Copper-64-Labeled Antibodies for PET Imaging

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In the imaging of tumors using radiolabeled monoclonal antibodies, the use of PET gives increased sensitivity over conventional gamma camera imaging techniques. Copper-64, a positron-emitting radionuclide, has been labeled to 1A3, an anticolorectal carcinoma monoclonal antibody, and its fragments 1A3-F(ab')$_2$ utilizing the bifunctional chelate Br-benzyl-TETA. The ^{64}Cu-labeled intact 1A3 and 1A3-F(ab')$_2$ have been evaluated as potential imaging agents for PET. Biodistribution studies of ^{64}Cu-benzyl-TETA-1A3 and ^{64}Cu-benzyl-TETA-1A3-F(ab')$_2$ in tumor-bearing hamsters were compared with those of ^{111}In-BrΦHBED-1A3, ^{111}In-BrΦHBED-1A3-F(ab')$_2$ and ^{125}I-labeled intact 1A3 and 1A3-F(ab')$_2$. Tumor uptake of ^{64}Cu-labeled intact 1A3 and fragments in the hamster model was superior to both ^{111}In- and ^{125}I-labeled intact 1A3 and fragments. Human dosimetry data for ^{64}Cu- and ^{123}I-labeled 1A3 and 1A3-F(ab')$_2$ were calculated from biodistribution data in rats. High kidney uptake of ^{64}Cu-benzyl-TETA-1A3-F(ab')$_2$ precludes clinical study at this time; however, the data shows that ^{64}Cu-benzyl-TETA-1A3 would be suitable for positron tomography imaging of colorectal cancer in patients.

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Antibodies labeled with positron-emitting isotopes have advantages over traditionally labeled antibodies due to the improved sensitivity of positron emission tomography (PET). This is particularly true in light of recent advances in PET imaging techniques, including whole-body imaging (*1*) and three-dimensional imaging (*2*) which increase the sensitivity of PET. In addition, these advances allow injection of less radiolabeled tracer for the acquisition of high quality images. The long-lived PET radionuclides available for antibody labeling include zirconium-89 ($t_{1/2}$ = 78.4 hr), bromine-76 ($t_{1/2}$ = 16.1 hr), iodine-124 ($t_{1/2}$ = 4.15 d) and copper-64 ($t_{1/2}$ = 12.7 hr) (*3*). We have chosen to investigate ^{64}Cu for several reasons including the availability of ^{64}Cu from the University of Missouri Research Reactor (MURR). The fact that ^{64}Cu is reactor produced makes it potentially more readily available than radionuclides that can only be produced by very large cyclotrons. Copper-64-labeled antibodies used in PET imaging of tumors could be utilized to determine individual radiation dosimetry prior to therapy with ^{67}Cu-labeled antibodies (*4*), as has been accomplished by Eary and co-workers (*5*) using low-dose ^{131}I-labeled antibodies prior to therapy with the iodinated antibody. Apelgot and co-workers have shown ^{64}Cu to be equally effective in DNA damage as ^{67}Cu (*6*), therefore ^{64}Cu-labeled antibodies in high doses may also be useful in radioimmunotherapy.

In order to use ^{64}Cu-labeled monoclonal antibodies (Mabs) for imaging, the copper must be chelated to the Mab through a bifunctional chelate. Copper binds weakly to native protein sites, therefore a bifunctional chelate which forms strong bonds with copper and is also covalently linked to the antibody must be utilized. Chelates of Cu(II) with ligands such as EDTA and DTPA are not stable in serum, possibly due to the lability of Cu(II) to ligand exchange (*7*). A macrocyclic bifunctional chelating agent, 6-bromoacetamidobenzyl-1,4,8,11-tetraazacyclotetradecane-N,N',N",N"'-tetraacetic acid (Br-benzyl-TETA or BAT) has been developed which forms complexes with Cu(II) that are stable in serum for several days (*8,9*). Copper-67-labeled benzyl-TETA-Lym-1, an anti-lymphoma Mab, has been investigated in tumor-bearing nude mice where the percent injected dose per gram (%ID/g) of tumor tissue reached a maximum of 14.7% at 3 days (*10*). Subsequently, this ^{67}Cu-labeled Mab was administered in humans as part of a study on radioimmunotherapy agents for lymphoma (*4*).

We have now conjugated 1A3, an anticolorectal carcinoma Mab (*11*), with the bifunctional chelate Br-benzyl-TETA and have radiolabeled the Mab conjugate with both ^{67}Cu and ^{64}Cu. Mab 1A3 reacts with antigen(s) extracted in the methanol phase of a Folch extract of human colon cancer cells (*12*). It does not bind to carcinoembryonic antigen (CEA), and extensive histological analysis suggested that the recognized antigen was strongly expressed in primary colon cancer tissue and weakly, or not at all, in normal colon tissue (*11,13*). We have evaluated copper-labeled intact 1A3 and 1A3-F(ab')$_2$ fragments using the model of Golden Syrian hamsters carrying GW39 human colon tumors. Biodistribution of the copper-labeled benzyl-TETA-1A3 and copper-labeled benzyl-TETA-1A3-

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$F(ab')_2$ in tumor-bearing hamsters were compared to that of ^{111}In-labeled 1A3 using the bifunctional chelate *N,N'-bis*(2-hydroxybenzyl)-1-(4-bromoacetamidobenzyl)-1,2-ethylenediamine-*N,N'*-diaceticacid(BrΦHBED), ^{111}In-BrΦHBED-1A3-$F(ab')_2$, as well as ^{125}I-1A3 and ^{125}I-1A3-$F(ab')_2$. Human dosimetry data for ^{64}Cu-labeled 1A3 and ^{64}Cu-labeled 1A3-$F(ab')_2$ were calculated from biodistribution data in adult, female, Sprague-Dawley rats.

