Synthesis of Fluorine-18-Labeled Biotin Derivatives: Biodistribution and Infection Localization

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Recently there has been much interest in the exploitation of the high binding affinity of avidin/biotin as a means of targeting drugs and radionuclides for in vivo applications. We are interested in broadening the application of the avidin/biotin complex to PET. To this end we set out to prepare ^{18}F-labeled biotin analogs. **Methods:** Two ^{18}F biotin derivatives, [3aS-(3aα,4β,6aα)]-hexahydro-2-oxo-1H-thieno[3,4-d]imidazole-4-(N-3-(1-[^{18}F]fluoropropyl))pentanamide (1) and [3aS-(3aα,4β,6aα)]-tetrahydro-4-(5-(1-[^{18}F]fluoropentyl)-1H-thieno[3,4-d]imidazol-2(3H)-(2) were prepared with high specific activity (NCA) and evaluated for their potential in infection localization. **Results:** Compound 1 binds to avidin and the biodistribution of these derivatives were studied in *Escherichia coli* infected rats. Half of the infected rats were treated with avidin 24 hr prior to intravenous injection of the ^{18}F-labeled biotin analogs. Biotin 1, without avidin pretreatment, showed a selectivity of 6.08 ± 1.12 for infection compared to normal muscle. With avidin pretreatment, selectivity increased slightly, giving an infection to normal muscle ratio of 6.39 ± 0.96. In contrast, the biodistribution of biotin 2 indicated more binding to normal muscle with an infection to normal muscle ratio of 0.58 ± 0.07. This lack of selectivity illustrates the importance of the side-chain amide group in infection localization. There was some defluorination of 1 and 2, as evidenced by increased ^{18}F bone uptake after 60 min: 2.94 ± 0.37 and 1.17 ± 0.21 %IG/g ± s.d., respectively. **Conclusions:** Biotin derivatives could be radiofluorinated with high specific activity. Biotin 1, is a potential positron tomography tracer for infection imaging.

Key Words: infection; PET; biotin; radiofluorine

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High-contrast tumor imaging can be achieved by allowing a nonradiolabeled antibody to localize and clear from the circulation prior to administration of a low molecular weight radiolabeled moiety with high affinity for the pretargeted antibody (*1–8*). One such method utilizes the high affinity of avidin, a cationic glycoprotein found in egg whites, for biotin, a naturally occurring vitamin (*1*). Avidin is capable of binding four biotin molecules to form the avidin/biotin complex (Kd = 10^{-15}M): an essentially irreversible process (*2*).

Two basic approaches for pretargeting tumors with the avidin/biotin system have been used in patients and animals. In the first method, avidin (streptavidin)-conjugated antibodies are injected and days later, when antibody-tumor binding is maximized, a radioactive biotin derivative is injected to localize the tumor (*1*). Unfortunately, incomplete clearance of unbound antibody from the blood circulation can obscure visualization of the target site. In the second method, blood background is reduced by injecting biotinylated antibodies followed by cold avidin three days later (*1*). The resultant circulating biotinylated antibody/avidin complexes are sequestered from the blood by the liver. Radioactive biotin is then injected, and binds to the antibodies already localized in the tumor.

In recent years, studies have demonstrated that the use of specific antibodies for pretargeting infection or tumors may not be necessary to achieve good images. A study by Morrel et al. (*9*) reported uptake of ^{111}In-labeled IgG and human serum albumin (HSA) in an *Escherichia coli* infected rat model. The accumulation of both labeled pro-

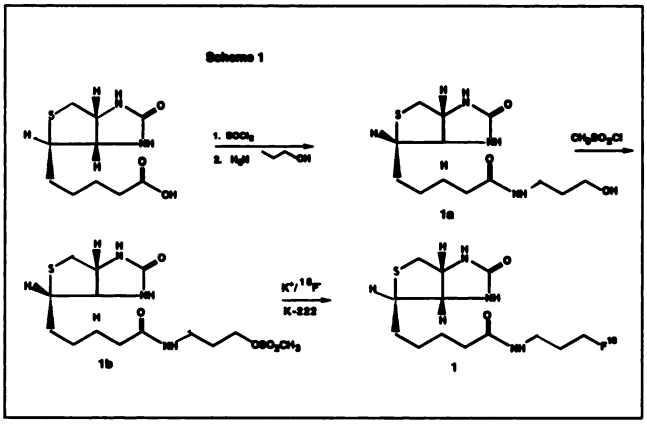

FIGURE 1. Scheme 1. Synthesis of 3aS-(3aα,4β,6aα)]-hexahydro-2-oxo-1H-thieno[3,4-d]imidazole-4-(N-3-(1-[^{18}F]fluoropropyl))-pentanamide (Biotin 1).

