DTPA-Coupled Antibodies Labeled with Yttrium-90

D. J. Hnatowich, F. Virzi, and P. W. Doherty

Department of Nuclear Medicine, University of Massachusetts Medical Center, Worcester, Massachusetts

Yttrium-90 has been described as one of the best radionuclides for tumor therapy when chelated to tumor-associated antibodies. This evaluation is based on the superior properties of this radionuclide (suitable half-life, pure beta-ray emitter of intermediate energy, stable daughter, and suitable chemical properties) and because it is available as a radionuclide generator product by decay of its 28-yr parent ^{90}Sr. We have determined that ^{90}Y obtained from one such generator is suitable for labeling antibodies coupled with DTPA. Furthermore, we have shown that the dissociation rate of [^{90}Y]DTPA-IgG in serum at 37°C is similar to that of [^{111}In]DTPA-IgG at about 8–9%/day. Biodistribution studies of ^{111}In- and ^{90}Y-labeled to DTPA-coupled IgG show that the labels distribute nearly identically at 1 hr postadministration, although differences in distribution are apparent at 24 hr. It is possible that these differences reflect the redistribution of the labels following catabolism at the site of localization.

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The results of past studies using radiolabeled antibodies for tumor diagnosis have encouraged some investigators to initiate clinical trials of radioimmunotherapy using iodine-131- (^{131}I) labeled antibodies administered i.v. (*1*) and intracavitary (*2*). The use of this radionuclide, however, is associated with several drawbacks. In vivo deiodination with the rapid appearance of activity in urine, sometimes amounting to 50% of the injected dose in 24 hr (*3*), may explain the extremely low levels of radioiodine found in patient tumors (*4*). In addition, ^{131}I emits several abundant gamma rays in its decay and is therefore not ideal for therapy.

In a recent evaluation of radionuclides for radioimmunotherapy, Wessels and Rogus (*5*) have selected yttrium-90 (^{90}Y) as one of the four best therapeutic radionuclides to be used in conjunction with tumor-associated antibodies. This selection was based on suitable half-life (64 hr), absence of gamma-ray emissions, stable daughter, intermediate beta-ray energy ($E_{\beta max}$ = 2.3 MeV), and chemical properties suitable for forming chelates with diethylenetriaminepentaacetic acid (DTPA) (*6*). In addition, the radionuclide may be obtained by decay of its parent, strontium-90 (^{90}Sr) (T 1/2 28 yr) by means of a radionuclide generator (*7,8*).

Our laboratory has been investigating DTPA-coupled antibodies labeled with indium-111 (^{111}In) (*9,10*) and technetium-99m (^{99m}Tc) (*11,12*). Techniques similar to those used in these earlier studies to label coupled antibody, to determine the extent of nonspecific binding to the protein and, most importantly, to evaluate the stability of the label, have now been applied to ^{90}Y.

MATERIALS AND METHODS

Evaluation of the radioactivity

The ^{90}Y used in these studies was obtained carrier-free as the chloride in 1.0*M* HCl* or as the ethylenediaminetetraacetic acid (EDTA) chelate in 0.005*M* EDTA from a ^{90}Sr-^{90}Y radionuclide generator. The generator was constructed as described by Skraba et al. (*7*). Dowex 50W × 8 (50–100 mesh) cation exchange resin was washed with conc. NaOH, rinsed well with distilled water, and packed into a 1 cm × 0.6 cm glass column. The column was fitted with inlet and outlet tubes and placed in a lead shield. Two millicuries of ^{90}Sr† in 0.01 ml of 1*M* HCl was added to 600 μl of 0.003*M* EDTA solution at pH 5.2 and containing 0.1% phenol as a bacteriostat. The pH value was adjusted to 6.0 with dilute NaOH and the solution added to the generator

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For reprints contact: D. J. Hnatowich, PhD, Dept. of Nuclear Medicine, University of Massachusetts Med. Ctr., Worcester, MA 01605.

column. The generator was then eluted with 0.003*M* EDTA, pH 5.0 at a flow rate of 1.0 ml/min. The elution yield was ~75% with the activity appearing primarily in the first 1 ml.