EXPERIMENTAL PROTOCOL

Materials and Methods

2-Iminothiolane and Sephadex G-25/50 were purchased from Sigma Chemical Co. Triethanolamine hydrochloride was purchased from Aldrich. Ultrapure ammonium dihydrogen phosphate and 99.9999% pure HCl (minimum 35% by assay) were purchased from Johnson Matthey and ammonium citrate (puriss) was purchased from Fluka. All solutions were made using distilled deionized water (Milli-Q®; >18 MΩ resistivity). Br-benzyl-TETA was prepared from nitro-benzyl-TETA as described by McCall et al. (*7*). The synthesis of p-nitrobenzyl-TETA was performed according to the method of Moi et al. (*8*). Sephadex G-25/50 was equilibrated in 0.1 *M* ammonium phosphate, pH 8, or 0.1 *M* ammonium citrate, pH 5.5, as described elsewhere (*14*). BrΦHBED was prepared as described by Mathias et al. (*15*). Mabs 1A3 (intact) and BB5 were purified from serum-free medium by Invitron (St. Louis, MO) using proprietary methods. The 1A3-$F(ab')_2$ fragments were also generated from the intact 1A3 by Invitron. All Mabs were judged to be at least 85% pure by analysis on gradient SDS-PAGE. Adult female Sprague-Dawley rats were purchased from Sasco (Omaha, NE), and Golden Syrian hamsters were purchased from Charles River (Wilmington, MA). All animal experiments were performed in compliance with guidelines specified by the Washington University Animal Studies Committee and the Jewish Hospital Animal Care Committee. Iodine-125-sodium iodide was purchased from E. I. DuPont de Nemours (Boston, MA) or Amersham (~16.4 mCi/mg). Indium-111-indium chloride (4.19×10^5 Ci/g) in sodium chloride (pH 1-2) was provided by Mallinckrodt, Inc.

Preparation and Purification of ^{64}Cu

Copper-64 was produced and purified at the University of Missouri-Columbia Research Reactor. The production of ^{64}Cu was via the fast neutron reaction, ^{64}Zn(n, p)^{64}Cu using naturally abundant (48%) ^{64}Zn. The zinc metal target (400–500 mg, 99.999% pure) was sealed in a quartz vial and irradiated (flux trap position) for 150 hr in a cadmium-lined can. Thermal neutron reactions (n, γ) producing ^{65}Zn and ^{69m}Zn were minimized since cadmium reduced the thermal neutron flux on the zinc target.

Following irradiation the ^{64}Cu was chemically separated from the zinc metal. The separation was done in a glove box with column chromatography in approximately 2 hr. The zinc metal was removed from the quartz vial, dissolved in 4–5 ml of concentrated HCl and evaporated to dryness. The zinc was then redissolved in 3 ml of 1 *M* acetic acid (pH 2.7), and applied to a Chelex 100 column (200–400 mesh, 6 ml bed volume) that had been preequilibrated with the 1 *M* acetic acid (pH 2.7). An additional 30 ml of 1 *M* acetic acid was rinsed through the Chelex column, which removed more than 95% of the zinc radionuclides. The ^{64}Cu retained on the column was rinsed with 5 ml of deionized water and eluted with 10 ml of 1 *M* HCl. This fraction was applied to an anion exchange column (AG1-X8, 100–200 mesh, 6 ml bed volume) that was pre-equilibrated with 1 *M* HCl. Any remaining zinc impurities were retained on the column while the ^{64}Cu was recovered in the eluate. The measured specific activity (graphite furnace atomic absorption) of the purified ^{64}Cu ranged from 72,000 to 240,000 Ci/g (end of irradiation).

Conjugation of Br-Benzyl-TETA to 1A3, BB5 and 1A3-$F(ab')_2$

A solution of 1A3, BB5 or 1A3-$F(ab')_2$ was prepared for conjugation using centrifuged Sephadex G-25/50 3 ml spin columns (*12*) equilibrated with 0.1 *M* ammonium phosphate, pH 8. To the buffered antibody, excess Br-benzyl-TETA in aqueous solution and freshly prepared 2-iminothiolane (2IT) in 50 m*M* triethanolamine hydrochloride were added in that order. Molar ratios of Br-benzyl-TETA:Ab were 20:1 and molar ratios of 2IT:Ab used were 10:1 for intact 1A3 and 1A3-$F(ab')_2$. The solution was incubated at 37° C for 30 min. Benzyl-TETA-Ab was isolated from the free ligand and 2IT using a Sephadex G-25/50 spin column (*12*) equilibrated in 0.1 *M* ammonium citrate, pH 5.5. The conjugated Ab was then stored at −80° C until it was used.

