Improved Imaging of Infections by Avidin-Induced Clearance of ^{99m}Tc-Biotin-PEG Liposomes

Peter Laverman, Samuel Zalipsky, Wim J.G. Oyen, Els Th.M. Dams, Gert Storm, Nasreen Mullah, Frans H.M. Corstens, and Otto C. Boerman

Department of Nuclear Medicine, University Hospital Nijmegen, Nijmegen; Department of Pharmaceutics, Utrecht Institute for Pharmaceutical Science, Utrecht University, Utrecht, The Netherlands; and Alza Corporation, Menlo Park, California

This article describes the preparation and optimization of biotin-polyethyleneglycol (PEG) liposomes and their application in experimental infection models to improve the scintigraphic imaging of infection and inflammation. **Methods:** Biotin was coupled to PEG-distearoylphosphatidylethanolamine (DSPE) and subsequently incorporated in the PEG liposomes. Biotinylated liposomes were radiolabeled with ^{99m}Tc-hydrazinonicotinamide. In vitro binding studies were performed to find the optimal biotin concentration in the liposomes. In rats the biodistribution of the ^{99m}Tc-biotin-PEG liposomes was compared with the biodistribution of normal (nonbiotinylated) ^{99m}Tc-PEG liposomes. Furthermore, in vivo studies in rats were performed to study both the effect of the biotin content and the optimal avidin dose for efficient clearance of the liposomes. Liposomes containing 0.5 or 1.0 mol% biotin-PEG-DSPE were compared in rats with a *Staphylococcus aureus* infection in the left calf muscle. Avidin was injected 4 h after injection of the liposomes. **Results:** Biotinylation of the liposomes did not affect their in vivo behavior. All biotin-PEG liposome formulations tested showed good in vitro avidin binding with 50% inhibitory concentrations ranging from 36 to 8 μmol/L. With avidin doses higher than 100 μg, both preparations rapidly cleared from the circulation. As a result, abscess-to-blood ratios increased 5-fold. To illustrate the potential of the avidin-induced clearance of radiolabeled PEG liposomes, we also studied the ^{99m}Tc-biotin-PEG liposomes in rabbits with a subcutaneous *S. aureus* abscess. The infection was visualized only after injection of 100 μg avidin. **Conclusion:** This study shows that biotin-coated ^{99m}Tc-PEG liposomes in combination with the injection of avidin can lead to improved imaging of infection or inflammation localized especially in regions with high blood-pool activity.

Key Words: PEG liposomes; biotin liposomes; infection; biotin–avidin

J Nucl Med 2000; 41:912–918

Long-circulating liposomes, coated with polyethyleneglycol (PEG), have proven to be excellent vehicles for radiolabels to image infection and inflammation scintigraphically (*1–6*). Although the mechanism of accumulation of PEG liposomes in infections is not fully understood, the uptake of liposomes in the abscess is thought to be driven mainly by high blood levels of the liposomes. Subsequently, liposomes accumulate in the inflammatory focus as made possible by locally enhanced vascular permeability (*7*). Thus, for scintigraphic imaging of infection and inflammation, long-circulating liposomes are preferred over conventional liposomes because long-circulating liposomes are cleared from the bloodstream much more rapidly. However, as a result of their long circulatory half-life, the background activity in the blood pool, and thus in nontarget tissue, remains relatively high, even at later time points. Such high background activity may hamper visualization of infection and inflammation in, or in the vicinity of, the heart and larger vessels. Fast clearance of the liposomes will reduce the amount of radiolabeled liposomes in the circulation, which will result in lower background activity. On the other hand, it will also reduce abscess uptake. Ideally, the liposomes should clear quickly from the circulation after sufficient accumulation in the abscess, thus resulting in high abscess-to-blood ratios.

Spar et al. (*8*) were among the first to consider that injection of a second antibody a few hours after the injection of the first radiolabeled antibody could lead to the formation of high-molecular-weight immune complexes that are rapidly cleared from the circulation by the liver. Using this approach they were able to improve target-to-background ratios. This strategy was modified by Ogihara-Umeda et al. (*9*), who used the well-defined biotin–avidin interaction (*10*) to complex circulating biotinylated liposomes. Injection of avidin a few hours after administration of the liposomes led to aggregation of the liposomes, resulting in rapid clearance from the circulation by cells of the mononuclear phagocytic system. They were able to show that, using such a strategy, the visualization of tumors could be improved with radiolabeled, conventional liposomes (*9,11*). However, their strategy has neither been adapted for PEG liposomes as drug carriers nor been used for imaging of infection and inflammation. Several methods to produce biotinylated liposomes have been described: direct incorporation of a biotinylated phospholipid into the lipid bilayer, biotin linked to the lipid bilayer through a spacer molecule, and biotin conjugated to the distal end of PEG-distearoylphosphatidylethanolamine (DSPE) molecules.

In this article we report on the preparation and optimiza-

Received May 13, 1999; revision accepted Aug. 24, 1999.