The [^{90}Y]EDTA chelate in the generator eluant was destroyed and the activity obtained in the ionic form by one of two methods. Initially, the eluant was evaporated to dryness in a glass test tube and charred in a Bunsen burner flame. To the black residue was added 1 mg of Norit and 200 μl of 4*M* HCl and the suspension was purified over a 1 cm × 0.7 cm column of AG 1 × 4 (50-100 mesh) anion exchange resin. The eluant from this column was evaporated to dryness and redissolved in 0.1 ml of 0.5*M* sodium acetate pH 6 to a clear, colorless solution. More recently, the EDTA chelate was destroyed by adding to the eluant an equal volume of conc. HNO_3/conc. H_2SO_4 (50:50, v/v) and evaporating the solution to dryness. The activity was reconstituted with at least 30 μl of 2*M* sodium acetate pH 8.

Solutions of ^{90}Y were checked for ^{90}Sr breakthrough by ascending thin layer chromatography using ITLC-SG‡ and saline pH 7.0 eluant. In this chromatographic system, ^{90}Y remains at the origin while ^{90}Sr migrates with the solvent front (*13*). This separation was confirmed by analyzing an equilibrium mixture of ^{90}Sr-^{90}Y. The origin and solvent-front halves of the ITLC were counted separately in a NaI(Tl) well counter. If a separation of ^{90}Sr and ^{90}Y was achieved, activity in the origin half, containing only ^{90}Y, would decrease with the half-life of ^{90}Y (64 hr). The activity in the solvent-front half, rather than decreasing, would increase as ^{90}Y grows in by decay of ^{90}Sr. This would continue until the ^{90}Sr-^{90}Y equilibrium is re-established and thereafter, the activity would decrease with the half-life of ^{90}Sr (28 yr).

In addition to breakthrough, another concern is whether the generator eluant is free of interfering trace metals. Since trace metals present in the eluant will compete with ^{90}Y for the chelation sites, difficulties in labeling free DTPA indicates trace metal interference. Therefore, the level of interfering trace metals may be estimated by determining the lower limit on the concentration of free DTPA which can be labeled with ^{90}Y contained in the appropriate volume of eluant. Since trace metals present in the radioactivity sources will be introduced in proportion to the volume of eluant used, these tests were always conducted on volumes which would contain proportionately the same ^{90}Y activity at the time of use.

The acetate complex of ^{90}Y was prepared by mixing equal volumes of the ^{90}Y solution with 1*M* sodium acetate at pH 5.0 and ~1-2 μCi (about 1 μl) of this activity was added to 100 μl of solutions containing free DTPA at 1.0, 0.1, and 0.01 μg/ml. After 1-2 hr, each of the three solutions were analyzed by ascending paper chromatography on Whatman No. 1 paper using 0.1*M* tris buffer, pH 7 for development. In this system, ^{90}Y only as the DTPA chelate migrates to the solvent front. After developing, each paper was counted on a radiochromatogram scanner interfaced to a multichannel analyzer operating in multiscale mode (*14*).

The ^{90}Y activities in this work were measured in an ionization chamber§ calibrated by using a commercial source of phosphorus-32 (^{32}P) as standard.¶ An aliquot of a generator eluant was placed in a test tube containing a volume of water equal to that in an identical test tube containing a known activity of ^{32}P. The activity of ^{90}Y was then determined by assuming that the beta-ray end point energies of ^{32}P and ^{90}Y (1.7 and 2.3 MeV, respectively) will provide equal counting efficiencies and counting both test tubes in a NaI(Tl) well counter. The entire ^{90}Y eluant was then placed in a vial normally used to contain the activity during measurement and the activity measured in the ionization chamber using the ^{32}P setting. The error introduced by measuring ^{90}Y in the ionization chamber in this manner was determined to be ±1.1%.

