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Inhibition of Renal Uptake of Indium-111-DTPA-Octreotide In Vivo

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Indium-111-DTPA-octreotide has been successfully used for imaging of somatostatin receptor-positive lesions. However, significant renal uptake of ^{111}In-DTPA-octreotide exists, reducing the scintigraphic sensitivity for detection of small tumors in the perirenal region and the possibilities for radiotherapy. The aim of the present study was to determine whether renal uptake of ^{111}In-DTPA-octreotide could be reduced in vivo in rats. **Methods:** Male Wistar rats (200–250 g) were placed in metabolic cages and injected with ^{111}In-DTPA-octreotide (0.2 MBq and 0.5 μg octreotide), in the presence or absence of re-uptake blockers. At time t = 20 hr after injection, rats were sacrificed and organs were isolated and counted for radioactivity. **Results:** Adding NH_4Cl or $NaHCO_3$ to the food, resulting in the production of more acid or alkaline urine respectively, resulted in less radioactivity in the kidneys 20 hr after injection compared to controls. Lysine in a single dose of 400 mg/kg resulted in an inhibition of kidney uptake of 40%. When lysine was injected 30 min before ^{111}In-DTPA-octreotide, the inhibition was 25%. Arginine had less effect on tubular uptake of ^{111}In-DTPA-octreotide than lysine (20% inhibition). Sodium maleate inhibited kidney uptake of ^{111}In-DTPA-octreotide most successfully. Acetazolamide (100 mg/kg), succinylacetone (100 mg/kg), cystine dimethylester (340 mg/kg) and increase in urinary flow did not influence ^{111}In-DTPA-octreotide retention in the kidneys. **Conclusion:** It appeared possible to reduce re-uptake of ^{111}In-DTPA-octreotide in the rat kidney in vivo. The most pronounced effects were seen after administration of sodium maleate or lysine but, because of the described toxic effects of maleate, we will study further only the effects of lysine in a clinical setting.

Key Words: indium-111-DTPA-octreotide; renal tubular re-uptake; sodium maleate; lysine; urine pH

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Indium-111-DTPA-octreotide is a radiopharmaceutical that binds to somatostatin receptors (subtypes 2, 3 and 5) present in certain tissues. It is being used for scintigraphic imaging of somatostatin receptor-positive lesions, such as gastrointestinal pancreatic tumors, neuroblastoma, pheochromocytoma, breast cancer, Hodgkin's lymphoma and small-cell lung cancer (*1,2*). This peptide is cleared from the body mostly by the kidneys, 50% within the first four hours after injection. However, a significant amount of the dose accumulates in the renal parenchyma (in humans about 7% dose, 4 hr after injection), reducing the scintigraphic sensitivity for detection of small tumors in the perirenal region in the abdomen (*3*).

In this study we investigated if this renal accumulation could be reduced in vivo in rats. The infusion of certain amino acids, particularly lysine and arginine, has been shown to block renal tubular peptide re-absorption (*4*). An infusion of synthetic amino acids, containing lysine and arginine among other amino acids, significantly reduced parenchymal uptake (up to 50%) of ^{111}In-DTPA-octreotide 4 hr after injection in eight patients, without an effect on glomerular filtration rate (*5*). Also in mice, reduction of renal tubular reabsorption of ^{111}In-labeled Fab fragment was affected by systemic administration of lysine (*6*). We have, therefore, tested the influence of a single dose of lysine or arginine on renal tubular uptake of ^{111}In-DTPA-octreotide in vivo in the rat. We also investigated the influences of: increased urine production; changes in the pH of the urine, the latter by addition of NaCl, $NaHCO_3$ or NH_4Cl to the food; and sodium maleate on the re-uptake process. Maleate is known to produce a generalized defect of renal tubular transport, causing an immediate and transient diuresis, natriuresis, glucosuria and proteinuria, probably by inhibition of renal cortical Na-K-ATPase and ATP production (*7–9*). In addition, we tested the compounds succinylacetone, acetazolamide and cys-

TABLE 1
Distribution of Indium-111-DTPA-Octreotide in Organs of Control Rats 20 Hours after Administration*

Organ	Dose (%ID/g)
Blood	0.0019 ± 0.0003
Kidneys	1.52 ± 0.15
Liver	0.061 ± 0.012
Pancreas	0.52 ± 0.12
Spleen	0.03 ± 0.005
Adrenals	0.76 ± 0.10

*0.2 MBq and 0.5 μg octreotide; mean ± s.d., n = 8-29.

tine dimethylester, because these compounds have been described to interfere with renal uptake processes (*10–12*).