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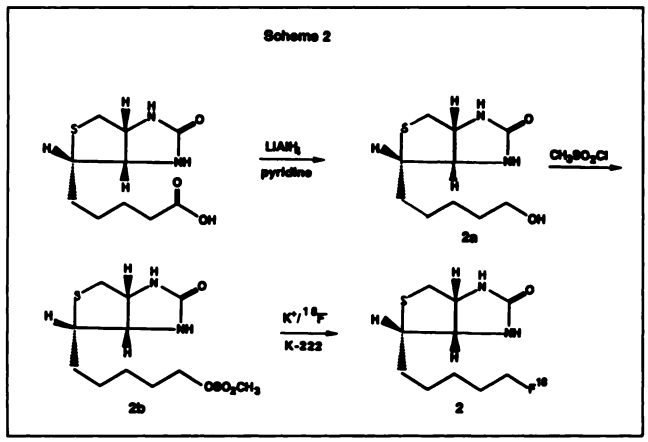

FIGURE 2. Scheme 2. Synthesis of 3aS-(3aα,4β,6aα)]-tetrahydro-4-(5-(1-[^{18}F]fluoropentyl)-1H-thieno[3,4-d]imidazol-2(3H)-one (Biotin 2).

teins was sufficient to produce clear images of the infection site. More recently a comparison of tumor localization using specific antibody and streptavidin was reported by Hnatowich (*10*). The biodistribution of [^{111}In]streptavidin was similar to that of ^{111}In-labeled specific antibody except for lower blood and higher kidney levels.

Application of the avidin/biotin system has been limited to single-photon radionuclides such as ^{111}In, ^{125}I and ^{99m}Tc. In general, the quality of single-photon, radioimmune images ranges from acceptable to poor. Our interest in new imaging agents for PET has led us to synthesize and evaluate some ^{18}F-labeled biotin derivatives. Utilization of short-lived positron emitting nuclides and PET should markedly improve resolution and sensitivity. In this preliminary study, we present the syntheses of two ^{18}F-labeled biotin derivatives, [3aS-(3aα,4β,6aα)]-hexahydro-2-oxo-1H-thieno[3,4-d]imidazole-4-(N-3-(1-[^{18}F]fluoropropyl))-pentanamide (Fig. 1) and [3aS-(3aα,4β,6aα)]-tetrahydro-4-(5-(1-[^{18}F]fluoropentyl)-1H-thieno[3,4-d]imidazol-2(3H)-one (Fig. 2), and their biodistributions in *Escherichia coli* infected rats.

Biotin 1 was chosen because it can be prepared easily from a sulfonate ester in good yield with high specific activity while maintaining the basic biotin structure. Biotin 2 was prepared and labeled in order to help elucidate the role of the side chain on biotin binding to avidin and infection sites.

MATERIALS AND METHODS

General

Analysis for carbon and hydrogen was performed by Galbraith Laboratories (Knoxville, TN). Melting points were measured with a Fisher-Johns apparatus and are uncorrected. Proton NMR spectra (d_6-DMSO) were acquired with a Bruker AM 500 instrument; the chemical shifts are reported in parts per million (delta) down field from tetramethylsilane. All reagents were purchased from Aldrich Chemical Co, (Milwaukee, WI) and were used without further purification. Acetonitrile was purchased from Pierce (Rockford, IL) and used as shipped.

Thin-layer chromatography was preformed on silica gel Gf 250 plates (Analtech, Inc., Newark, DE). Chromatograms of the radiolabeled compounds were counted with a Bioscan System 200 (Washington, D.C.). High-pressure liquid chromatography was performed on a Resolvex C18 column (4.6 mm × 25 cm, Fisher Scientific Co.) at a flow rate of 1 ml/min using water/acetonitrile (60:40) solvent system. Separation was monitored with a UV detector (λ = 185 nm) and a NaI(Tl) radioactivity detector.