Preparation and labeling of coupled antibody

Human IgG** and an $F(ab')_2$ antibody, designated 19-9 and directed against the CA 19-9 antigen†† (*15*) were both coupled with DTPA using the cyclic anhydride (*9,10*). Coupled antibodies were either labeled before or after purification from free DTPA on a 18 cm × 0.3 cm column of Sephadex G50. The antibodies were either labeled with ^{90}Y or ^{111}In added as the acetate in 0.5*M* acetate pH 6.0 buffer.

We had earlier shown that when ^{111}In as the acetate is added to DTPA-coupled antibody solutions, the activity does not bind nonspecifically to the protein but rather labels the DTPA groups exclusively. This was demonstrated in hydrolyzed control experiments in which ^{111}In was added to a solution containing coupled antibody and a solution containing uncoupled antibody and free DTPA at the same concentrations. The protein was labeled in the former but not the latter case (*9*). The same test was applied in this study to ^{90}Y; a solution containing 20 mg/ml of IgG in 0.05*M* bicarbonate pH 8.2 buffer was divided in half; half was coupled with DTPA at a 1:1 molar ratio using the cyclic anhydride while the other half was added to a solution of the hydrolyzed anhydride. Without purifying the antibody solutions of free DTPA, ^{90}Y as the acetate was added to each solution to a specific activity of 1 μCi/μg of protein. After 2 hr of incubation at room temperature, each solution was analyzed by high performance liquid chromatography (HPLC) using a TSK-125 size-exclusion protein column‡‡ and an in-line radioactivity detector interfaced to the multichannel analyzer (*14*).

The kinetics of labeling DTPA-coupled proteins with ^{90}Y was investigated by adding ^{90}Y as the acetate to a solution of DTPA-coupled IgG at 20 mg/ml and free

DTPA so that the specific activity was 1 μCi/μg of protein. Samples were removed periodically for analysis by HPLC as above. The radioactivity traces were analyzed to determine the percentage of activity present as labeled protein.

In vitro stability studies

One of the important questions concerning the use of DTPA-coupled proteins labeled with ^{90}Y is whether the label is stable in serum at 37°C. Using an anti-human transferrin and an anti-19-9 affinity column we have been able to show that in the case of ^{111}In, about 9%/day of the label is lost to DTPA-coupled antibodies in serum and that this loss is accounted for by the transcomplexation of ^{111}In to transferrin (*16*). The identical methods were used in this study to assess the serum stability of [^{90}Y]DTPA-antibodies. Anti-human transferrin columns were prepared by conjugating 4 mg of goat anti-human transferrin§§ with 1 g of cyanogen bromide activated Sepharose C1-4B using standard methodology (*17*). A sample for analysis was added to a 1 cm × 0.3 cm column of this resin and the resin rinsed with 1 ml of 0.2*M* phosphate-buffered saline (PBS). The fraction of the added activity which eluted was determined by counting the eluant in a well counter against a standard. The columns were regenerated between uses with 1 ml of 3*M* sodium thiocyanate and re-equilibrated with PBS. The 19-9 affinity columns were prepared by covalently coupling 19-9 IgG to cyanogen bromide-activated Sepharose C1-4B (*17*). After preparing several 2 cm × 0.3 cm columns of this resin, each column was washed with a minimum of 4 ml of 0.05*M* sodium citrate pH 4.0 containing 1% BSA and 0.001% thimerosal. The antigen, obtained from conditioned well media harvested from confluent SW 1116 cells derived from a human colorectal adenocarcinoma (*18*), was added and each column was washed with 1 ml of the citrate buffer, the sample to be analyzed was added and the column washed again with 1 ml of the citrate buffer. The eluant was counted in a well counter along with a standard to determine the fraction of added activity which was bound. Finally, the column was regenerated by washing with 1 ml of 3*M* sodium thiocyanate followed by the citrate buffer.