For correspondence or reprints contact: Peter Laverman, Department of Nuclear Medicine, University Hospital Nijmegen, P.O. Box 9101, NL-6500 HB Nijmegen, The Netherlands.

tion of 2 different types of biotinylated PEG liposomes. First, PEG liposomes were prepared by incorporating biotinylated *N*-(biotinoyl)dipalmitoyl-L-α-phosphatidylethanolamine (DPPE) in the lipid bilayer. Second, a biotin-PEG-DSPE conjugate was prepared and thereafter incorporated in the lipid bilayer of PEG liposomes. Whereas in the first formulation the biotin is attached to the surface of the PEG liposomes, in the second formulation biotin is attached to the distal end of the surface-grafted PEG chains. In vitro avidin-binding studies were performed to determine the optimal biotin concentration in the liposomes. Furthermore, in vivo studies in rats with a *Staphylococcus aureus* infection were performed to determine the optimal avidin dose for efficient clearance of the liposomes. Additionally, the application of biotinylated liposomes was studied in an experimental model of infection in rabbits.

MATERIALS AND METHODS

Reagents

Partially hydrogenated egg phosphatidylcholine with an iodine value of 35 was a gift from Lipoïd GmbH (Ludwigshafen, Germany). DSPE and the PEG-2000 derivative of DSPE were purchased from Avanti Polar Lipids (Alabaster, AL). *N*-Hydroxysuccinimidyl-hydrazino-nicotinate hydrochloride (S-HYNIC) was synthesized as described by Abrams et al. (*12*) with minor modification (*13*). The hydrazinonicotinamide derivative of DSPE (HYNIC-DSPE) was prepared as described (*14*). Avidin was from Sigma Chemical Co. (St. Louis, MO). Biotin-DPPE was purchased from Pierce Chemical Co. (Rockford, IL). Biotin-PEG-DSPE was synthesized according to Wong et al. (*15*).

Liposome Preparation

PEG liposomes were prepared as described (*14*). Briefly, a lipid mixture of egg phosphatidylcholine (60 mol%), cholesterol (33 mol%), HYNIC-DSPE (2 mol%), and various amounts of biotin-PEG-DSPE (0.1, 0.5, or 1.0 mol%) or biotin-DPPE (0.5 mol%) was prepared in methanol and chloroform (10:1). PEG-DSPE was added to the mixture to adjust the total amount of biotin-PEG-DSPE and PEG-DSPE to 5.0 mol%. After evaporation of the organic solvents, the resulting lipid film was dispersed in phosphate-buffered saline (PBS), pH 7.4, at room temperature. Liposomes were sized by multiple extrusion through 2 stacked polycarbonate membranes with a medium-pressure extruder (Lipex Biomembranes, Inc., Vancouver, British Columbia, Canada). After sizing, the suspension was dialyzed against PBS overnight at 4°C with 4 buffer changes to remove unconjugated S-HYNIC. Liposomes were stored in PBS at 4°C.

The mean particle size of the liposomal preparations was determined by a dynamic light-scattering system (Malvern 4700; Malvern, Worcestershire, UK). The mean size of the liposomes was 90 nm with a polydispersity index of 0.1.

Radiolabeling

Labeling of the liposomes was performed as described (*14*) with minor modifications. Briefly, 250 μL liposomes (100 μmol phospholipid/mL) were added to a mixture of 10 mg *N*-tris(hydroxymethyl)-methylglycine (Tricine; Fluka, Buchs, Switzerland) and 10 μg stannous sulfate in 250 μL saline and $^{99m}TcO_4^-$ in saline (10 MBq/μmol phospholipid). The mixture was incubated for 15 min at room temperature. Radiochemical purity was determined using instant thin-layer chromatography on silica gel strips (Gelman Science, Inc., Ann Arbor, MI) with 0.15 mol/L citrate buffer, pH 5.0, as the mobile phase.

Avidin-Binding Assay

An avidin-binding assay was performed analogously to the assay described by Corley and Loughrey (*16*) with minor modifications. Briefly, 96-well plates (Costar, Cambridge, MA) were coated with avidin (1 μg/well in PBS) overnight at 4°C. Plates were washed 3 times with a mixture of PBS and 0.5% (weight/volume) bovine serum albumin and then washed once with PBS. The remaining protein-binding sites were blocked with a mixture of PBS and 0.5% (weight/volume) bovine serum albumin for 1 h at 37°C. Wells were rinsed 3 times with the same buffer and then rinsed twice with PBS. Subsequently, wells were incubated with serially diluted radiolabeled liposomes and incubated at 37°C for 1 h. Wells were washed 3 times with 0.5% (weight/volume) bovine serum albumin in PBS and washed once with PBS and counted in a shielded-well scintillation γ counter (Wizard; Pharmacia-LKB, Uppsala, Sweden).

Dose-Finding Studies

Rat Model of Infection. An abscess was induced in the left calf muscle of young, male, randomly bred Wistar rats (Harlan B.V., Horst, The Netherlands [220–240 g]) with approximately 1×10^9 colony-forming units of *S. aureus* (ATCC 25923; American Type Culture Collection, Rockville, MD) in a 0.1-mL 1:1 suspension of autologous blood and normal saline. During the procedure, animals were anesthetized with ether. After 24 h, when swelling of the muscle was apparent, the radiolabeled liposomes were injected intravenously through the tail vein. Avidin was administered through the tail vein 4 h after injection of the radiolabeled liposomes unless stated otherwise.

γ Camera Imaging and Biodistribution Studies. Twenty-four hours after *S. aureus* inoculation, 2 sets of 12 rats received 15 MBq ^{99m}Tc-biotin-PEG liposomes (0.5 or 1.0 mol% biotin-PEG-DSPE) through the tail vein. Four hours after injection of the liposomes, each set was divided in 4 groups (3 rats/group) and received 10, 30, 100, or 300 μg avidin intravenously. Rats were anesthetized with a mixture of enflurane (Ethrane; Baxter Pharmaceutical Products, New Providence, NJ), nitrous oxide, and oxygen and placed prone on a γ camera (Orbiter; Siemens Medical Systems, Hoffman Estates, IL) equipped with a parallel-hole, low-energy collimator. Images were recorded 30 min after injection of the avidin. The images (300,000 counts) were stored digitally in a 256 × 256 matrix.