Synthesis of Biotin 1

Biotin 1a. (+)Biotin (1g, 4.09 mmole) was added to 20 ml of a stirred 50/50 mixture of thionyl chloride and benzene and the mixture was refluxed under N_2 for 2 hr. Excess thionyl chloride was removed by distillation and the residue was cooled to room temperature under N_2, dissolved in dry THF (10 ml) and added to a stirred solution of 3-amino-1-propanol (1 g) in THF (20 ml) at 25°C. The solvent was removed by roto-evaporation, and excess amino propanol was removed at 120°C under vacuum (1 torr). The crude material was purified by chromatography on a silica gel column (30 g) eluted with CH_2Cl_2/MeOH/HCO_2H (20:80:0.5). Crystallization from MeOH gave 0.80g (65%) of product. This product showed a single spot (R_f = 0.45) on TLC with CH_2Cl_2/MeOH/HCO_2H (20:80:0.5); mp = 130–132°C. ^{1}H NMR (d_6-DMSO) d2.05 (t,J=6.2 Hz, 2H, CH_2-O), 3.2–3.5 (m,6H, N-CH_2-), 6.35 (s, 1H, O=CNH-ring), 6.40 (s, 1H, O=CNH-ring), 7.75 (t, J=5.6 Hz, 1H, O=CNH). Anal. Calcd. for $C_{13}H_{23}N_3O_3S$: C, 51.80,H, 7.70. Found: C, 51.69; H, 7.51.

Biotin Mesylate 1b. Biotin 1a (100 mg, 0.33 mmole) was dissolved in 3 ml of dry pyridine by heating and stirring under N_2. The solution was cooled to room temperature, and 0.7 ml of a 0.87 M solution of methansulfonyl chloride in CH_2Cl_2 was added. After 30 min, the mixture was chromatographed on silica gel using acetone/acetonitrile (90:10). The product (86 mg, 69%) showed a single spot on TLC (R_f = 0.49) with MeOH/CH_2Cl_2/HCO_2H (10: 90:0.5) that was visualized with $MoO_3 \cdot H_3PO_4$.

Biotin 1. A 10-ml reactivial containing KF (48 mg), Kryptofix (300 mg), biotin mesylate (200 mg), and 7 ml of acetonitrile was heated at 110°C for 30 min. The solvent was roto-evaporated and the residue was chromatographed on silica gel (25 g) using CH_2Cl_2/MeOH/HCO_2H (85:15:0.5). This product showed a single spot (R_f = 0.38) by TLC with the same solvent. ^{1}H NMR (d_6 - DMSO) d3.2–3.5 (m,6H, N - CH_2-), 4.4 (dt,J = 52, 6.5 Hz, 2H, CH_2-F), 6.35 (s, 1H, O = CNH-ring), 6.40 (s, 1H, O = CNH-ring), 7.75 (t, J = 5.6 Hz, 1H, O = CNH). Anal. Calcd. for $C_{13}H_{22}N_3O_2SF$: C, 54.90,H, 7.80. Found: C, 52.19; H, 8.13.

Synthesis of Biotinol 2

Biotinol 2a. To a stirred suspension of lithium aluminum hydride (LAH) (1.0g, 25.8 mmole) in anhydrous ether (125 ml), a hot pyridine (25 ml) solution of (+)biotin (1.0g, 4.01 mmole) was added dropwise. After addition, the reaction mixture was stirred at room temperature for 30 min and then heated to reflux for an additional 30 min. The excess LAH was then destroyed by careful dropwise addition of water. Water and organic solvents were removed by roto-evaporation. The residue was acidified (pH 2) with 6 N HCl and the water was removed by roto-evaporation. The residue was extracted with chloroform (5 × 20 ml) and the chloroform layer evaporated in vacuo to give a white solid. Recrystallization from methanol gave 0.44 g (50%) of the reduced product; mp 164–167°C (reference 11, mp 164–169°C). ^{1}H NMR (d_6-DMSO) d 4.35 (t, J = 6 Hz, 2H, O-CH_2-), 6.35 (s, 1H, O=CNH-ring), 6.40 (s, 1H, O=CNH-ring).