Human IgG coupled with an average of one DTPA group per molecule and 19-9 F(ab')$_2$ antibody coupled with an average of 1.9 groups per molecule were both labeled with [^{111}In]acetate and purified from free activity on a 18 cm × 0.3 cm column of Sephadex G50. The final specific activities were 0.5 μCi/μg and 1.2 μCi/μg for IgG and 19-9, respectively. Each protein was also labeled with [^{90}Y]acetate and, after 2 hr, purified on a 18 cm × 0.3 cm column of P30‡‡. The final specific activities were 1.0 μCi/μg and 1.7 μCi/μg for IgG and 19-9, respectively. The radiochemical purity of each preparation was determined by HPLC to be greater than 90%.

Fresh human serum from five healthy volunteers was obtained and filtered through a 0.22 μm Millipore filter into four sterile vacutainers, such that each vacutainer contained 0.2 ml. The labeled antibodies, in 2–8 μl, were then added (final antibody concentration 1 μg/ml), the vacutainers were purged with N_2, resealed, and placed in a shaking water bath at 37°C. Samples were removed at 0, 22, and 40 hr for analysis on the affinity columns. Ten columns were prepared with each resin type and 2 hr was needed to complete the analysis at each time point. The pH of each serum sample was monitored at each time point and was found to remain within the range 7.2 ± 0.2.

Finally, we have investigated the effect of ^{90}Y activity on the binding of [^{111}In]DTPA-19-9 to the anti-19-9 affinity column to establish the effect of radiolysis on the labeled antibody. DTPA-19-9 F(ab')$_2$ was labeled with ^{111}In to a specific activity of 11 μCi/μg in 0.9% saline pH 7 at an antibody concentration of 2.5 mg/ml. The radiochemical purity was found to be 100% by HPLC analysis. A solution of ^{90}Y was added to the ^{111}In-labeled antibody solution such that the final concentration of ^{90}Y was 2.0 mCi/ml. Affinity chromatography was then performed on ^{111}In-labeled antibody with and without ^{90}Y. The analyses were performed immediately and after 24 hr at room temperature using an intrinsic germanium gamma-ray detector¶¶ and the multichannel analyzer. The counting system was capable of accurately determining the intensity of the 245 keV gamma-ray in the decay of ^{111}In even in the presence of ^{90}Y.

Animal studies

An IgG antibody was coupled with an average of three DTPA groups per molecule; one half of the preparation was labeled with ^{111}In while the other half was labeled with ^{90}Y. After purification over G50, both preparations were stored overnight at 4°C in 0.05*M* bicarbonate pH 7.6 buffer. Ten healthy CD-1 male mice were injected by way of the tail vein with 0.1 ml (10 μg of IgG) of each preparation. Half the animals were killed at 1 hr post-administration and the remainder were killed at 24 hr. Tissues were removed, rinsed in saline, patted dry, and weighed.

Tissue samples containing ^{90}Y were counted in a well-type, gamma-ray detector in the same manner as ^{111}In rather than by liquid scintillation but only after establishing validity. A sample of liver uniformly labeled with ^{90}Y was cut into several pieces of different size. Each piece was weighed and placed in the bottom of a test tube. The test tubes were then counted in a NaI(Tl) well counter both with and without the addition of the same amount of water (1 ml) to each tube sufficient to cover all tissues. The relationship of tissue counts to tissue weight was then determined. As a further check on the counting procedure, several samples of liver, kidney, and bone (representing different counting