After recording the final images, rats were killed by an intraperitoneal injection of 30 mg sodium phenobarbital, and the biodistribution of the radiolabel was determined quantitatively. A blood sample was obtained by cardiac puncture. After cervical dislocation, samples of infected left calf muscle, uninfected contralateral muscle, lung, spleen, kidney, liver, and small intestine were dissected, weighed, and counted in a shielded-well scintillation γ counter. To correct for physical decay and to permit calculation of the uptake of the radiolabeled liposomes, injection standards were counted simultaneously. Results are expressed as percentage injected dose per gram (%ID/g) tissue. Abscess-to-contralateral muscle and abscess-to-blood ratios were calculated.

Rabbit Model of Infection

A subcutaneous infection was induced in rabbits as described (*17*). Briefly, 3 female New Zealand White rabbits (De Vaan B.V.,

Bergharen, The Netherlands) weighing 2.5–2.8 kg were inoculated subcutaneously in the neck with 0.5×10^{11} colony-forming units of *S. aureus* (ATCC 25923) in a 0.4-mL 1:1 suspension of autologous blood and normal saline. The rabbits' necks were shaved before the procedure; during the procedure the animals were anesthetized with a subcutaneous injection of a 0.6-mL mixture of 0.315 mg/mL of fentanyl and 10 mg/mL of fluanisone (Hypnorm; Janssen Pharmaceutical, Buckinghamshire, UK). Seventy-two hours later, when swelling in the neck region was palpable, rabbits were injected with 37 MBq ^{99m}Tc-biotin-PEG liposomes (0.5 mol% biotin-PEG-DSPE). Fours hours later, the rabbits were immobilized and injected intravenously with 100 μg avidin. Anterior and lateral images were recorded with a γ camera just prior to and 30 min after the injection of avidin.

Statistical Analysis

All mean values are expressed as mean ± SEM. Statistical analysis was performed using a 1-way ANOVA. The level of significance was set at $P < 0.05$.

RESULTS

To investigate the in vitro avidin-binding capacity, the liposomal formulations were tested in an in vitro binding assay. As shown in Figure 1, the liposomes with 0.5 mol% biotin-DPPE incorporated in the lipid bilayer showed only limited binding to the immobilized avidin. The 50% inhibitory concentration (IC_{50}) was at least 1000-fold higher than was the IC_{50} of the 3 biotin-PEG-DSPE liposomes. The 3 liposome preparations with biotin-PEG-DSPE showed a much higher avidin-binding affinity. The formulation with 0.1 mol% biotin-PEG-DSPE incorporated in the lipid bilayer displayed an IC_{50} of 36 μmol/L (data not shown). The preparations with 0.5 and 1.0 mol% biotin-PEG-DSPE showed a higher avidin-binding affinity with IC_{50} values of 8 and 11 μmol/L, respectively. Because of the poor avidin-binding capacities of both the PEG liposomes with biotin-DPPE incorporated in the lipid bilayer and the PEG liposomes with 0.1 mol% biotin-PEG-DSPE, these formulations were not studied further in vivo.

To investigate whether the incorporation of biotin-PEG-DSPE in the liposomes had influenced the in vivo behavior of the long-circulating liposomes, ^{99m}Tc-biotin-PEG liposomes (1.0 mol% biotin-PEG-DSPE) were compared with radiolabeled nonbiotinylated PEG liposomes in rats with an *S. aureus* infection in the left calf muscle. The results of the tissue biodistribution studies are summarized in Table 1. No significant differences were found in biodistribution, except for uptake in the liver and the small intestine at 24 h after injection. Uptake in the liver was slightly higher for the biotinylated PEG liposomes, whereas uptake in the small intestine was lower for the biotin-PEG liposomes. These results indicate that the biotin-PEG-DSPE incorporation in the lipid bilayer had no major influence on the in vivo behavior of the liposomes.

Further studies to investigate the effect of the biotin content in the liposomes and the effect of the avidin dose on the clearance of the liposomes after injection of avidin were performed in rats with an *S. aureus* infection. Results of the tissue biodistributions of both the 0.5 and 1.0 mol% ^{99m}Tc-biotin-PEG-DSPE liposome preparations are presented numerically in Table 2 and presented in part graphically in Figure 2. Injection of 10 μg avidin did not affect the long-circulating characteristics of both preparations. Administration of 30 μg avidin instead of 10 μg did not alter the biodistribution of the 0.5 mol% biotin-PEG liposomes. However, it did so in the case of the 1.0 mol% biotin-PEG liposomes. The uptake in all organs decreased significantly, whereas the liver and spleen uptake increased 2-fold. A further increase to 100 μg avidin led to a significant decrease of activity in the blood, muscle, lungs, and kidneys, findings similar to those observed in the rats injected with 0.5 mol% biotin-PEG liposomes, with also a sharp increase of liver