MATERIALS AND METHODS

Radiolabeling and Radiopharmaceutical Quality Control

[DTPA-D-Phe^1]octreotide and $^{111}InCl_3$ (DRN 4901, 370 MBq/ml in HCl, pH 1.5–1.9) were obtained from Mallinckrodt Medical (Petten, The Netherlands). The radiolabeling procedure was performed as described earlier (*13*).

Tissue Distribution and Specific Binding of Indium-111-DTPA-Octreotide

Male Wistar rats (200–250 g) were placed in metabolic cages 24 hr before the start of the experiment. Rats were fed either dry rat chow ad libitum or 35 g/day chow suspension in water (35 g = 14 g chow in 21 ml water). Drinking water was always available ad libitum.

At time t = 0, rats were anaesthetized with ether and injected with ^{111}In-DTPA-octreotide (0.2 MBq and 0.5 μg octreotide), preceeded or not by injection of uptake-blocking compounds (listed below), into the dorsal vein of the penis (volume 200 μl). In order to study nonspecific binding, some rats were injected subcutaneously with 0.5 mg octreotide in 1 ml 0.05 *M* acetic acid in 154 m*M* NaCl, 40 min before injection of ^{111}In-DTPA-octreotide.

Twenty hours after injection of the radiolabeled product, rats were sacrificed with ether and organs were isolated. Tissue distribution was studied by measuring radioactivity in isolated organs as well as in blood samples.

Statistical evaluation was performed using one-way analysis of variance followed by comparison among class means and Student's t-test, corrected for multiple pairwise comparisons between means. Results are expressed as mean ± s.d.

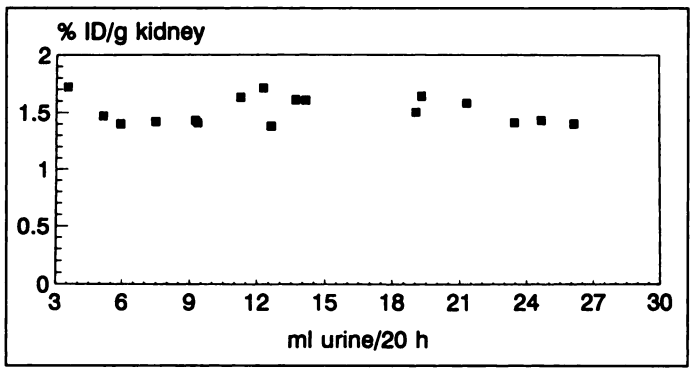

FIGURE 1. Influence of urine production during the experiment on kidney uptake of ^{111}In-DTPA-octreotide (n = 17).

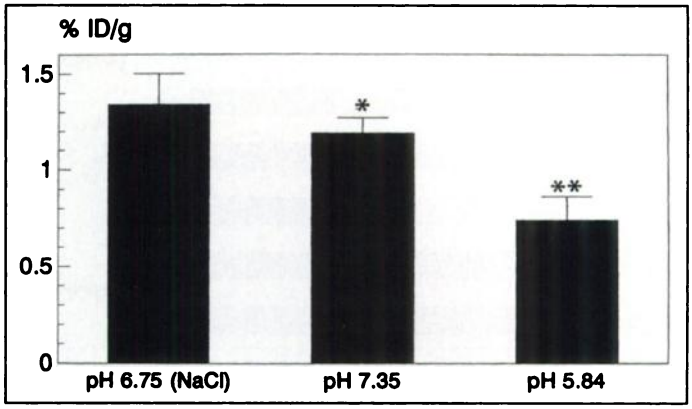

FIGURE 2. Influence of urinary pH on kidney uptake of ^{111}In-DTPA-octreotide. *p < 0.05; **p < 0.005 versus control (NaCl), all groups n = 3.