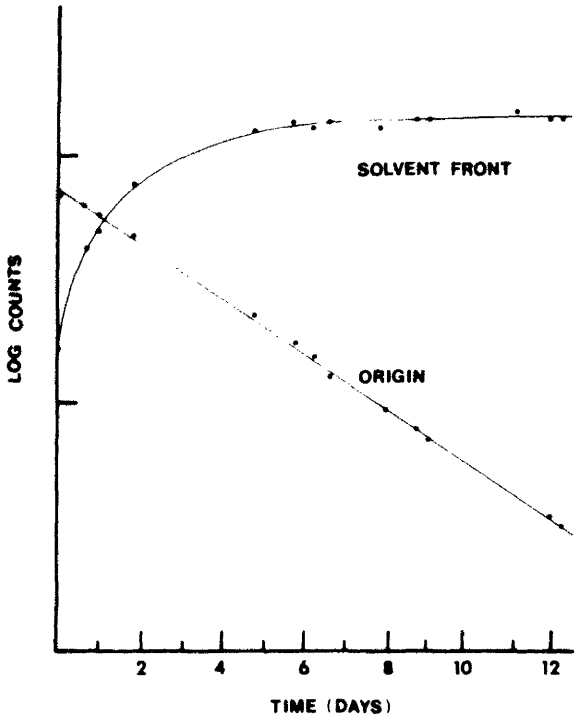

FIGURE 1
Counts compared with time obtained by counting the solvent front and origin halves of ITLC strip used to separate equilibrium mixture of ^{90}Sr-^{90}Y

geometries) were counted by the same procedure, then were digested in hot conc. HNO_3 and recounted.

After establishing that tissue samples containing ^{90}Y may be accurately counted in a well-type, gamma-ray detector, this counting method was used in the animal studies. Each tissue was counting in the well counter along with a standard of the injectate, also contained in a test tube with the same volume of water.

RESULTS AND DISCUSSION

Evaluation of the radioactivity

Thin layer chromatography was used to measure the ^{90}Sr breakthrough in the generator eluants. Figure 1 illustrates the results of analyzing a ^{90}Sr-^{90}Y equilibrium mixture and demonstrates that a separation is achieved; activity at the origin of the ITLC strip decays to background levels with the half-life of ^{90}Y while activity at the solvent front increases initially as the ^{90}Sr-^{90}Y equilibrium is re-established and remains constant thereafter, as expected for initially pure ^{90}Sr.

That the counting rates of the origin and solvent-front halves of the ITLC are nearly equal (within a factor of four) in Fig. 1 demonstrates that the counter used in these studies detects ^{90}Sr and ^{90}Y with nearly equal efficiencies. Therefore, following ITLC analysis, the ^{90}Sr breakthrough of a generator eluant is approximately equal to the fraction of the total counts which appear in the solvent front half. Analyzed in this manner, the ^{90}Sr breakthrough averaged 0.05% (N = 8). However, since ^{90}Y as the EDTA chelate also migrates to the solvent front, the presence of any EDTA will result in the overestimate of breakthrough. Therefore, this value must be regarded as an upper limit on breakthrough of the generator.

The extent of interfering trace metals in the purchased activity and in the generator eluant was determined by using these ^{90}Y sources to label solutions of free DTPA at low concentrations. The purchased ^{90}Y activity varied in chelation ability from batch to batch such that 30–100% of the activity was chelated at 0.1 μg/ml of DTPA. The generator-produced sources were comparable showing an average of 45% chelation to 0.1 μg/ml of DTPA. By comparison, when the chelation-grade ^{111}In*** used in this investigation is applied in this test, typically 80–90% of the activity is chelated in the 0.01 μg/ml DTPA solution. Nevertheless, as demonstrated below, the levels of trace metals present in the ^{90}Y generator eluant was not sufficient to interfere under the conditions of this study.