Radiolabeling of Benzyl-TEAT-Ab with ^{64}Cu and ^{67}Cu

Radiolabeled antibody was prepared by evaporating the ^{64}Cu or ^{67}Cu (in 1 N HCl) to dryness and reconstituting in 0.250 ml 0.1 N ultrapure HCl. Copper-64 or ^{67}Cu-acetate was prepared by mixing an aliquot of the ^{64}Cu- or ^{67}Cu-chloride solution with ammonium acetate (0.1 *M*, pH 6, final pH = 5.5) that was added to benzyl-TETA-Mab in ammonium citrate (0.1 *M*, pH 5.5). The mixture was incubated at room temperature for 15 min and purified on a 3-ml Sephadex G-25/50 spin column (*12*) equilibrated in 0.1 *M* ammonium citrate, pH 5.5.

Isotopic Dilution Method

The isotopic dilution method was used to measure the number of chelates (benzyl-TETA) attached to intact 1A3 and 1A3-$F(ab')_2$. The intact 1A3 or 1A3-$F(ab')_2$ was conjugated with Br-benzyl-TETA as described previously. Copper-64-acetate was added to 7–10 solutions containing amounts ranging from 0 to 0.1 μmol of nonradioactive $CuCl_2$. The ^{64}Cu-acetate, of differing specific activities, was then added to the benzyl-TETA-1A3 (1.0 mg/sample) or benzyl-TETA-1A3-$F(ab')_2$ (0.8 mg/sample) and labeled as previously described. The percent labeling efficiency was plotted as a function of total micromoles of copper added to the reaction. From the plot, the point of 50% reduction of the original antibody labeling efficiency using no-carrier-added copper was determined. This point approximates the number of micromoles of chelate attached to the antibody.

Radiolabeling of BrΦHBED-1A3 with ^{111}In

Indium-111-citrate was prepared by mixing ^{111}In-chloride in sodium citrate (0.1 *M*, pH 8). The ^{111}In-citrate was then added to the purified BrΦHBED-1A3 (*15*) and incubated for 1 hr at room temperature. The labeling purification was carried out using Sephadex G-50/50 spin columns equilibrated in 0.01 *M* sodium citrate, pH 8 (*15*).

Iodination

Iodinated 1A3 was prepared by the iodogen method (*16*). Iodine-125-1A3 was separated from unbound ^{125}I by use of

Sephadex G-25/50 spin columns (*12*). The ^{125}I-1A3 specific activity was 1–3 μCi/μg.

FPLC of ^{64}Cu-Benzyl-TETA-Antibodies

Fast protein liquid chromatography (FPLC) was performed with a Pharmacia/LKB chromatograph using a Superose 12 size-exclusion column eluted at 0.4 ml/min with 0.1 *M* $NaHCO_3$/0.15 *M* NaCl, pH 7.2. The eluate was monitored for UV absorption at 280 nm and 0.5 ml fractions were collected to determine the amount of radioactivity per fraction. Solutions of ^{64}Cu-benzyl-TETA-1A3 and ^{64}Cu-benzyl-TETA-1A3-F(ab')$_2$ were chromatographed prior to injection (25 μl of 1–2 μg/μl antibody).

Immunoreactivity Assay

The immunoreactivity (IR) of each radiolabeled antibody preparation was determined prior to in vivo use in a direct binding assay using GW39 human colon cancer cell suspensions as targets. Conditions of antigen excess were used in this assay (*17*). IR values were determined using regression analysis and linear extrapolation of the data to binding at infinite antigen excess.

Animal Model

The animal biodistribution experiments were carried out in 6-wk-old immunocompetent male Golden Syrian hamsters implanted with GW39 human colon carcinoma in the musculature of their right thigh (*18*). Tumors were harvested, minced, washed and stored in liquid nitrogen. Upon thawing, cell suspensions were routinely ≥90% viable. Tumor cell suspensions (50%, v/v; 0.5 ml ca. 2.5×10^8 cells) were injected in the right thigh of hamsters and allowed to grow for 2 days before radiolabeled antibody was injected. All labeled Mabs were injected intracardially. In one set of experiments, hamsters were co-injected with 35–50 μg of ^{67}Cu-benzyl-TETA-1A3 and ^{125}I-1A3, and in another experiment hamsters were co-injected with 25 μg of ^{111}In-BrΦHBED-1A3 and 25 μg ^{125}I-1A3. Copper-64-benzyl-TETA-1A3 and ^{64}Cu-benzyl-TETA-1A3-F(ab')$_2$ were each administered singly in other hamster experiments. The hamsters were killed by exsanguination at various times up to 120 hr postinjection of radiolabeled antibodies. The entire tumor, bladder, heart stomach, kidneys, spleen and thyroid were removed, as well as samples of blood, bone, skin, muscle, liver, lung and intestine. All organs were rinsed in PBS, blotted, weighed and then counted in an automated gamma scintillation NaI(T1) well-type counter.