Methods and Compounds Used to Reduce Renal Uptake of Indium-111-DTPA-Octreotide

Rats were fed either dry rat chow or, to increase urine production, 35 g/day chow suspension in water (35 g = 14 g chow in 21 ml water). Rats were given intravenous injections of: positively charged amino acids L-lysine (400 mg/kg) or L-arginine (460 mg/kg), that can bind to negatively charged sites on renal tubular membranes; and compounds that have been described to interfere with renal uptake processes—sodium maleate (200, 400 and 600 mg/kg), acetazolamide (100 mg/kg), succinylacetone (100 mg/kg) and cystine dimethylester (340 mg/kg). Also, NaCl (control), $NaHCO_3$ or NH_4Cl was added to the food, during the 48 hr before the start of the experiment, in order to induce changes in the pH of the urine.

All compounds injected intravenously were administered at physiological pH in volumes of 200 μl immediately before ^{111}In-DTPA-octreotide injection, unless otherwise stated.

RESULTS

In Table 1, the distribution of radioactivity in organs of control rats is shown, expressed as percentage injected dose (%ID)/g tissue, 20 hr after administration of ^{111}In-DTPA-octreotide. Under the conditions used in our experiments, excretion into the urine is ≥70% dose within 20 hr. Radioactivity in the kidneys is 1.52% ID/g at this time.

In Figure 1, the influence of increase in urine production on tubular reabsorption of ^{111}In-DTPA-octreotide is depicted. Rats that eat dry rat chow (set of left points in Fig. 1) produce less

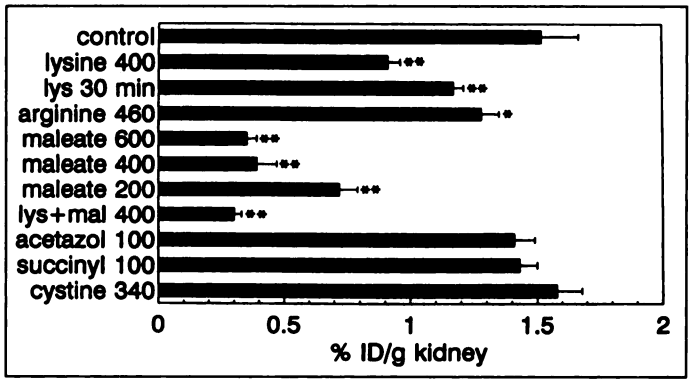

FIGURE 3. Influence of several compounds on kidney uptake of ^{111}In-DTPA-octreotide. Doses are given in mg/kg. control: n = 29, lysine: n = 8, lys 30 min = lysine injected 30 min before radiolabeled compound (n = 3), arginine: n = 3, maleate 600 mg/kg: n = 3, maleate 400 mg/kg: n = 8, maleate 200 mg/kg: n = 3, lysine + maleate: n = 4. Acetazol = acetazolamide (n = 3), succinyl = succinyl acetone (n = 3), cystine = cystine dimethylester (n = 3). *p < 0.01, **p < 0.001 versus control.

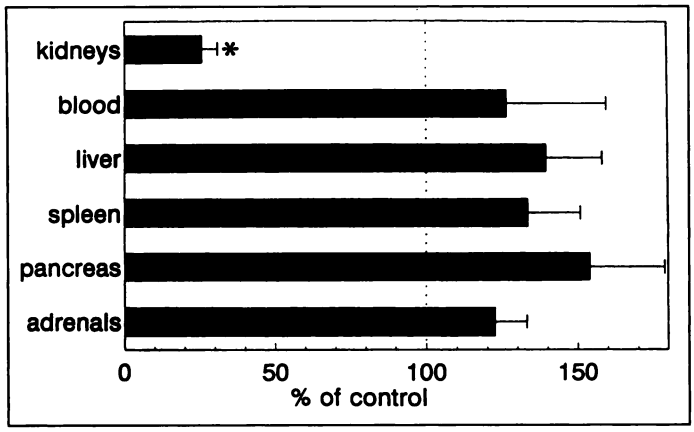

FIGURE 4. Influence of 400 mg/kg sodium maleate on the uptake of ^{111}In-DTPA-octreotide in several organs (n = 8).

urine (range 3.2–13.5 ml/20 hr) than rats that eat food suspended in water (set of points further to right in Fig. 1; range 18.8–26.6 ml/20 hr). However, the increase in urine production did not lower the amount of radioactivity in the kidneys (dry food: 1.49% ± 0.22% ID/g; food suspension: 1.53% ± 0.13% ID/g; not significantly different).