Preparation and labeling of coupled antibody

The absence of nonspecific binding of ^{90}Y to antibody was confirmed by comparing the HPLC profile of a DTPA-coupled IgG preparation with that of its hydro-

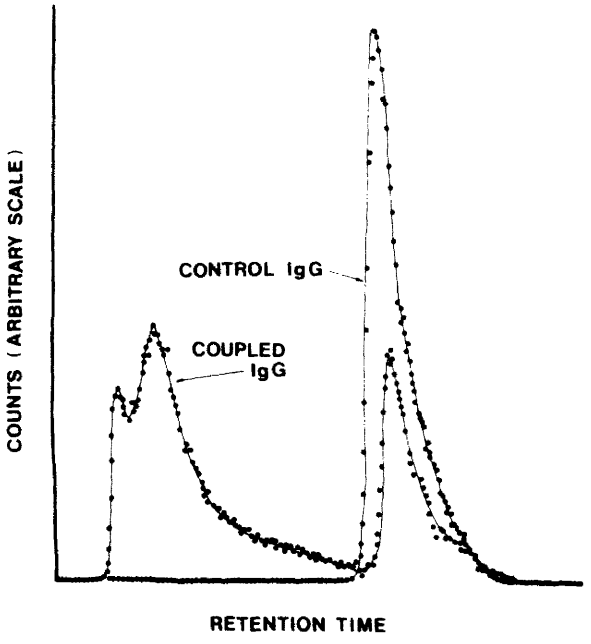

FIGURE 2
Radioactivity HPLC traces of DTPA-coupled and uncoupled IgG antibody after labeling with ^{90}Y. Activity is seen to elute with protein at early retention times only in former case

lyzed control, both labeled with ^{90}Y. The radioactivity traces obtained by the analysis of both solutions are presented in Fig. 2 and they show in both a radioactivity peak appearing at long retention times due to labeled free DTPA. In the case of the coupled IgG, about 69% of the activity is bound to protein and elutes early whereas there is no detectable activity bound to protein in the case of the hydrolyzed control. As a further check, a similar experiment was performed with uncoupled protein free of all DTPA; once again no activity was observed to be protein-bound.

The results of an investigation of the kinetics of labeling DTPA-coupled proteins with ^{90}Y is presented in Fig. 3 which shows that about 2 hr at room temperature are required for maximum labeling.

In vitro stability studies

Both anti-human transferrin and anti-19-9 affinity chromatography was performed on DTPA-coupled IgG and the $F(ab')_2$ fragment of 19-9, labeled with ^{90}Y and ^{111}In and incubated in fresh serum. The results of this investigation are presented in Table 1. It is apparent that ^{111}In and ^{90}Y behave very similarily. An average of about 4%/day of ^{111}In transcomplexed to transferrin as determined by anti-transferrin affinity chromatography

TABLE 1
Analysis by Anti-Human Transferrin and CA 19-9 Affinity Chromatography of Serum Incubates of IgG and 19-9 $F(ab')_2$ Coupled with DTPA and Labeled with ^{90}Y and ^{111}In*

Time	Anti-transferrin column	Anti-19-9 column
T = 0 hr		
^{111}In-19-9	3.3 (3.3)	81 (6.9)
^{111}In-IgG	5.5 (3.4)	3.4 (3.4)
^{90}Y-19-9	4.9 (4.6)	83 (4.8)
^{90}Y-IgG	8.4 (7.2)	4.4 (5.3)
T = 22 hr		
^{111}In-19-9	12 (2.9)	71 (5.1)
^{111}In-IgG	9.9 (1.7)	3.6 (3.3)
^{90}Y-19-9	7.0 (3.4)	73 (6.3)
^{90}Y-IgG	11 (5.3)	11 (5.9)
T = 40 hr		
^{111}In-19-9	13 (2.9)	58 (5.8)
^{111}In-IgG	13 (7.3)	6.6 (4.2)
^{90}Y-19-9	10 (8.3)	57 (7.4)
^{90}Y-IgG	12 (15)	14 (14)

* Expressed as percent bound with 1 s.d. in parenthesis (N = 5).