Biotinol mesylate (2b). To a solution of (+)Biotinol (2a) (100 mg, 0.43 mmole) in pyridine (3 ml) was added 0.6 mL of a 0.87 M solution of thionyl chloride in CH_2Cl_2. The mixture was stirred for 30 min followed by chromatography on silica gel using acetone/acetonitrile 90:10. The product (96 mg, 75%) showed a single spot on TLC (R_f = 0.42) with EtOAc/CH_2Cl_2/acetone/HCO_2H (10:80:10:0.5) that was visualized with $MoO_3 \cdot H_3PO_4$.

Biotin 2. This compound was prepared from 2b in the same manner as described for 1 above. Biotin 2 was purified on silica gel using EtOAc/CH_2Cl_2/acetone/HCO_2H (10:80:10:0.5) (Rf = 0.48); mp 110–112°C. ^1H NMR (d_6-DMSO) d 4.6 (dt, J = 55, 6.5 Hz, F-CH_2-), 6.35 (s, 1H, O=CNH-ring), 6.40 (s, 1H, O=CNH-ring). Anal. Calcd. for $C_{10}H_{16}N_2OSF$: C, 56.31,H, 7.57. Found: C, 56.59; H, 7.79.

Preparation of ^{18}F-Labeled 1 and 2

Fluorine-18-fluoride was produced with a cyclotron by the $^{18}O(p,n)^{18}F$ nuclear reaction on ^{18}O-enriched water in a silver plated target at 17 MeV, 20mA-hr. (Scanditronix, Sweden, MC17F) (*12*).

[3aS-(3aα,4β,6aα)]-hexahydro-2-oxo-1H-thieno[3,4-d]imidazole-4-(N-3-(1-[^{18}F]fluoropropyl))pentanamide (1). A 5-ml reactivial containing ^{18}F in $H_2{}^{18}O$ (50 mCi, 1 mL), Kryptofix (3 mg), and K_2CO_3 (1 mg) was evaporated to dryness at 100°C under N_2. The contents were dried by addition of acetonitrile (4 × 2 ml) with heating at 110°C under a stream of N_2. Biotin mesylate 1b (2 mg) in acetonitrile (1 ml) was added and the vial was sealed and heated at 110°C for 10 min. Solvent was removed and the labeled product purified by chromatography on a short column of silica gel (10 g) using MeOH/CH_2Cl_2/HCO_2H (20:80:0.5). The time required for synthesis and purification was 90 min (from the end of bombardment) and the radiochemical yield was 3.4 to 5.9 mCi (12–21%, EOS). HPLC analysis of the final product showed a single radioactive peak with a retention time (R_t) 2.8 min corresponding to that of the [^{19}F]fluoro-biotin 1. The chemical purity of 1 was >98%.

3aS-(3aα,4β,6aα)]-tetrahydro-4-(5-(1-[^{18}F]fluoropentyl)-1H-thieno-[3,4-d]imidazol-2(3H)-one (2). This compound was prepared in the same manner as 1. Biotin 2 was purified on silica gel (10 g) using EtOAc/CH_2Cl_2/acetone/HCO_2H (10:80:10:0.5). The radiochemical yield was 5.6 mCi (20%, EOS) and radiochemical purity was determined to be greater than 98%. The chemical purity of 2 was >98% by HPLC.

Competitive Binding of Fluorine-18 Labeled 1 and 2 to Avidin versus d-Biotin

A preliminary measure of the binding characteristics of the fluoro analogs of biotin were performed. The binding of these novel ^{18}F-labeled biotin analogs to avidin was evaluated by measuring the binding of ^{18}F-labeled biotin analogs to avidin in the presence of varying concentrations of unlabeled d-biotin. Serial dilutions of d-biotin were prepared in phosphate buffered saline pH 7.4 (PBS). A fixed quantity of the no-carrier added ^{18}F-labeled biotin 1 or 2 (10–100μCi) was mixed with each d-biotin dilution so that the range of concentrations of cold biotin were three orders of magnitude greater and less than the concentration of avidin (10^{-16}M). A 200 μl aliquot of the ^{18}F-biotin/cold biotin solution was added to 200 μl of avidin in PBS. The solution was vortexed and allowed to incubate for 1 hr after which time a 5μl aliquot of the solution was analyzed by instant thin-layer chromatography (Gelman ILTC-SG) using 0.1 M acetate buffer pH 6.0. In this chromatographic system the ^{18}F-biotin analogs bound to avidin remain at the origin while the unbound ^{18}F-biotin analogs move to the solvent front. Percent binding of ^{18}F-biotin analog was calculated as counts [(counts at origin/total counts) * 100]. For each ^{18}F-biotin analog, potential impurities remaining at the origin were determined and the results of binding corrected accordingly.