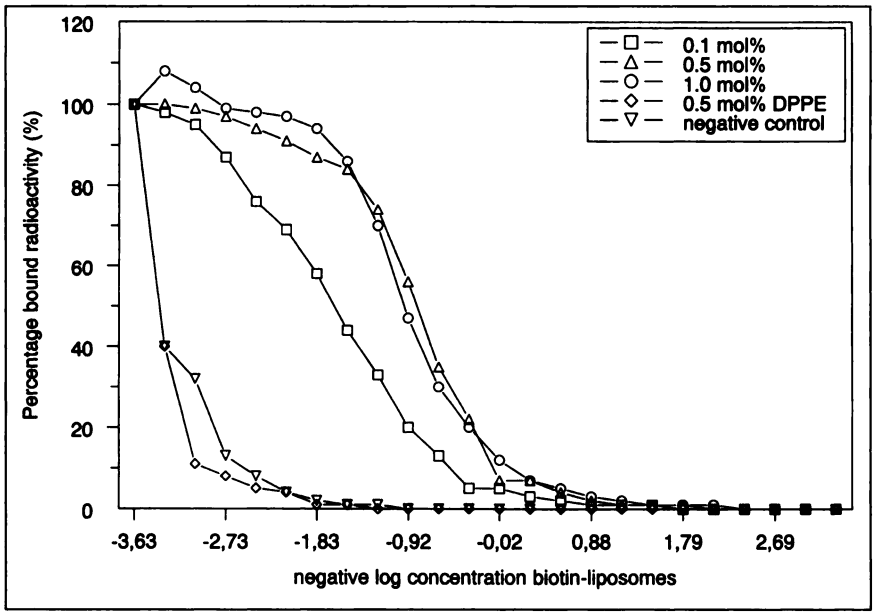

FIGURE 1. In vitro binding to avidin of 3 biotin-PEG liposome preparations and liposome formulation in which biotin is coupled directly to DPPE. PEG liposomes without biotin served as negative control.

TABLE 1

Biodistribution, Abscess-to-Muscle Ratios, and Abscess-to-Blood Ratios of ^{99m}Tc-Biotin-PEG Liposomes and ^{99m}Tc-PEG Liposomes in Rats with *S. aureus* Infection in Left Calf Muscle 5 and 24 Hours After Injection

Parameter	5 h after injection			24 h after injection		
	Biotin-PEG liposomes	*P*	PEG liposomes	Biotin-PEG liposomes	*P*	PEG liposomes
Blood	3.05 ± 0.09	NS	2.91 ± 0.09	1.23 ± 0.20	NS	1.22 ± 0.12
Muscle	0.06 ± 0.00	NS	0.06 ± 0.01	0.05 ± 0.00	NS	0.04 ± 0.00
Abscess	1.10 ± 0.04	NS	1.04 ± 0.08	1.34 ± 0.20	NS	1.64 ± 0.25
Lung	0.69 ± 0.08	NS	0.72 ± 0.04	0.36 ± 0.08	NS	0.35 ± 0.04
Spleen	3.21 ± 0.23	NS	3.07 ± 0.25	5.02 ± 0.63	NS	3.86 ± 0.37
Kidney	0.67 ± 0.08	NS	0.84 ± 0.05	0.71 ± 0.09	NS	0.74 ± 0.06
Liver	0.82 ± 0.06	NS	0.70 ± 0.03	0.90 ± 0.09	<0.05	0.66 ± 0.02
Small intestine	0.68 ± 0.03	NS	0.64 ± 0.04	0.52 ± 0.05	<0.01	0.93 ± 0.10
Abscess-to-muscle ratio	20.9 ± 2.6	NS	18.7 ± 3.5	29.9 ± 3.5	NS	36.9 ± 5.3
Abscess-to-blood ratio	0.36 ± 0.01	NS	0.36 ± 0.03	1.10 ± 0.08	NS	1.32 ± 0.09

NS = not significant.
Data are expressed as %ID/g ± SEM (n = 5 rats/group).

uptake. In rats with 1.0 mol% biotin-PEG liposomes, activity levels in the lungs and kidneys decreased even further with increasing avidin dose, whereas splenic uptake rose to 14.5 ± 0.4 %ID/g. Administration of 300 μg avidin did not further enhance the blood clearance in rats injected with 0.5 mol% biotin-PEG liposomes, whereas the 1.0 mol% liposomes were cleared slightly more efficiently. However, in these rats, abscess uptake was reduced as well.

Because of the efficient clearance of the ^{99m}Tc-biotin-PEG liposomes from the circulation, through the administration of avidin, abscess-to-blood ratios increased markedly with increasing avidin doses. After administration of 100 μg

TABLE 2

Biodistribution, Abscess-to-Muscle Ratios, and Abscess-to-Blood Ratios of ^{99m}Tc-Biotin-PEG Liposomes with Various Concentrations of Biotin-PEG-DSPE Incorporated in Lipid Bilayer After Injection of Different Avidin Doses 4 Hours After Injection of ^{99m}Tc-Biotin-PEG Liposomes and 30 Minutes After Injection of Avidin