Dosimetry

Adult Sprague-Dawley rats with an average weight of 150 g were used in dosimetry studies of ^{64}Cu-benzyl-TETA-1A3 intact and F(ab')$_2$, and ^{123}I-1A3 intact and F(ab')$_2$. The amount of antibody injected ranged from 25 to 50 μg/rat. For each compound, groups of four or five rats were killed at 1, 3, 6, 12, 24 and 36 hr postinjection. Samples of blood, liver, kidney, spleen, red marrow, stomach, small intestine, upper large intestine and lower large intestine were removed, weighed and counted. The %ID/g and %ID/organ were calculated for these organs. The assumption was made that the rat biodistribution which determined %ID/organ at various time points postinjection is the same as the human biodistribution. Iodine-125-1A3 and ^{125}I-1A3-F(ab')$_2$ antibodies were injected to determine the rat biodistribution for calculations of ^{123}I-labeled 1A3 and 1A3-F(ab')$_2$ dosimetry based on the assumption that the biodistributions were the same. The organ values were decay-corrected using the appropriate decay constant and converted into μCi/organ/mCi injected. The μCi/organ values were decay-corrected and plotted versus the time of death in hours. For each organ, the number of μCi-h/organ/mCi were determined by measuring the area under the curves. The total body μCi-h was determined by the formula $1000 \cdot T_{1/2}(hr) \cdot 1.44$ ($T_{1/2}$ refers to physical half-life) (*19*). In the dose calculation for the total body, it is assumed there is no excretion. The total body absorbed radiation dose is therefore a conservative estimate, since some excretion of activity occurs within the 36-hr time interval of the study. The μCi-h/organ values were totaled and subtracted from the total body μCi-h. The remaining μCi-h quantity was then attributed to uniformly distributed activity in "other" tissue. The absorbed fraction (S values), rads/μCi-h, were obtained from the MIRD tables for ^{64}Cu and ^{123}I (*19*). The absorbed dose was calculated by multiplying the organ uptake in μCi-h/mCi values by the appropriate S value (rads/μCi-h), with the resulting organ dose in rads/mCi administered.

RESULTS

The labeling efficiency of ^{64}Cu to intact 1A3 and 1A3 fragments ranged from 60% to 75% using spin column purification techniques. The isotope dilution method using ^{64}Cu and cold $CuCl_2$ indicated 1.8 chelates were attached to intact 1A3 and 1.4 chelates were attached to 1A3-F(ab')$_2$.

The immunoreactivity (IR) values for ^{64}Cu-benzyl-TETA-1A3 ranged from 85% to 95%, whereas the IR value for the ^{64}Cu-labeled fragments was 66%. Copper-labeled BB5, used as a negative control antibody, had an IR of <20%. IR values for ^{111}In- or ^{125}I-labeled intact 1A3 and 1A3-F(ab')$_2$ were routinely >85%.

The antibodies 1A3 and 1A3-(Fab')$_2$ radiolabeled with ^{64}Cu and ^{67}Cu (conjugated with benzyl-TETA), ^{111}In (BrΦHBED conjugated) and ^{125}I were evaluated and compared in the hamster tumor model. In all studies, tumors were implanted 2 days before injection of the radiolabeled antibodies. Biodistribution studies were carried out at time points to 120 hr postinjection for the intact 1A3 and out to 36 hr for 1A3-F(ab')$_2$. The average percent injected dose bound per gram of tissue and tumor-to-nontumor ratios were calculated for each experiment. A plot of tumor uptake versus time for ^{67}Cu-benzyl-TETA-1A3, ^{64}Cu-benzyl-TETA-1A3 and ^{64}Cu-benzyl-TETA-1A3-F(ab')$_2$ is shown in Figure 1. The results demonstrated that by 24 hr after Mab injection maximal tumor binding had been achieved for the Cu-labeled antibodies in this model system. Hamster biodistribution and tumor-to-nontumor ratios comparing radiolabeled intact 1A3 uptake at 24 hr from representative experiments are depicted in Figures 2 and 3. At the 24-hr time point, tumor uptake of ^{64}Cu-benzyl-TETA-1A3 (14.4 ± 4.80) was significantly better than ^{111}In-BrΦHBED-1A3 (8.6 ± 2.9 %ID/g tumor) ($p < 0.0004$) and ^{125}I-1A3 (10.4 ± 4.4 %ID/g tumor) ($p < 0.03$). A small, but significant difference ($p < 0.03$) was found in liver uptake when ^{64}Cu-benzyl-TETA-1A3 was compared to ^{111}In-BrΦHBED-1A3 (1.5 ± 0.1 versus 1.8 ± 0.6 %ID/g liver, respectively), while no significant difference was found in liver uptake when ^{64}Cu-benzyl-TETA-1A3 and

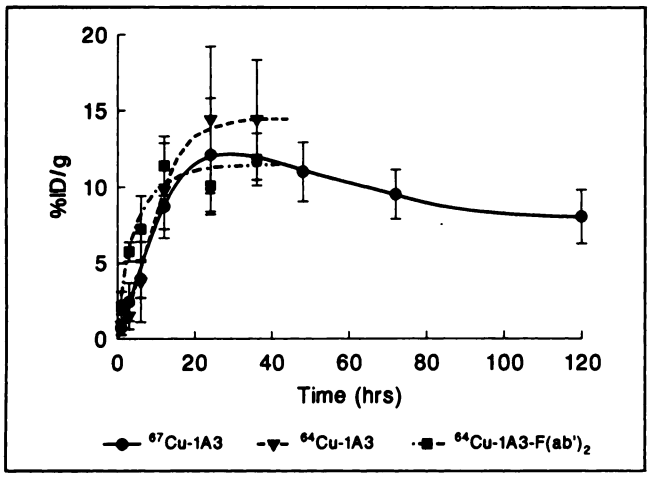

FIGURE 1. Tumor uptake (%ID/g) versus time for ^{67}Cu-benzyl-TETA-1A3, ^{64}Cu-benzyl-TETA-1A3 and ^{64}Cu-benzyl-TETA-1A3-F(ab')$_2$.