In Figure 2, the effect of changes in pH of the urine, induced by addition of electrolytes to the food, on tubular reabsorption is shown. Control rats received 1 mmole/day NaCl in their food suspension, resulting in a normal urinary pH (6.75 ± 0.22). To induce production of more acidic or alkaline urine, 1 mmole NH_4Cl or $NaHCO_3$ was added to the food. This resulted in urine pH values of 5.84 ± 0.18 and 7.35 ± 0.04. Figure 2 shows that both alkalinization and acidification of the urine resulted in a lower dose in the kidneys 20 hr after administration of the radiolabeled compound.

Figure 3 shows the effects of several compounds, injected intravenously (just prior to ^{111}In-DTPA-octreotide administration, unless otherwise stated), on the kidney dose 20 hr after administration of ^{111}In-DTPA-octreotide. Administration of lysine in a single dose of 400 mg/kg resulted in an inhibition of the kidney uptake of 40% ($p < 0.001$ versus control). When lysine was injected 30 min before ^{111}In-DTPA-octreotide, the inhibitory effect was less pronounced at 25% ($p < 0.001$ versus control). Arginine, also a positively charged amino acid, had less effect on tubular re-uptake of ^{111}In-DTPA-octreotide than lysine (20% inhibition).

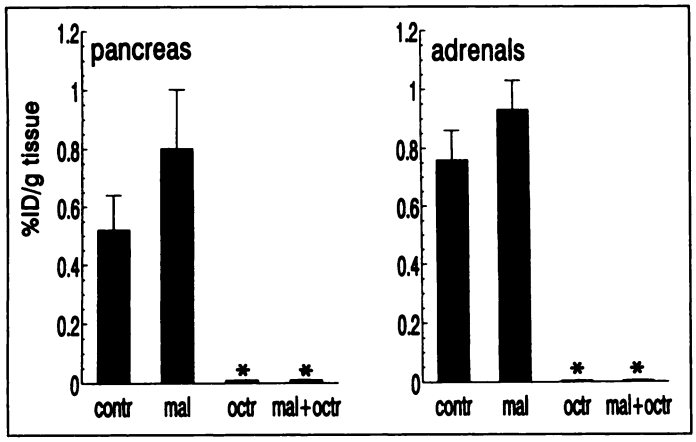

FIGURE 5. Uptake of ^{111}In-DTPA-octreotide in pancreas and adrenals after pretreatment with 0.5 mg unlabeled octreotide 40 min before ^{111}In-DTPA-octreotide administration, whether or not in combination with sodium maleate (400 mg/kg). *$p < 0.001$ versus control. All groups n = 3.

Sodium maleate was the most effective compound tested. As shown, the effects of sodium maleate were dose dependent. It appeared however, that rats given sodium maleate ate less of their food than control rats. They ate 81.6% of control when given 600 mg/kg maleate, and 77.5% of control when given 400 mg/kg maleate.

A combination of lysine and maleate (both 400 mg/kg) reduced the kidney dose of ^{111}In-DTPA-octreotide more than both compounds alone ($p < 0.005$ versus sodium maleate and $p < 0.001$ versus lysine alone). Rats receiving this combination ate 90.9% of the control. The combination of lysine and sodium maleate together with administration of electrolytes ($NaHCO_3$ or NH_4Cl) in the food, did not change kidney uptake of ^{111}In-DTPA-octreotide significantly compared to the combination of lysine and sodium maleate alone (not shown). Figure 3 shows the effects of several other tested compounds as well, including acetazolamide (100 mg/kg), succinylacetone (100 mg/kg) and cystine dimethylester (340 mg/kg). These compounds had no significant effect on the amount of radioactivity in the kidneys 20 hr after ^{111}In-DTPA-octreotide administration.