Parameter	Avidin (μg)			
	10	30	100	300
0.5 mol% biotin-PEG-DSPE				
Blood	4.44 ± 0.27	3.21 ± 0.75	0.72 ± 0.12	0.68 ± 0.03
Muscle	0.07 ± 0.01	0.07 ± 0.01	0.03 ± 0.00	0.03 ± 0.01
Abscess	1.72 ± 0.10	1.68 ± 0.10	1.34 ± 0.16	1.52 ± 0.28
Lung	1.09 ± 0.09	0.85 ± 0.17	0.26 ± 0.02	0.25 ± 0.02
Spleen	4.59 ± 0.54	5.77 ± 0.19	7.13 ± 0.92	7.54 ± 0.65
Kidney	1.16 ± 0.03	0.85 ± 0.12	0.43 ± 0.04	0.46 ± 0.05
Liver	1.53 ± 0.26	3.21 ± 0.65	6.86 ± 0.29	6.41 ± 0.74
Small intestine	0.75 ± 0.06	0.64 ± 0.08	0.58 ± 0.02	0.74 ± 0.03
Abscess-to-muscle ratio	26.2 ± 1.4	24.6 ± 2.19	40.9 ± 6.0	57.9 ± 14.3
Abscess-to-blood ratio	0.39 ± 0.05	0.56 ± 0.10	1.99 ± 0.43	2.26 ± 0.44
1.0 mol% biotin-PEG-DSPE				
Blood	4.57 ± 0.23	2.00 ± 0.06	0.70 ± 0.09	0.52 ± 0.03
Muscle	0.11 ± 0.01	0.06 ± 0.01	0.09 ± 0.01	0.05 ± 0.01
Abscess	2.35 ± 0.13	2.34 ± 0.15	1.80 ± 0.50	1.31 ± 0.09
Lung	1.04 ± 0.14	0.55 ± 0.06	0.36 ± 0.03	0.27 ± 0.02
Spleen	4.50 ± 0.38	8.58 ± 0.79	14.5 ± 0.4	12.6 ± 1.3
Kidney	1.71 ± 0.10	1.05 ± 0.05	0.78 ± 0.03	0.61 ± 0.02
Liver	3.50 ± 0.06	9.27 ± 0.46	9.34 ± 0.10	8.11 ± 0.15
Small intestine	1.34 ± 0.02	1.45 ± 0.25	0.77 ± 0.10	1.18 ± 0.03
Abscess-to-muscle ratio	22.6 ± 1.4	38.0 ± 5.9	21.0 ± 7.5	28.1 ± 1.7
Abscess-to-blood ratio	0.52 ± 0.01	1.17 ± 0.05	2.51 ± 0.41	2.51 ± 0.20

Data are expressed as %ID/g ± SEM (n = 3 rats/group).

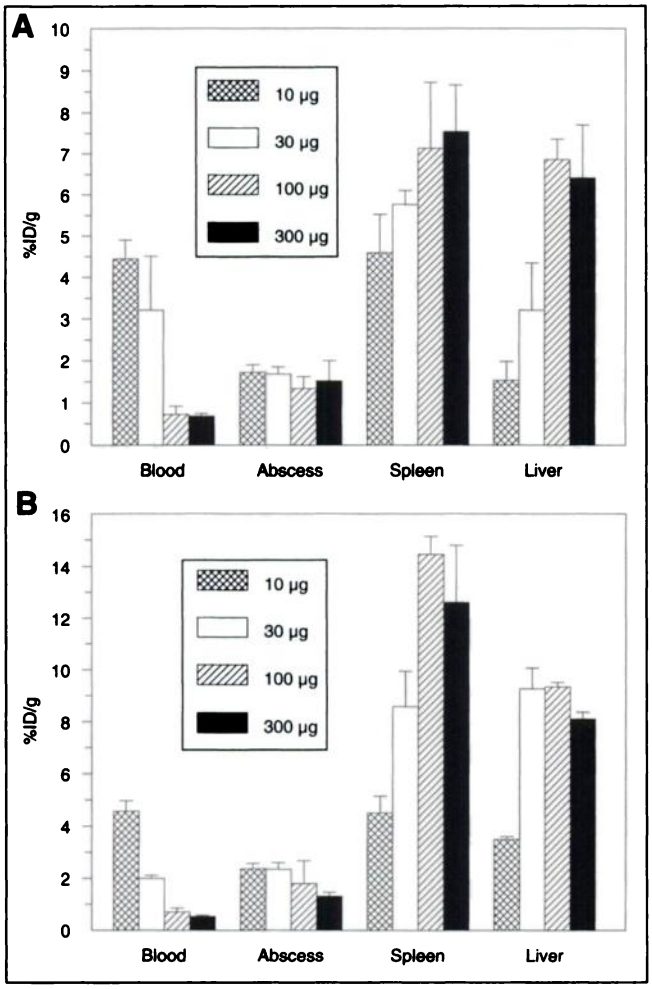

FIGURE 2. Biodistributions of 0.5 mol% ^{99m}Tc-biotin-PEG liposomes (A) and 1.0 mol% ^{99m}Tc-biotin-PEG liposomes (B) in rats with an *S. aureus* infection in left calf muscle.

avidin, abscess-to-blood ratios increased to values as high as 1.99 ± 0.43 and 2.51 ± 0.41 for the 0.5 and 1.0 mol% biotin-PEG liposomes, respectively, representing a 5-fold increase compared with the ratios obtained with biotinylated PEG liposomes (0.36 ± 0.01) 5 h after injection without subsequent administration of avidin. Ratios obtained with the biotin-PEG liposomes after injection of 100 µg avidin 4 h after injection were 2-fold higher than were the ratios obtained 24 h after injection (1.32 ± 0.09) with the nonbiotinylated PEG liposomes. Administration of 300 µg avidin did not lead to a significant increase of the abscess-to-blood ratios compared with the 100 µg dose.