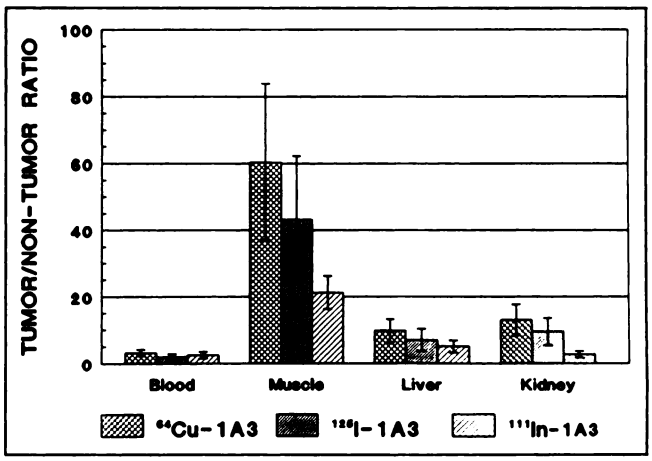

FIGURE 3. Comparison of 24 hr postinjection tumor-to-non-tumor ratios of selected tissues for ^{64}Cu-benzyl-TETA-1A3, ^{125}I-1A3 and ^{111}In-BrΦHBED-1A3.

^{125}I-1A3 were compared. The tumor-to-muscle ratio was significantly higher for ^{64}Cu-benzyl-TETA-1A3 (60.4 ± 23.5) than either ^{111}In-BrΦHBED-1A3 (21.2 ± 4.9) ($p < 0.002$) or ^{125}I-1A3 (43.2 ± 19.2) ($p < 0.04$). Tumor-to-blood ratios for ^{64}Cu-benzyl-TETA-1A3 (3.21 ± 0.94) were also higher than the values for ^{125}I-1A3 (1.87 ± 0.87) and ^{111}InBrΦHBED-1A3 (2.55 ± 0.98) at the 24-hr time point. Copper-64-benzyl-TETA-1A3 tumor-to-tissue ratios for 11 other harvested tissues were as good or better than those of ^{125}I-1A3 or ^{111}In-Br HBED-1A3.

Biodistribution data in hamsters and tumor-to-nontumor ratios for muscle, blood, kidney and liver comparing ^{64}Cu-, ^{111}In- and ^{125}I-1A3-F(ab')$_2$ are shown in Figures 4 and 5. Although ^{64}Cu-benzyl-TETA-1A3-F(ab')$_2$ was only 66% immunoreactive, the tumor uptake at 24 hr in hamsters was more than three times that for ^{111}In-Br HBED-1A3-F(ab')$_2$ (10.1 ± 1.89 versus 2.39 ± 0.76 %ID/g) and more than twice that of ^{125}I-F(ab')$_2$ (4.26 ± 2.01 %ID/g). The tumor uptake of ^{64}Cu-benzyl-TETA-1A3-F(ab')$_2$ reached a maximum at 12 hr postinjection (11.4 ± 1.95 %ID/g), and this level was maintained through the 36-hr time point (Fig. 1). The tumor-to-nontumor ratios for the clearance organs were optimum at 24 hr. When ^{64}Cu-benzyl-TETA-1A3-F(ab')$_2$ tumor-to-nontumor ratios were compared to ^{111}In-BrΦHBED-1A3-F(ab')$_2$, significant improvements were seen with the copper-labeled Mab for blood ($p < 0.01$) and 11 other tissues, except in the case of the tumor-to-kidney ratios which were similar. Tumor-to-nontumor ratios were comparable or improved when ^{64}Cu-benzyl-TETA-1A3-F(ab')$_2$ was compared to ^{125}I-1A3-F(ab')$_2$, except for liver and kidney ratios which were superior for the iodinated fragments. The liver and blood clearance (%ID/g) of ^{64}Cu-labeled fragments at 24 hr (0.81 ± 0.047 and 1.15 ± 0.12, respectively) were an improvement over the intact antibody (1.51 ± 0.12 and 4.45 ± 0.54). The kidneys were the primary route of excretion of ^{64}Cu-benzyl-TETA-1A3-F(ab')$_2$. Although

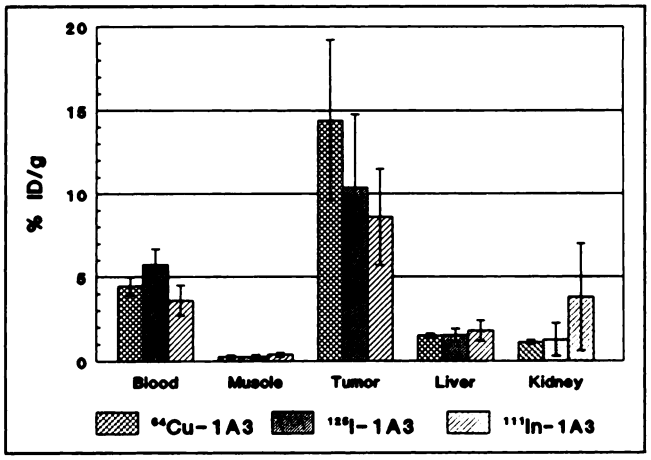

FIGURE 2. Biodistribution in hamsters of ^{64}Cu-benzyl-TETA-1A3, ^{125}I-1A3 and ^{111}In-BrΦHBED-1A3 at 24 hr postinjection.