Biodistribution Studies in Rats

A clinical isolate of *E. coli* was stored at −70°C in a freezing media containing 20% glycerol and 80% dextrose phosphate until use. Aliquots of bacteria were defrosted and colony counts performed on serial dilutions grown overnight on BBL Brucella agar plates. Based on the colony count, freshly thawed bacteria suspensions were washed and diluted with saline to a final concentration of 8 × 10^{10} organisms/ml. Aliquots (0.1 ml) of bacterial suspensions (8 × 10^9 organisms/ml) were injected into the right thigh muscle of 48 male Sprague-Dawley rats (125–150 g, Charles River Breeding Laboratories, Burlington, MD). Twenty-four rats were used for each labeled biotin compound. Twenty-four hours after injection, swelling in the right thigh readily appeared, and 24 rats each received 1.67 mg of avidin in saline via the tail vein. Forty-eight hours after bacterial inoculations, 80 to 150 mCi of ^{18}F-biotin 1 or 2 was administered. Rats were sacrificed at 5 and 60 min postinjection, six rats per time point. Blood samples were obtained by cardiac puncture. Syringes were weighed before and after injection to determine the volume delivered. The activity per unit volume was obtained from standards. A total of eleven different tissues were excised, weighed and counted. These tissues included blood, bone, lung, liver, adrenal, spleen, kidneys, heart, muscle (thigh), teste and infected muscle (thigh). The excised tissues were blotted, weighed and counted in a scintillation well counter. The raw counts were decay corrected. Results were expressed as percent injected dose per gram and infection-to-normal muscle ratios (mean ± s.d.). Biodistribution results were evaluated by analysis of variance (one-way ANOVA). The effect of avidin pretreatment on biodistribution was evaluated by student t-test.

RESULTS

Biodistribution

Table 1 shows the biodistribution of ^{18}F-labeled biotin 1 at 5 and 60 min after injection in rats with and without avidin pretreatment (24 hr). Analysis of variance demonstrated a significant main effects of organs ($p < 0.001$) at 5 and 60 min on biotin 1 accumulation. At 5 min, accumulation of 1 in kidney was greater than in all other organs (4.5-fold higher than blood) ($p < 0.001$). Accumulation of biotin 1 in liver and spleen were greater than in normal muscle ($p < 0.02$). Sufficient clearance of radioactivity had occurred within 60 min in all other organs relative to infection except for bone ($p < 0.001$). At 60 min, the selectivity for infected compared to normal muscle was 6.08 ± 1.12 (Table 2); accumulation in infected muscle was greater than kidney ($p < 0.05$), blood ($p < 0.01$), spleen ($p < 0.01$), liver ($p < 0.001$) and normal muscle ($p < 0.001$). The distribution of biotin 1 in all organs did not change significantly with avidin-pretreatment at 5 min. At 60 min with pretreatment, accumulation in bone ($p < 0.01$) was less than without avidin-pretreatment. Compared to untreated rats, accumulation of biotin 1 in avidin-treated rats was greater in kidney ($p < 0.02$), blood ($p < 0.05$), and normal muscle ($p < 0.002$).