The biodistribution of the radiolabeled biotin-PEG liposomes (0.5 and 1.0 mol%) was also studied scintigraphically. As depicted in Figure 3, for both preparations, no enhanced clearance was observed after injection of 10 µg avidin, meaning that the heart was still quite visible. Administration of 30 µg avidin only marginally enhanced the clearance of the 0.5 mol% biotin-PEG liposomes, whereas the 1.0 mol% group showed major avidin-induced clearance, visible as a strongly reduced activity level in the heart. Almost complete clearance of radioactivity from the heart was achieved after injections of 100 and 300 µg avidin. The abscess was visible at all avidin dose levels, and no decrease of radioactivity in the abscess was observed. Apparently, the liposomes cleared mainly to the liver and spleen as shown by increased activity in these organs.

Avidin-induced clearance of PEG liposomes may be useful in situations in which visualization of the abscess is hampered by high background radioactivity. This application is illustrated in rabbits with a subcutaneous *S. aureus* abscess. The infection was located in the neck, superimposed over the heart region, and anterior imaging with ^{99m}Tc-biotin-PEG liposomes did not visualize the infection. However, after administration of 100 µg avidin, the infection became visible (Fig. 4). The lateral images show that this uptake is caused by accumulation in the abscess and not by residual radioactivity in the heart region.

DISCUSSION

This study aimed to optimize the use of radiolabeled PEG liposomes in infection imaging by reducing the background

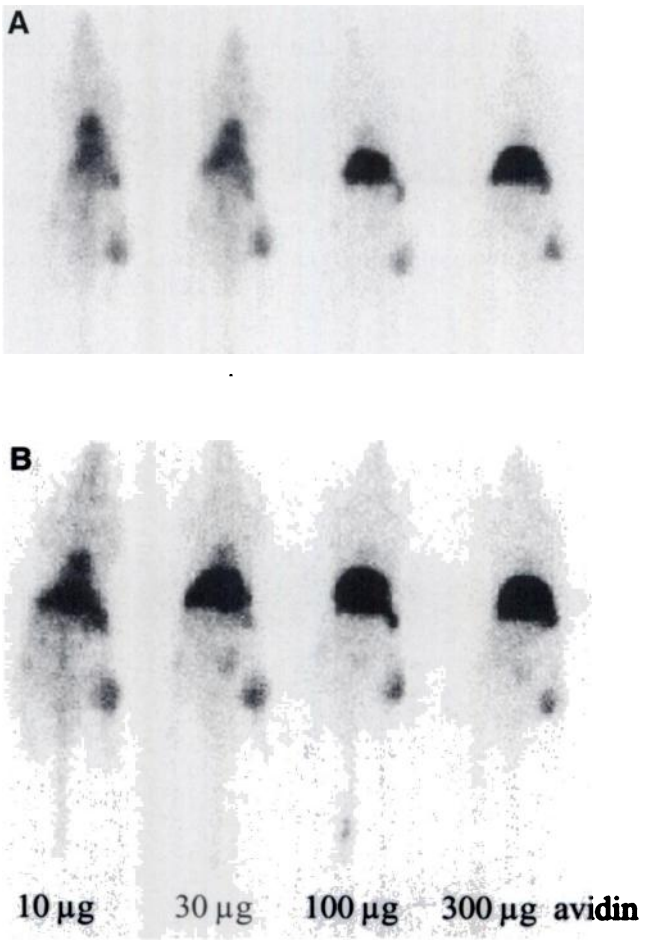

FIGURE 3. Scintigraphic images of rats with unilateral *S. aureus* abscess in calf muscle recorded 4 h after injection of 0.5 mol% ^{99m}Tc-biotin-PEG liposomes (A) or 1.0 mol% ^{99m}Tc-biotin-PEG liposomes (B) and 30 min after administration of various doses of avidin.

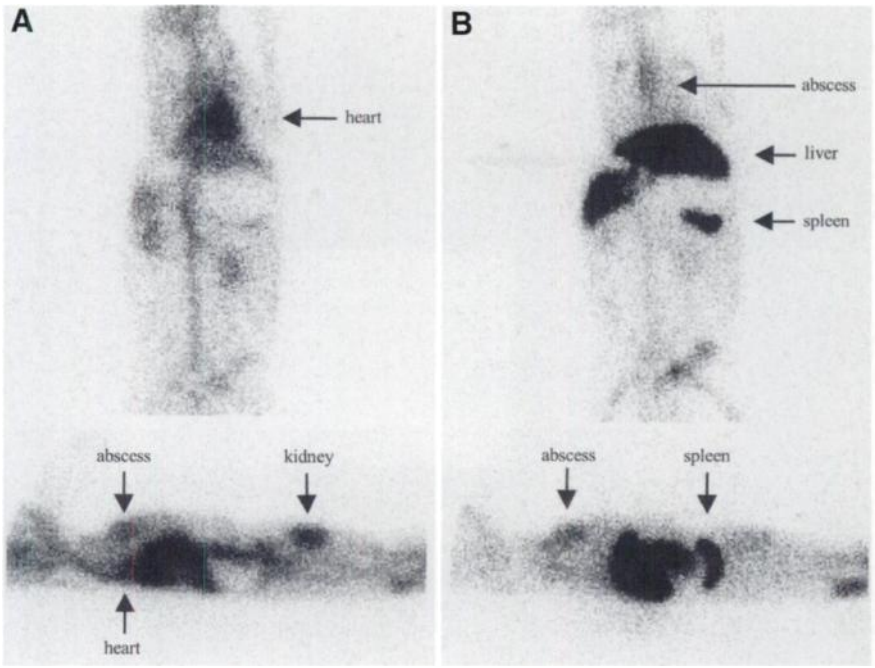

FIGURE 4. Scintigraphic images of rabbits with subcutaneous *S. aureus* infection in neck 4 h after injection of 0.5 mol% ^{99m}Tc-biotin-PEG liposomes and 30 min after administration of 100 μg avidin. Images before (A) and after (B) avidin injection are shown. Top panel shows anterior images, and bottom panel shows left lateral images.