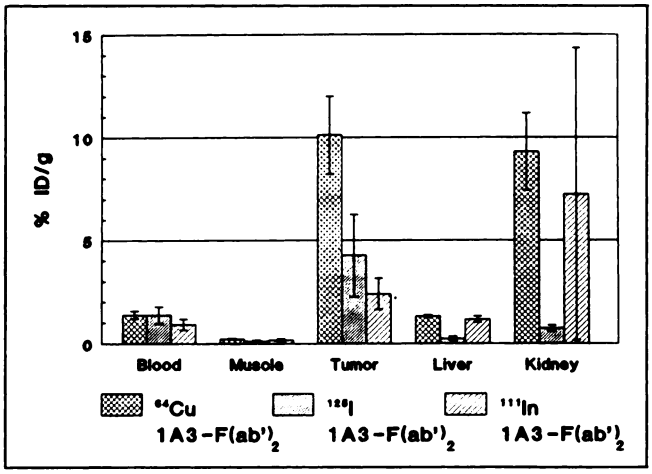

FIGURE 4. Biodistribution in hamsters at 24 hr postinjection comparing ^{64}Cu-benzyl-TETA-1A3-F(ab')$_2$, ^{125}I-1A3-F(ab')$_2$ and ^{111}In-BrΦHBED-1A3-F(ab')$_2$.

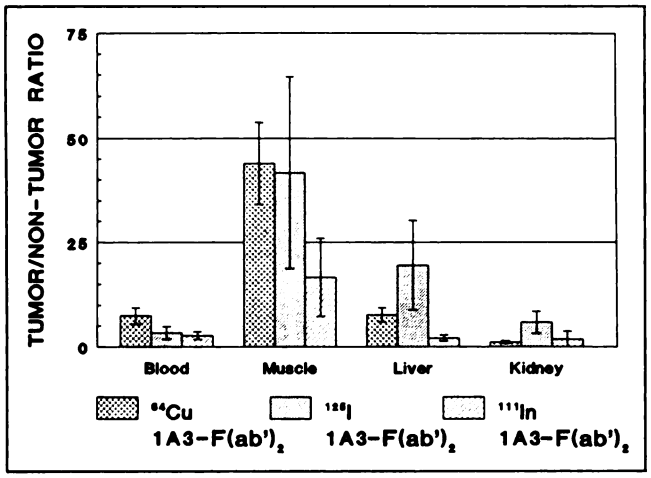

FIGURE 5. Tumor-to-nontumor ratios at 24 hr postinjection of selected tissues for ^{64}Cu-benzyl-TETA-1A3-F(ab')$_2$, ^{125}I-1A3-F(ab')$_2$ and ^{111}In-BrΦHBED-1A3-F(ab')$_2$.

there was significant clearance of ^{64}Cu-fragment from the kidneys from 12 to 24 h, the %ID/g remained high (10.0 ± 1.92).

The results of dosimetry studies for ^{64}Cu-benzyl-TETA-1A3 intact and F(ab')$_2$ compared to ^{123}I-1A3 intact and F(ab')$_2$ are presented in Table 1. The human absorbed doses (rad/mCi) that were obtained are presented for kidney, liver, lower large intestine (LLI), upper large intestine (ULI) and bone marrow. The absorbed doses for ^{64}Cu-labeled 1A3 and F(ab')$_2$ in the liver are similar (0.41 rad/mCi and 0.35 rad/mCi), but both are higher than the ^{123}I-labeled intact and F(ab')$_2$ (0.16 rad/mCi and 0.09 rad/mCi). The kidney absorbed dose is similar to the liver with ^{64}Cu-benzyl-TETA-1A3 (0.42 rad/mCi and 0.41 rad/mCi, respectively), but the kidney dose for ^{64}Cu-benzyl-TETA-1A3-F(ab')$_2$ is much higher than the liver (4.41 rad/mCi versus 0.35 rad/mCi). Additionally, the kidney dose for ^{64}Cu-labeled 1A3-F(ab')$_2$ is much higher than that for ^{123}I-1A3-F(ab')$_2$ (4.41 rad/mCi and 0.19 rad/mCi, respectively). This dose would limit the amount of ^{64}Cu-benzyl-TETA-1A3-F(ab')$_2$ that could be administered to patients. With ^{64}Cu-labeled intact 1A3 and 1A3-F(ab')$_2$ absorbed doses for ULI (0.31 rad/mCi and 0.30 rad/mCi) and LLI (0.52 rad/mCi and 0.52 rad/mCI) are similar. The ^{123}I-1A3 intact and F(ab')$_2$ absorbed doses for ULI (0.13 rad/mCi and 0.12 rad/mCi) and LLI (0.13 rad/mCi and 0.11 rad/mCi) are also similar, but considerably lower than the corresponding copper labeled antibodies. The thyroid is additionally included for the iodinated antibodies. The absorbed dose is significantly higher for this organ than any other organ for iodinated antibodies. The thyroid absorbed dose for ^{123}I-1A3 intact is significantly lower than the ^{123}I-1A3-F(ab')$_2$ (0.31 rad/mCi and 2.51 rad/mCi). If iodinated antibodies were used clinically, potassium iodide (KI) would be administered to block the thyroid uptake. Overall, the absorbed doses for ^{64}Cu-labeled intact 1A3 and 1A3-F(ab')$_2$ are higher than those of iodinated intact antibody and antibody fragments.