Figure 4 shows the effects of 400 mg/kg sodium maleate on the uptake of ^{111}In-DTPA-octreotide in several other organs 20 hr after administration, expressed as % of control values (Table 1). In contrast to the kidneys the dose in all other organs, including the blood, was increased after sodium maleate administration, though not significantly (range 121–155% of control values). In order to study if this increased uptake of ^{111}In-DTPA-octreotide represented specific binding, some rats were pretreated with 0.5 mg unlabeled octreotide before ^{111}In-DTPA-octreotide administration, combined or not with administration of sodium maleate (400 mg/kg). Unlabeled octreotide competitively inhibited the binding of ^{111}In-DTPA-octreotide to the somatostatin receptors, as is shown in Figure 5 for pancreas and adrenals. These organs both contain somatostatin receptors, the amount of radioactivity after unlabeled octreotide pretreatment was less than 2% of the control values. It is further shown that the increase in radioactivity in pancreas and adrenals after sodium maleate administration is inhibited by unlabeled octreotide, showing that the increase in organ radioactivity consisted of specific binding of ^{111}In-DTPA-octreotide to the somatostatin receptors.

DISCUSSION

Peptides in plasma are filtered through the glomerular capillaries in the kidneys and subsequently reabsorbed almost completely (≥90%) by the proximal tubular cells via receptor-mediated endocytosis. First, the ligand binds to a carrier on the membrane. The carrier-ligand complex is internalized in an intracellular vesicle, the vesicle content becomes acidified, releasing the ligand from its receptor. Then the ligand is routed to the lysozomes where degration takes place. These steps require energy (*14*). Lysozomal degradation has also been described for ^{111}In-DTPA-octreotide. Its labeled degradation products are trapped in the lysozomes, because of the charged DTPA-complex (*15*). This study demonstrates that the uptake of ^{111}In-DTPA-octreotide by the renal tubular cells after glomerular filtration can be reduced, in favor of the scintigraphic sensitivity of detection for small tumors in the perirenal region and of radiotherapy.

Increases in urine production, as shown in Figure 1, were induced by giving food suspension in water. However, reuptake of ^{111}In-DTPA-octreotide in the renal tubules is apparently a very efficient process that is not influenced by increased urine production.

Addition of electrolytes to the diet, resulting in a change in pH of the urine, reduced kidney uptake of ^{111}In-DTPA-octreotide (Fig. 2). Membranes of renal tubular cells contain negatively charged sites, to which positively charged amine or guanine residues of peptides can bind (*4*). Changing the pH of the (primary) urine may influence these binding processes, decreasing the efficiency of the re-uptake process of ^{111}In-DTPA-octreotide.

Decreased binding to negatively charged membranes of renal tubular cells of ^{111}In-DTPA-octreotide after administration of the positively charged amino acids lysine and arginine can be explained by the same phenomenon (*5*). In our study the effect of lysine was more pronounced than that of arginine (Fig. 3), in accordance with the findings of Mogensen and Solling (*4*). They reported that compounds with a positively charged group preferentially located terminally in the molecule inhibited instantaneously the tubular protein reabsorption. This finding of instantaneous inhibition is in concert with our finding that administration of lysine 30 min before ^{111}In-DTPA-octreotide injection was less effective in reduction of kidney uptake than administration just prior to injection of the radiolabeled compound.

Hammond et al. (*5*) reported that an infusion of synthetic amino acids containing lysine and arginin, among other amino acids, significantly reduced renal uptake of ^{111}In-DTPA-octreotide 4 hr after injection, without effect on glomerular filtration rate. Also in mice, inhibition of the renal tubular reabsorption process, in this case of ^{111}In-labeled Fab fragment, could be effected by systemic administration of lysine (*6*). This is in accordance with our findings in rats.

The most pronounced effect on renal uptake of ^{111}In-DTPA-octreotide was exerted by sodium maleate (Fig. 3). Sodium maleate has been used to study renal tubular dysfunction comparable to Fanconi's syndrome in humans (*7,8*). Maleate forms maleyl-CoA by reacting with succinyl-CoA, thereby reducing the cellular CoA supply and inhibiting the citric acid cycle in tubular cells (*16*). The resulting reduced ATP supply or the reaction of the maleyl-CoA with membrane proteins inhibits a variety of renal transport systems, including peptide reabsorption (*16,17*). The effect of sodium maleate was dose dependent, and the combination of lysine and sodium maleate resulted in even greater inhibition of the re-uptake process.