activity and thus obtaining higher target-to-nontarget ratios. The optimization was based on the second antibody and the biotin–avidin system. Both of these approaches have been used widely to improve diagnostic imaging and radioimmunotherapy (*8,10*). In previous studies, radiolabeled biotin-coated liposomes without PEG modification have been used for tumor imaging (*9,11*). In a series of experimental models of infection we have shown (*1–6*) that long-circulating PEG liposomes provide excellent localization in infections and in sites of noninfectious inflammation. To try to further improve image quality, we adapted the biotin–avidin system to PEG liposomes.

To optimize avidin-induced clearance of biotin-PEG liposomes, the biodistributions of the preparations were studied in a rat model of bacterial infection. No major differences were observed between radiolabeled (standard) nonbiotinylated PEG liposomes and radiolabeled biotinylated PEG liposomes. Furthermore, the optimal avidin dose for effective clearance of liposomes from the circulation was determined. Major clearance of the liposomes was achieved at avidin doses of 100 μg or higher. After injection of avidin, blood levels were reduced by a factor of 7–9, in accordance with the results of Goins et al. (*18*) that were obtained in rabbits with experimental colitis after administration of biotin-coated liposomes.

Increasing the avidin dose from 100 to 300 μg did not further improve abscess-to-blood ratios. Apparently, there is an optimum in avidin concentration to form biotin–avidin aggregates. Increasing the avidin concentration will not further improve aggregation of the liposomes but, rather, will lead to competition of the avidin molecules to bind a biotin molecule. In this study, 100 μg avidin was sufficient to induce efficient liposome aggregation.

The study in rabbits with subcutaneous *S. aureus* infection clearly illustrates the potential of the biotin-coated radiolabeled PEG liposomes to visualize infection in the vicinity of the heart and major blood vessels. The infectious focus was obscured by the activity in the blood pool before injection of the avidin but became visible after avidin-induced clearance. Obviously, the visualization of infections in, or in the vicinity of, the liver and spleen cannot be improved with this approach because with avidin there is a shift of activity from the blood to these organs. In spite of this disadvantage, we consider the biotin–avidin approach particularly promising to improve the visualization of typical infections, such as infected vascular grafts and endocarditis.

In this study we showed that radiolabeled PEG liposomes containing biotin-DPPE have only limited affinity for avidin, whereas Ogihara-Umeda et al. (*9*) observed good avidin-binding capacity using the same biotinylation method with liposomes without PEG coating. Most likely, the PEG coating of the liposomes hampered the biotin–avidin interaction. Loughrey et al. (*19*) provided direct evidence that incorporation of PEG-2000–DSPE in biotin-DSPE liposomes causes a 67% decrease in avidin-binding affinity. In their efforts to improve avidin binding, they found that the avidin-binding capacity of PEG liposomes improved only marginally when a 6-carbon spacer arm between the biotin and the DSPE was used. Apparently, the bulky PEG moieties still caused steric hindrance, thus hampering the binding of streptavidin to the biotin molecule (*16,20*). A new approach to avoid PEG-mediated steric hindrance effects has been described by Blume et al. (*21*). They encountered the same problem when preparing antibody-coated PEG liposomes: Steric hindrance of the PEG tails reduced the antigen-binding affinity of the Stealth liposomes (*21*). This phenomenon was avoided by coupling the antibodies to the terminus of the PEG-DSPE molecule. Zalipsky et al. (*22*) and

Gabizon et al. (*23*) have reported the same strategy. Apparently, coupling the biotin to the end of the PEG tails greatly improves the interaction of biotin with avidin.

In preclinical studies we have shown that optimal liposome accumulation in the abscess is observed 24 h after injection. However, when the avidin is administered 24 h after injection of the liposomes, no accelerated clearance of the liposomes was induced (data not shown). Therefore, in all our studies, the avidin was injected 4 or 5 h after injection of the radiolabeled liposomes. Possibly, the biotinylated liposomes are susceptible to biotinidase activity in the circulation (*24*).

CONCLUSION

This study shows that the use of avidin 4–5 h after administration of biotin-coated ^{99m}Tc-PEG liposomes leads to improved imaging of infection and inflammation localized in regions in or near the blood pool. This high blood-pool activity can be strongly reduced by injection of avidin. Because the uptake of the biotin liposomes in the abscess is optimal after 24 h, further studies are necessary to develop biotin liposomes that will be cleared by avidin after such a time span.

ACKNOWLEDGMENTS

The authors thank Gerrie Grutters and Bianca Lemmers-de Weem (Central Animal Laboratory, University of Nijmegen, The Netherlands) for assistance in the animal experiments and Emile Koenders for technical assistance. The study was supported by grant NGN 55.3665 from the Technology Foundation (Technologiestichting STW), The Netherlands.