FPLC analysis of a sample of ^{64}Cu-benzyl-TETA-1A3 (citrate buffer, pH = 5.5) left at room temperature overnight, demonstrated that greater than 98% of the radioactivity is still associated with one fraction having a molecular weight corresponding to the intact conjugated antibody (Fig. 6A). FPLC activity balance measurements indicated that all radioactivity was recovered. Rat blood samples taken at time points ranging from 1 to 36 hours showed that 100% of the radioactivity was associated with the fraction corresponding to the intact conjugated antibody. FPLC of a sample of ^{64}Cu-benzyl-TETA-1A3-F(ab')$_2$ (citrate buffer, pH = 5.5) demonstrated that only 80% of the radioactivity corresponded to the molecular weight of the 1A3-F(ab')$_2$ (Fig. 6B). The remainder of the sample had a molecular weight corresponding to a smaller fragment which is possibly Fab. FPLC of plasma samples from rats injected with ^{64}Cu-benzyl-TETA-1A3-F(ab')$_2$ at 1–36-hr time points showed only one radioactive peak corresponding to the molecular weight of ^{64}Cu-labeled 1A3-F(ab')$_2$.

DISCUSSION

The data in this study demonstrate that by using the bifunctional chelate Br-benzyl-TETA ^{64}Cu or ^{67}Cu could be stably complexed to both intact 1A3 and 1A3-F(ab')$_2$. The labeling yields are high (>65% labeling efficiency) and ^{64}Cu-benzyl-TETA-1A3 (in citrate buffer, pH = 5.5) was stable in vitro at room temperature and was also stable in vivo as shown by FPLC of rat blood samples. In the

TABLE 1
Human Absorbed Doses (rad/mCi) for ^{64}Cu-1A3 Intact and F(ab')$_2$ Fragments Compared to ^{123}I-1A3 Intact and F(ab')$_2$ Fragments

Organ	Dose (rad/mCi)			
	^{64}Cu-benzyl-TETA-1A3	^{64}Cu-benzyl-TETA-1A3-F(ab')$_2$	^{123}I-1A3	^{123}I-1A3-F(ab')$_2$
Liver	0.41	0.35	0.16	0.09
Kidney	0.42	4.14	0.12	0.19
ULI	0.31	0.30	0.13	0.12
LLI	0.52	0.52	0.13	0.11
Marrow	0.28	0.32	0.13	0.10
Thyroid	—	—	0.31	2.51

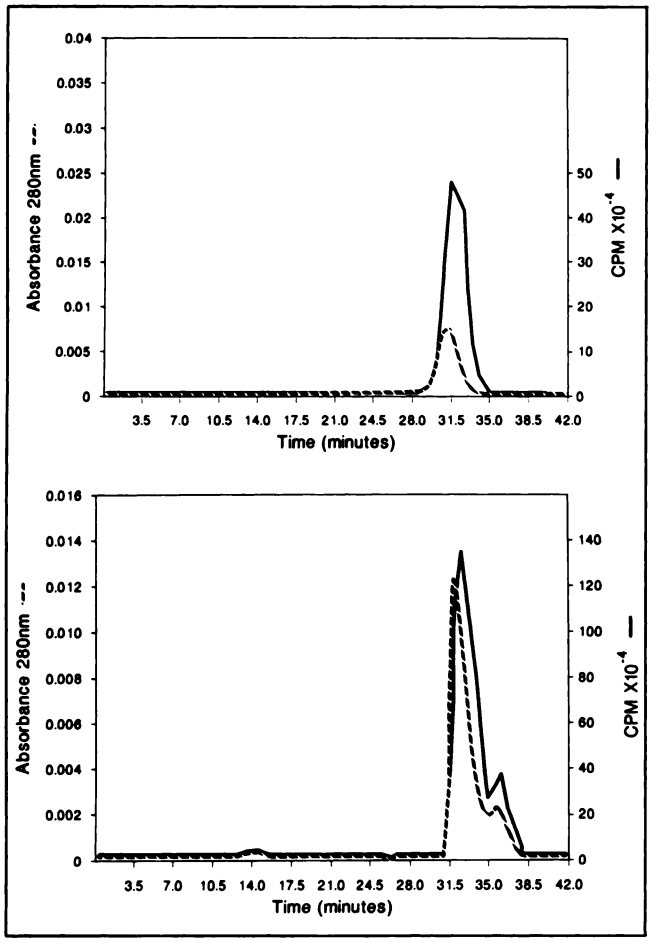

FIGURE 6. (A) FPLC trace of ^{67}Cu-benzyl-TETA-1A3 maintained at room temperature overnight. The column was calibrated with several molecular weight markers. Intact IgG (mol wt 150,000 Da) eluted with a retention time of 30.0 min. (B) FPLC trace of ^{64}Cu-benzyl-TETA-1A3-F(ab')$_2$ injectate for hamster studies. Column calibration with molecular weight markers indicated that F(ab')$_2$ fragment (mol wt 100,000 Da) eluted with a retention time of 32.5 min and the Fab fragment (mol wt 50,000 Da) eluted with a retention time of 36.0 min.

hamster model, the tumor uptake of Cu-labeled intact 1A3 was far superior to both ^{111}In-BrΦHBED-1A3 and ^{125}I-1A3. The %ID/g of ^{67}Cu-labeled benzyl-TETA-1A3 in tumor tissue reached a maximum at 1 day post-injection of 12.1 ± 3.71 in hamsters (Fig. 2), whereas the maximum %ID/g tumor uptake of ^{111}In-labeled BrΦHBED-1A3 (7.94 ± 1.28) occurred at 3 days postinjection (*15*). Indium-111-BrΦHBED-1A3 is currently being evaluated in a Phase II clinical trial. Iodinated intact 1A3 had a maximum tumor uptake at 24 hr, but the %ID/g in tumor tissue (10.4 ± 4.41) was significantly less ($p < 0.03$) than that of ^{64}Cu-labeled 1A3 (14.4 ± 4.8). For patient studies it is preferable to image as soon as possible postinjection of the Mab.