TABLE 1
Tissue Distribution of ^{18}F-Biotin 1 in *E. coli* Infected Rats

	%IG/g ± s.d.			
	Without avidin		With avidin	
Tissue	5 min	60 min	5 min	60 min
Blood	1.07 ± 0.31	0.14 ± 0.02	1.13 ± 0.11	0.19 ± 0.04
Heart	1.00 ± 0.34	0.14 ± 0.02	0.99 ± 0.12	0.09 ± 0.01
Lung	1.21 ± 0.13	0.11 ± 0.02	1.07 ± 0.21	0.13 ± 0.02
Liver	1.39 ± 0.16	0.13 ± 0.03	1.29 ± 0.17	0.16 ± 0.02
Spleen	1.40 ± 0.30	0.14 ± 0.03	1.52 ± 0.32	0.19 ± 0.0
Kidney	4.78 ± 0.80	0.23 ± 0.05	4.01 ± 0.77	0.32 ± 0.04
Adrenal	1.00 ± 0.20	0.09 ± 0.02	0.85 ± 0.13	0.11 ± 0.02
Testes	0.57 ± 0.02	0.16 ± 0.03	0.50 ± 0.11	0.19 ± 0.04
Normal muscle	0.83 ± 0.09	0.78 ± 0.02	0.82 ± 0.12	0.10 ± 0.02
Infected muscle	1.22 ± 0.36	0.43 ± 0.10	0.99 ± 0.11	0.65 ± 0.14
Bone	1.10 ± 0.16	2.94 ± 0.37	1.19 ± 0.23	2.29 ± 0.14

The biodistribution of biotin 2 in rat tissues is shown in Table 3. Five min after injection without avidin, accumulation of biotin 2 in liver, kidney and adrenal was greater than blood ($p < 0.001$), heart ($p < 0.001$), spleen ($p < 0.001$), muscle ($p < 0.001$) and bone ($p < 0.001$). At 60 min accumulation of biotin 2 in bone was greater than all other organs ($p < 0.001$) and accumulation in adrenal and lung was greater than kidney ($p < 0.05$), liver ($p < 0.02$), normal muscle ($p < 0.001$) and blood ($p < 0.001$). The infected/normal tissue ratio was 0.58 ± 0.07 indicating more binding to normal muscle than to the infection (Table 4). At 5 min with avidin pretreatment, there was a decrease in accumulation of biotin 2 in adrenal ($p < 0.01$), kidney ($p < 0.001$), spleen ($p < 0.001$) and heart ($p < 0.05$) compared to untreated rats. At 60 min there was greater accumulation in blood ($p < 0.05$), heart ($p < 0.05$), spleen ($p < 0.001$), kidney ($p < 0.01$) and normal muscle ($p < 0.05$) relative to untreated rats. Avidin pretreatment did not change the infected to normal tissue ratio.

DISCUSSION

Since the report of using labeled IgG for localizing sites of infection (*13*), there has been considerable interest in using other proteins as substitutes for antibodies. In tumor models, Hnatowich et al. (*10*) injected streptavidin followed by ^{111}In-labeled biotin and achieved tumor visualization. We have investigated the possibility of using ^{18}F-labeled biotin for imaging sites of infection. PET should offer improvements in imaging because of more rigorous attenuation correction leading to more accurate quantitative capabilities.

In this study we demonstrated that ^{18}F-labeled biotin derivatives 1 and 2 could be readily prepared. The amide containing analog (1) binds to avidin and can be displaced with cold d-biotin. In contrast, the alkyl analog shows no specific binding. A scatchard transformation displacement curve yields a kd for biotin 1 of 3.12×10^{-14} M with a Bmax of 5.57×10^{-16} M.

In vivo, compound 1 shows a high selectivity (6.08 + 1.12) for infected compared to normal muscle at 60 min postinjection. This ratio increased only slightly by administration of avidin 24 hr prior to injection of ^{18}F-labeled Compound 1. Compound 2 does not localize at the site of infection.

The relatively high infected/normal tissue ratio obtained with biotin 1 at less than one hr without the use of pretar-

TABLE 2
Infected-to-Normal Tissue Ratios of ^{18}F-Biotin 1 in *E. coli* Infected Rats

	Without avidin		With avidin	
Tissue	5 min	60 min	5 min	60 min
Blood	1.30 ± 0.86	3.09 ± 0.54	0.90 ± 0.14	3.75 ± 0.75
Heart	1.21 ± 0.26	6.21 ± 0.57	1.08 ± 0.18	7.59 ± 1.34
Lung	1.00 ± 0.23	4.21 ± 0.69	1.06 ± 0.30	4.83 ± 0.91
Liver	0.89 ± 0.25	3.48 ± 0.67	0.83 ± 0.16	4.05 ± 0.65
Spleen	0.94 ± 0.49	2.98 ± 0.30	0.64 ± 0.11	3.34 ± 0.72
Kidney	0.26 ± 0.05	1.96 ± 0.24	0.28 ± 0.06	2.04 ± 0.35
Adrenal	1.06 ± 0.24	5.20 ± 1.20	1.20 ± 0.22	5.74 ± 1.14
Testes	2.14 ± 0.60	2.87 ± 0.50	2.00 ± 0.37	3.35 ± 0.56
Normal muscle	1.54 ± 0.69	6.08 ± 1.12	1.24 ± 0.20	6.39 ± 0.96
Bone	1.06 ± 0.33	0.16 ± 0.04	0.90 ± 0.18	0.28 ± 0.07