REFERENCES

1. Boerman OC, Storm G, Oyen WJG, et al. Sterically stabilized liposomes labeled with indium-111 to image focal infection. *J Nucl Med.* 1995;36:1639–1644.
2. Oyen WJG, Boerman OC, Storm G, et al. Detecting infection and inflammation with technetium-99m-labeled Stealth liposomes. *J Nucl Med.* 1996;37:1392–1397.
3. Oyen WJG, Boerman OC, Storm G, et al. Labelled Stealth liposomes in experimental infection: an alternative to leukocyte scintigraphy? *Nucl Med Commun.* 1996;17:742–748.
4. Boerman OC, Oyen WJG, Storm G, et al. Technetium-99m labelled liposomes to image experimental arthritis. *Ann Rheum Dis.* 1997;56:369–373.
5. Dams ETM, Oyen WJG, Boerman OC, et al. Technetium-99m-labeled liposomes to image experimental colitis in rabbits: comparison with technetium-99m-HMPAO-granulocytes and technetium-99m-HYNIC-IgG. *J Nucl Med.* 1998;39:2172–2178.
6. Laverman P, Boerman OC, Oyen WJG, Dams ETM, Storm G, Corstens FHM. Liposomes for scintigraphic detection of infection and inflammation. *Adv Drug Deliv Rev.* 1999; 37:225–235.
7. Maeda H, Seymour LW, Miyamoto Y. Conjugates of anticancer agents and polymers: advantages of macromolecular therapeutics in vivo. *Bioconj Chem.* 1992;3:351–362.
8. Spar IL, Goodland RL, Desiderio MA. Immunological removal of circulating ^{131}I-labeled rabbit antibody to rat fibrinogen in normal and tumor-bearing rats. *J Nucl Med.* 1964;5:428–443.
9. Ogihara-Umeda I, Sasaki T, Nishigori H. Active removal of radioactivity in the blood ciruculation using biotin-bearing liposomes and avidin for rapid tumour imaging. *Eur J Nucl Med.* 1993;20:170–172.
10. Hnatowich DJ, Virzi F, Rusckowski M. Investigations of avidin and biotin for imaging applications. *J Nucl Med.* 1987;28:1294–1302.
11. Ogihara-Umeda I, Sasaki T, Toyama H, Oda K, Senda M, Nishigori H. Rapid tumor imaging by active background reduction using biotin-bearing liposomes and avidin. *Cancer Res.* 1994;54:463–467.
12. Abrams MJ, Juweid M, ten Kate CI, et al. Technetium-99m-human polyclonal IgG radiolabeled via the hydrazino nicotinamide derivative for imaging focal sites of infection in rats. *J Nucl Med.* 1990;31:2022–2028.
13. Claessens RAMJ, Boerman OC, Koenders EB, Oyen WJG, van der Meer JWM, Corstens FHM. Technetium-99m labelled hydrazinonicotinamido human non-specific polyclonal immunoglobulin G for detection of infectious foci: a comparison with two other technetium labelled imunnoglobulin preparations. *Eur J Nucl Med.* 1996;23:414–421.
14. Laverman P, Dams ETM, Oyen JWG, et al. A novel method to label liposomes with ^{99m}Tc by the hydrazino nicotinyl derivative. *J Nucl Med.* 1999;40:192–197.
15. Wong JW, Kuhl TL, Israelachvili JN, Mullah N, Zalipsky S. Direct measurement of tethered ligand-receptor interaction potential. *Science.* 1997;275:820–822.
16. Corley P, Loughrey HC. Binding of biotinated-liposomes to streptavidin is influenced by liposome compostion. *Biochim Biophys Acta.* 1994;1195:149–156.
17. Sharar SR, Winn RK, Murry CE, Harlan JM, Rice CL. A CD18 monoclonal antibody increases the incidence and severity of subcutaneous abscess formation after high-dose *Staphylococcus aureus* injection in rabbits. *Surgery.* 1991;110:213–220.
18. Goins B, Awasthi VD, Klipper R, Phillips WT. Use of a technetium-99m-(Tc-99m)-labeled biotin-liposome/avidin system in a rabbit colitis model to improve early image detection [abstract]. *J Nucl Med.* 1998;39:125P.
19. Loughrey HC, Ferraretto A, Cannon A-M, et al. Characterisation of biotinylated liposomes for in vivo targeting applications. *FEBS Lett.* 1993;332:183–188.
20. Ahmad I, Allen TM. Antibody-mediated specific binding and cytotoxicity of liposome-entrapped doxorubicin to lung cancer cells *in vitro*. *Cancer Res.* 1992;52:4817–4820.
21. Blume G, Cevc G, Crommelin MDJA, Bakker-Woudenberg IAJM, Kluft C, Storm G. Specific targeting with poly(ethylene glycol)-modified liposomes: coupling of homing devices to the ends of the polymeric chains combines effective target binding with long circulation times. *Biochim Biophys Acta.* 1993;1149:180–184.
22. Zalipsky S, Mullah N, Harding JA, Gittelman J, Guo L, DeFrees SA. Poly(ethylene glycol)-grafted liposomes with oligopeptide or oligosaccharide ligands appended to the termini of the polymer chains. *Bioconj Chem.* 1997;8:111–118.
23. Gabizon A, Horowitz AT, Goren D, et al. Targeting folate receptor with folate linked to extremities of poly(ethyleneglycol)-grafted liposomes: in vitro studies. *Bioconj Chem.* 1999;10:289–298.
24. Thoma RW, Peterson WH. The enzymatic degradation of soluble bound biotin. *J Biol Chem.* 1954;210:569–579.