Antibody fragments are often preferred over intact antibodies for several reasons. The biological half-life of fragments is shorter, therefore fragments clear the blood faster than intact antibodies. The Fc region of intact antibodies is most likely to trigger allergic responses after a single injection of the Mab (*20*). Since there are Fc receptors on most cells of normal tissue including liver, the use of fragments in which the Fc portion has been removed should eliminate appreciable nonspecific Mab binding to normal tissues (*20*). Additionally, the fragments clear primarily through the kidneys and accumulate less in the liver than intact antibodies (*21*). The liver uptake of labeled intact antibodies is of special concern, because colorectal tumors often metastasize to the liver. For imaging purposes, any nonspecific uptake of the labeled antibody by the liver may obscure metastases that may be present. Thus, the use of Mab fragments should optimize colorectal carcinoma imaging studies.

In this study, we focused on 1A3-F(ab')$_2$ fragments which we have routinely produced with both high purity (≥90%) and good IR values (≥90%), in contrast to Fab fragments which, in preliminary experiments, have shown poor IR values (≤40%) (Connett JM, *unpublished results*). Preliminary results on ^{64}Cu-benzyl-TETA-1A3-F(ab')$_2$ are promising. Tumor uptake at 24 hr and tumor-to-muscle ratios far exceeded values for ^{125}I-1A3-F(ab')$_2$ and ^{111}In-BrΦHBED-1A3-F(ab')$_2$, but the accumulation and retention of ^{64}Cu-benzyl-TETA-1A3-F(ab')$_2$ in the kidney was high. FPLC analysis of ^{64}Cu-benzyl-TETA-1A3-F(ab')$_2$ prior to injection indicated 20% of the radioactivity was associated with a molecular weight fraction that corresponds to ^{64}Cu-benzyl-TETA-1A3-Fab that clears exclusively through the kidney (*22*). Further attempts will be made to purify the ^{64}Cu-benzyl-TETA-1A3-F(ab')$_2$ from this labeled impurity, which may reduce the uptake by the kidneys. It may be possible to alter the biodistribution by altering the ratios of benzyl-TETA, 2IT and antibody. Since the labeling yields are high, it may be possible to use smaller amounts of benzyl-TETA and 2IT. The immunoreactivity and kidney clearance may be improved by changing the number of chelates attached to the fragment.

The dosimetry study indicated that ^{64}Cu-benzyl-TETA-1A3 gave reasonable absorbed doses and should be a candidate for PET studies. For a 10-mCi injection of the radiolabeled intact 1A3, which would allow adequate imaging statistics for PET, the absorbed dose to the LLI would be 4.4 rads. In contrast, the same absorbed dose to the kidney would result by administering only 1 mCi of ^{64}Cu-benzyl-TETA-1A3-F(ab')$_2$, making the ^{64}Cu-labeled fragments unacceptable for use in a clinical trial. The dosimetry results also showed that both intact ^{123}I-1A3 and ^{123}I-1A3-F(ab')$_2$ have low radiation absorbed doses for all organs, except the thyroid for ^{123}I-1A3-F(ab')$_2$. Since the thyroid can be blocked, ^{123}I-1A3-F(ab')$_2$ could also be chosen for clinical use with traditional gamma scintigraphy.

CONCLUSIONS

The data presented in this paper suggest that the biodistribution of ^{64}Cu-benzyl-TETA-1A3 would make it a

radiopharmaceutical suitable for PET imaging of colorectal carcinoma. Dosimetry calculations indicate that up to 10 mCi of the ^{64}Cu-benzyl-TETA-1A3 could be administered clinically with reasonable absorbed doses. With the use of new, high efficiency PET imaging techniques (*1,2*), high quality whole-body PET images are likely to be obtained with a 10-mCi dose. Another approach would be to utilize ^{123}I-labeled 1A3 fragments. For ^{123}I-1A3-F(ab')$_2$, the absolute target uptake is less than that for ^{64}Cu-benzyl-TETA-1A3, although the dosimetry (on a per millicurie basis) is considerably more favorable. It is interesting to note that ^{64}Cu is reactor-produced, while ^{123}I is cyclotron produced. If ^{64}Cu were to be routinely produced it is likely to be less expensive than ^{123}I and it would have the advantage of PET versus SPECT imaging. Additionally, based on data using ^{125}I and ^{131}I, the labeling yields are expected to be similar for ^{64}Cu and ^{123}I.

In conclusion, we have developed and evaluated a ^{64}Cu-labeled anticolorectal antibody that appears very promising for utilization with PET for the evaluation of colon cancer. An amendment to our IND for ^{111}In-BrΦHBED-1A3 (*23*) to include ^{64}Cu-benzyl-TETA-1A3 administration in colorectal cancer patients is currently underway.

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