	6.4±0.3 (1.2)	74±5
TETA-F(ab') ₂	24	9.3±2.4	12.8±0.6 (0.7)	8.8±0.3 (1.1)	9.6±0.4 (1.0)	99±4
	96	4.2±1.6	5.6±0.4 (0.7)	5.8±0.6 (0.7)	5.7±0.4 (0.7)	56±5

*% Injected dose/g normalized for 25 g body weight; n=5. Tumor to tissue ratios shown in parentheses.

^b% Injected dose remaining.

Labeling studies with samarium were carried out by incubating ¹⁵³Sm chloride solution in 0.05 - 0.1 M HCl, with antibody-chelator conjugate (1 to 3 average ligands per antibody) in 0.1 M NaHCO₃ buffer, pH 8.3, and 0.1 to 0.2 M acetate as co-buffer, for 2 to 18 hours, at ambient temperature. Incubation time depends on the total final reaction volume e.g., 2 h incubation was sufficient for 50 μL, but ~18 h were required for 200 μL. Unbound/non-specifically bound radioactivity was chased with 5 to 10 μl of 0.1 M EDTA, pH 7.0 (10 mM EDTA final concentration). The radiolabeled conjugates were

purified by size exclusion chromatography (DuPont Zorbax GF-250 column) using 0.1 M phosphate buffer, pH 7.0, as eluant, and online UV (280 nm) and radioactivity (NaI/Tl) detection systems. Fractions (6 drops) were collected and analyzed for their radioactivity content on a dose calibrator to determine labeling efficiencies.

In-vitro stability in serum of the various ^{153}Sm conjugates was determined using methods described above for the copper conjugates. Radiolabeled conjugates were also incubated with normal saline at 37°C , and analyzed in a similar fashion for comparison. The conjugate with 4-ICE showed no improvement in serum stability over the DTPADA conjugate. Semi-rigid CDTPA was somewhat better. The CTTHA conjugate, with even more available coordinating groups than DTPADA and CDTPA showed better stability than DTPADA, but apparently did not show any improvement over CDTPA. We have observed similar behavior with ^{89}Y labeled immunoconjugates. Only the rigid macrocycle DOTA-NHS, in which one of the carboxylate groups was used up for conjugation to the antibody offered a clear and significant improvement in serum stability. These results suggest that the ligand rigidity, and the number of available coordinating groups both are important factors that contribute to improved stability of rare-earth metal labeled antibody conjugates. More than eight coordinating groups seem to adversely affect serum stability, probably due to steric crowding.

HPLC purified monomeric labeled antibody fractions were pooled, and diluted with saline for animal injections. Both normal (Balb/c) as well as human tumor (SW 948 cells) xenografted nude mice were used. Five mice were used for each preparation per time point. Each mouse received $1\text{-}5\ \mu\text{Ci}/25\text{-}50\ \mu\text{g}$ antibody/ $200\ \mu\text{L}$ via tail vein. The animals were sacrificed after 24, 48, or 96 hours, and various organs were dissected and counted for their radioactivity content. Percent of the injected dose (normalized to 25 g) taken up into selected organs is listed in Table 4.

Semi-rigid 4-ICE, CDTAMA, and CDTPA conjugates were less stable in-vivo than the DTPADA conjugate. CTTHA-1NHS with more available coordinating groups than DTPADA, also showed less in-vivo stability than the DTPADA conjugate. The rigid DOTA-1NHS conjugate gave the best tumor uptake, and was also the most stable. In-vivo stability of the ^{153}Sm labeled conjugates in tumor mice thus showed the following trend: DOTA > DTPA >

Table 4. Biodistribution^a of ¹⁵³Sm Labeled Anticolon Carcinoma Monoclonal Antibody 17-1A IgG in Human Tumor (SW 948 cells) Xenografted Nude Mice

Conjugate	Time (h)	Tumor	Blood	Liver	Kidney	Whole body retention ^b
SmCl ₃ (control)	96	0.25±0.08	0.03±0.01	55.6±7.6	1.0±0.2	--
		---	(8.3)	(0.00)	(0.25)	
DTPA-DA	96	4.3±0.5	2.9±0.6	8.6±0.8	1.8±0.4	76±3
		---	(1.5)	(0.5)	(2.4)	
4-ICE	24	3.5±0.5	1.7±0.1	23.4±1.6	4.6±0.7	80±4
		---	(2.1)	(0.15)	(0.76)	
	96	1.7±0.2	0.04±0.01	17.4±2.0	2.6±0.2	74±2
		---	(43)	(0.10)	(0.65)	
CDTPA-NHS	24	1.9±0.6	1.4±0.2	31.3±7.6	2.5±0.5	90±3
		---	(1.36)	(0.06)	(0.76)	
	96	1.3±0.3	0.13±0.02	26.1±6.5	1.8±0.8	78±7
	---	(10)	(0.05)	(0.72)		
CTTHA-NHS	24	3.5±0.5	6.6±0.5	26.0±8.3	2.7±0.2	92±3
		--	(0.53)	(0.13)	(1.3)	
	96	4.1±0.4	2.6±0.2	25.9±2.3	1.9±0.2	80±5
	---	(1.58)	(0.16)	(2.16)		
DOTA-NHS	24	7.9±1.1	12.2±1.3	13.7±0.5	4.8±0.6	93±5
		---	(0.65)	(0.58)	(1.65)	
	96	9.6±1.0	4.2±0.5	15.1±1.1	3.6±0.2	81±3
	---	(2.3)	(0.64)	(2.67)		

^a% Injected dose/g, normalized for 25g body weight; n=5. Tumor to tissue ratios shown in parentheses.

^b% Injected dose remaining.

CTTHA > 4-ICE ~ CDTPA. As with the in-vitro studies, the in-vivo results also suggest that both the rigidity and the number of available coordinating groups, together, are important for improved stability of rare-earth metal labeled immunoconjugates. However, further improvements in ¹⁵³Sm distributions is required, before this isotope can be used effectively for cancer immunotherapy.

Suitable ligand structures, to achieve such improvements, are presently under development (Srivastava, 1994, unpublished work).

CONCLUSION

Copper-67 and samarium-153 are two of the most attractive radionuclides for radioimmunotherapy. Two to three-day half lives, intermediate-energy beta emissions, imageable photons, and suitable chemical characteristics make these radionuclides ideal for this application. Production of high specific activity ^{67}Cu and ^{153}Sm , as well as the development of stable chelates for conjugating these radiometals to tumor-associated monoclonal antibodies have been addressed in this investigation. Based on preliminary in-vitro and in-vivo studies (in nude mice xenografted with human colon carcinoma), both ^{67}Cu and ^{153}Sm appear very promising for tumor radioimmunotherapy and warrant continued investigation.

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