TABLE 3
Tissue Distribution of ^{18}F-Biotin 2 in *E. coli* Infected Rats

Tissue	%IG/g ± s.d.			
	Without avidin		With avidin	
	5 min	60 min	5 min	60 min
Blood	0.88 ± 0.07	0.30 ± 0.02	0.85 ± 0.04	0.25 ± 0.03
Heart	0.80 ± 0.06	0.25 ± 0.03	0.96 ± 0.10	0.20 ± 0.03
Lung	1.38 ± 0.21	0.58 ± 0.10	1.59 ± 0.16	0.55 ± 0.12
Liver	1.63 ± 0.27	0.42 ± 0.03	1.91 ± 0.22	0.55 ± 0.13
Spleen	1.70 ± 0.68	0.26 ± 0.01	0.99 ± 0.10	0.51 ± 0.09
Kidney	1.62 ± 0.18	0.57 ± 0.04	2.70 ± 0.48	1.39 ± 0.21
Adrenals	1.56 ± 0.14	0.92 ± 0.21	2.00 ± 0.24	0.72 ± 0.12
Testes	0.59 ± 0.06	0.20 ± 0.03	0.74 ± 0.11	0.15 ± 0.02
Normal muscle	0.62 ± 0.09	0.35 ± 0.09	0.69 ± 0.10	0.28 ± 0.04
Infected muscle	0.49 ± 0.05	0.20 ± 0.02	0.44 ± 0.05	0.18 ± 0.02
Bone	0.73 ± 0.09	1.17 ± 0.21	0.86 ± 0.12	1.10 ± 0.20

geting is in marked contrast to the results of previous studies with tumors and infection. In the study by Hnatowich (*10*) in which unlabeled streptavidin was administered followed by ^{111}In-labeled biotin, normal tissue levels were reduced and the tumor/normal tissue ratio was comparable to that obtained with specific antibody. Tumor/blood and tumor/liver ratios were 10.6 and 2.2 for streptavidin versus 1.5 and 0.5 for specific antibody. When labeled biotin was used without preinjection of streptavidin, biodistribution showed minimal accumulation in all tissues. Similar results were obtained with a mouse model of *E. coli* infection (*8*). Pretargeting with streptavidin followed by ^{111}In-labeled biotin gave a threefold increase in infection/normal tissue ratio (ratio = 13) compared to that obtained from either ^{111}In-labeled IgG (ratio = 4.5) or ^{111}In-labeled streptavidin (ratio = 4.4). Infection/blood and infection/liver ratios were 6.2 and 9.3 for streptavidin pretreatment versus 0.3 (0.8) and 1.1 (1.2) for ^{111}In-labeled IgG (^{111}In-streptavidin). Administration of ^{111}In-labeled biotin without pretreatment of streptavidin, showed minimal accumulation in all tissues. In our study without avidin pretreatment an infection/normal tissue ratio of 6.08 ± 1.12 was achieved with biotin 1 and our values of 3.09 ± 0.54 and 3.48 ± 0.67 for infection/blood and infection/liver compare favorably with results using labeled IgG or labeled streptavidin. A comparison of these infected thigh-to-normal tissue ratios are given in Table 5.

The significance of ^{18}F-biotin 1 localization in infection, is illustrated by a comparison of infection/normal tissue ratios of other labeled agents (Table 6). All values are reported for mice or rats with *E. coli* infection of the thigh. The agents: ^{67}Ga-citrate, labeled polyclonal IgG, antinucleus antibody (TNT-1), serum albumin and streptavidin gave infection/normal tissue ratios between 2.6 to 4.5 within 4 to 6 hr of injection (*14*). These findings are in accordance with the view that all proteins will have approximately the same uptake in inflammatory lesions via the common mechanism of leaky capillaries. For ^{111}In-biotin, with its lower affinity as evidenced by minimal uptake, pretargeting with streptavidin increased target-to-background ratio since normal tissue concentration is decreased. The greater accumulation for ^{18}F-biotin may be explained by enhanced vascular permeability naturally occurring in infected tissues.