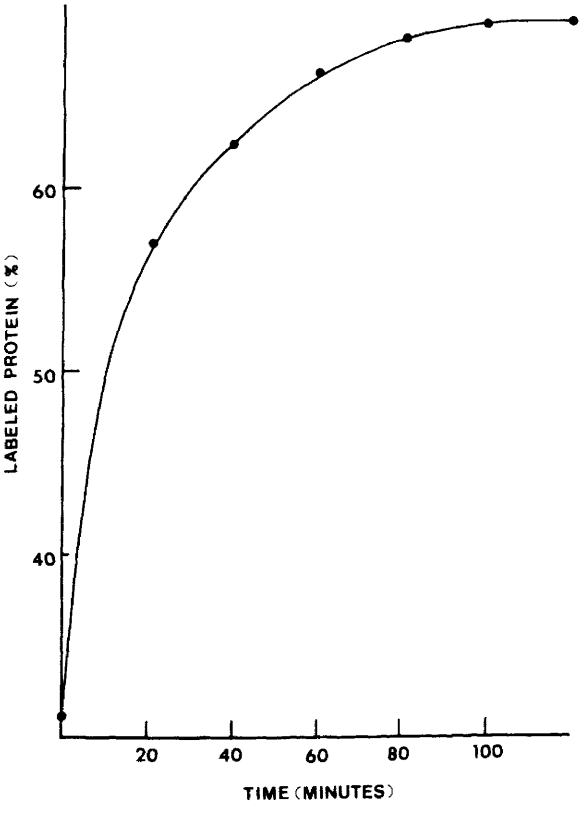

FIGURE 3
Percentage of ^{90}Y activity chelated to DTPA-coupled IgG antibody compared with time after adding activity

while for ^{90}Y this value was 2%/day. Also, an average of about 11%/day of ^{111}In left the antibody as determined by anti-19-9 affinity chromatography while for ^{90}Y this value was 13%/day.

In all cases the recoveries (i.e., the sum of activities in all washed compared with the applied activity) was greater than 85% and averaged 95% with the exception of ^{90}Y-labeled 19-9 on the anti-19-9 affinity column where these values averaged about 25%. In this case only, the released ^{90}Y bound irreversibly to the resin after the [^{90}Y]DTPA chelate dissociated in the sodium thiocyanate final wash.

A previous analysis of the dissociation of [^{111}In]-DTPA-19-9 in patient sera showed a rate of about 9%/day attributable entirely to transcomplexation to transferrin (*16*). The discrepancy with the above results reflects the limitations inherent in the present experiment primarily due to low counting rates. Nevertheless, the similar results obtained with both labels show that the rate of ^{90}Y dissociation is similar to that of ^{111}In and about 8–9%/day.

Anti-19-9 affinity chromatography was also used to determine the effect of ^{90}Y radiolysis on the immunoreactivity of the 19-9 antibody. The binding of ^{111}In-19-9 to the affinity column was measured immediately and after 24 hr in a solution containing ^{90}Y at 2.0 mCi/ml. The identical experiment was also performed for ^{111}In-19-9 in the same buffer but without ^{90}Y. In this manner, it was determined that initially 71 and 64% of the ^{111}In activity was bound to the column with and without the

^{90}Y while at 24 hr these values were 56 and 52%, respectively. Some decrease in retention is expected for ^{111}In-labeled antibody over 24 hr at room temperature and was observed, however, the presence of ^{90}Y at this concentration did not alter the binding of the ^{111}In-labeled antibody.

Animal studies

Animal tissue samples were counted for ^{90}Y by using a well-type, gamma-ray detector. The validity of this counting method was established by demonstrating that the relationship between counting rate and tissue weight was linear over the appropriate range. However, this was found to be the case only if the tissues were counted while immersed in water. Without the water, the relationship was nonlinear for tissues weighing about 50 mg or less. It is unlikely that bremsstrahlung production is important in this counting situation because of the absence of high Z materials in the samples, and therefore it is probably the beta rays which are being detected. Consequently, this behavior is most likely the result of beta-ray self-absorption (Wessels BW, private communication). The water apparently effects beta-ray self-absorption such that the relationship between count rate and tissue size becomes linear.

As a further check on the counting procedure, tissue samples were recounted after digestion. The recount showed an average increase in counts of 36 and 26% for liver and kidney, respectively, and an average decrease of 27% for bone. Because these differences are minor, corrections were not applied to the biodistribution results.

The specific activities of the ^{111}In- and ^{90}Y-labeled IgG antibodies used in animals were 1.2 $\mu C/\mu g$ and 0.3 $\mu Ci/\mu g$, respectively. Prior to use, each sample was made 2% in human serum albumin (HSA) and analyzed by size-exclusion HPLC. Good recoveries indicated the absence of colloidal activity in these preparations and the radiochemical purities were found to be 82 and 100% for ^{111}In and ^{90}Y, respectively. The results of animal biodistribution studies are presented in Table 2 in percent of the injected dose per g (wet weight) of tissue normalized to a 25 g animal.

Despite identical stabilities in serum and despite nearly identical biodistributions at 1 hr postadministration, the biodistribution of ^{90}Y and ^{111}In at 24 hr postadministration are clearly different (Table 2). The most likely explanation for the differences observed at 24 hr is redistribution of the label following catabolism of the antibody at the sites of localization. following catabolism, ^{111}In and ^{90}Y may be released to the circulation at different rates and possibly in different chemical forms such that they distribute differently.

An encouraging result of this investigation is that ^{90}Y-labeled to DTPA-coupled antibodies is stable in serum at 37°C and shows a dissociation rate comparable to that of ^{111}In-labeled antibodies. In the case of ^{111}In, this dissociation has not prevented the clinical use of antibodies labeled with this isotope (*16*). It is possible, therefore, that the stability of ^{90}Y-labeled antibodies observed in this work may be suitable for therapeutic uses.

TABLE 2
Biodistribution of ^{90}Y- and ^{111}In-Labeled DTPA-Coupled IgG in Normal Mice at 1- and 24-hr Postadministration*

Organ	1 hr (N = 4)		24 hr (N = 5)	
	^{111}In	^{90}Y	^{111}In	^{90}Y
Blood	22.4 (2.2)	24.5 (1.5)	8.7 (0.3	6.8 (0.8)
Heart	2.9 (0.4)	1.9 (0.3)	1.7 (0.1)	1.5 (0.4)
Lung	3.9 (2.2)	5.5 (2.4)	4.3 (0.5)	3.4 (0.9)
Liver	20.6 (2.1)	17.4 (6.3)	19.1 (0.7)	13.5 (3.9)
Spleen	3.7 (0.5)	3.6 (1.4)	3.9 (0.6)	2.5 (0.5)
Kidney	6.3 (1.1)	6.6 (1.7)	17.8 (1.8)	5.0 (0.5)
Muscle	0.5 (0.1)	0.5†	0.8 (0.1)	0.6 (0.4)
Bone	1.1 (0.2)	1.0 (0.2)	1.6 (0.3)	2.6 (0.7)

* Expressed as percent injected dose per g wet weight normalized to a 25 g animal. Mean value with 1 s.d. in parenthesis.
† N = 2.

FOOTNOTES

* Oak Ridge National Laboratories, Oak Ridge, TN.
† DuPont NEN Medical Products, N. Billerica, MA.
‡ Gelman Instrument Co., Ann Arbor, MI.
§ Model CRC-16, Capintec, Inc., Ramsey, NJ.
¶ $^{32}PO_43$-, Mallinckrodt, Inc., St. Louis, MO.
** Sigma Chemical Co., St. Louis, MO.
†† Centocor Inc., Malvern, PA.
‡‡ BioRad, Richmond, CA.
§§ U.S. Biochemical Corp., Cleveland, OH.
¶¶ Canberra Industries, Inc., Meriden, CT.
*** Medi-Physics, Inc., Richmond, CA.

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