Biotin in bacteria and higher animals is a precursor of the regulatory enzyme, acetyl CoA carboxylase, which serves

TABLE 4
Infected-to-Normal Tissue Ratios of ^{18}F-Biotin 2 in *E. coli* Infected Rats

Tissue	Without avidin		With avidin	
	5 min	60 min	5 min	60 min
Blood	0.56 ± 0.04	0.68 ± 0.06	0.51 ± 0.07	0.71 ± 0.05
Heart	0.61 ± 0.03	0.81 ± 0.05	0.46 ± 0.03	0.91 ± 0.05
Lung	0.36 ± 0.05	0.43 ± 0.20	0.28 ± 0.02	0.33 ± 0.06
Liver	0.31 ± 0.04	0.48 ± 0.33	0.23 ± 0.02	0.33 ± 0.05
Spleen	0.70 ± 0.04	0.78 ± 0.08	0.44 ± 0.05	0.35 ± 0.04
Kidney	0.30 ± 0.02	0.35 ± 0.03	0.16 ± 0.02	0.13 ± 0.02
Adrenals	0.31 ± 0.02	0.23 ± 0.05	0.22 ± 0.03	0.24 ± 0.03
Testes	0.84 ± 0.10	1.04 ± 0.11	0.59 ± 0.03	1.15 ± 0.06
Normal muscle	0.80 ± 0.12	0.58 ± 0.07	0.65 ± 0.10	0.64 ± 0.07
Bone	0.67 ± 0.05	0.17 ± 0.03	0.51 ± 0.03	0.17 ± 0.03

TABLE 5
Comparison of Infected Thigh-to-Normal Tissue Ratios

Tissue	^{111}In-IgG*	^{111}In-streptavidin*	Streptavidin/ ^{111}In-biotin*	^{18}F-biotin 1
Normal muscle	4.5	4.4	13.0	6.1
Blood	0.3	0.8	6.2	3.1
Liver	1.1	1.2	9.3	3.5
Kidney	0.8	0.3	0.8	2.0

*Reference 8.

as a carrier of carbon dioxide between remote catalytic sites (*15*). Studies with bacterial *E. coli* have shown biotin-dependent carboxylases proceeds through a carboxylated enzyme intermediate in which the site of carboxylation is at the ureido-O position on biotin (*16*). Although the amount of biotin required by some bacteria for maximal growth is small, about 1×10^{-12} mg per ml of culture, the amount needed by normal tissue may be sufficiently less. Speculatively, the greater accumulation of biotin 1 may be related to the biochemical role of biotin, since the structure of biotin 1 is similar to authentic biotin.

Our study shows that with the appropriate ^{18}F-labeled biotin derivative such as biotin 1, infection localization can be achieved simply by injection of the radioactive biotin. In less than 1 hr imaging can be performed. The lengthy approach of pretargeting with cold biotinylated antibodies and avidination of the biotinylated tumor-bound antibodies by an excess of cold avidin can be avoided. Hence, the disadvantages of the multistep protocol: repeated injections at defined time intervals and the immunogenicity of avidin can be eliminated when the reagent is used for infection localization. It will be interesting if similar behavior is observed in animal tumor models.

TABLE 6
Comparison of *E. coli* Infected Thigh-to-Normal Thigh Ratios for Various Radiopharmaceuticals

Agents	Infected/Normal muscle ratio	Time	Reference
^{67}Ga-citrate	2.6 ± 0.6	4	14
^{99m}Tc-HSA	4.1 ± 0.6	4	14
^{125}I-TNT-1	3.3 ± 0.5	4	14
^{99m}Tc-TNT-1	3.4 ± 0.08	4	14
^{99m}Tc-IgG	3.0 ± 1.1	4	14
^{111}In-IgG	4.5	6	8
^{111}In-streptavidin	4.4	6	8
Streptavidin + ^{111}In-Biotin	13	3	8
^{111}In-Biotin	minimal	3	8
^{18}F-Biotin 1	6.1 ± 1.1	1	

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