The compounds cystine dimethylester, succinylacetone and acetazolamide had no effect on reabsorption of ^{111}In-DTPA-octreotide. Increased excretion of peptides into the urine of rats in vivo, without causing any renal abnormalities, has been described after administration of cystine dimethylester (*12*), but in our study a single dose did not influence ^{111}In-DTPA-octreotide reabsorption. The same holds for succinylacetone, a compound that depresses oxygen consumption in the rat renal tubule (*10*) and inhibits peptide re-uptake (*11*) without damage to mitochondria. Acetazolamide is known to increase intracellular pH, but did not influence ^{111}In-DTPA-octreotide reabsorption in our study.

The increasing effect of sodium maleate on the dose of ^{111}In-DTPA-octreotide in other organs than the kidneys may be explained by described inhibiting effects on the renal glomerular filtration rate (*18*). However, although the increasing effect was consistent, it is not significantly different from the control situation because of rather large standard deviations. One may conclude from our findings, however, that during inhibition of glomerular filtration of ^{111}In-DTPA-octreotide, a longer residence time in the plasma will occur leading to a higher uptake in the organs. The effect of sodium maleate on organ uptake was also found in the case of ^{161}Tb-DTPA-octreotide (*19*). Uptake in all organs, except for the kidneys, was significantly increased after sodium maleate administration.

The effect of sodium maleate on glomerular filtration rate may not explain the inhibition of kidney uptake of ^{111}In-DTPA-octreotide. Hysing et al. (*18*) studied the effects of sodium maleate on protein reabsorption at a glomerular filtration rate in dogs by altering the renal arterial perfusion pressure. Under these conditions, they still found the reduction of reabsorption by sodium maleate, showing that the effect of sodium maleate on tubular reabsorption is not caused by a decrease in glomerular filtration rate alone.

Figure 5 shows higher uptake of ^{111}In-DTPA-octreotide into the pancreas and adrenals after maleate administration, with both organs containing somatostatin receptors. This increase in binding can be completely prevented with unlabeled octreotide, showing that the higher uptake after maleate represented the higher specific binding of ^{111}In-DTPA-octreotide to the somatostatin receptors.

Although maleate had inhibitory effects on renal re-uptake of ^{111}In-DTPA-octreotide, it may not be suitable for administration to humans because of the toxic effects of this compound on the kidneys. Conflicting data have been published. Worthen (*7*) described abnormalities in the proximal tubules within 2 hr after injection to rats, the degree related to the dose of sodium maleate (480–1440 mg/kg) given. Normal renal histology returned after intervals of 24 to 72 hr, depending on the dose administered. Harrison and Harrison (*8*) also did not find evidence of permanent injury in rat kidneys after a dose of 160 mg/kg BW daily for a period of 2 to 3 wk. Hysing et al. (*18*) found that the maleate stopped protein reabsorption in dogs without a significant increase in brush border and lysosome marker enzymes in the urine. However, Verani et al. (*20*) observed in kidneys of rats treated with 200 or 400 mg/kg maleic acid by infusion (1 hr) an immediate injury that progressed to necrosis by 24 hr after administration. No dose relationship was seen. We could not find histological abnormalities in kidneys of rats after a single dose of 400 mg/kg maleate (not shown). However, the cited findings caused us to abandon the idea of human administration.

It appeared possible to reduce re-uptake of ^{111}In-DTPA-octreotide in the rat kidney in vivo. The most pronounced effects were seen after administration of sodium maleate or lysine but, because of possible toxic effects of maleate, we will further study the effects of lysine in clinical studies only.

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Detecting Infection and Inflammation with Technetium-99m-Labeled Stealth® Liposomes

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The performance of ^{99m}Tc Stealth® liposomes was investigated in various rat models. **Methods:** Preformed polyethyleneglycol-containing liposomes with encapsulated reduced glutathione, were radiolabeled using the lipophilic ^{99m}Tc-HMPAO. The labeled liposomes were intravenously administered to rats with focal *S. aureus* or *E. coli* infection, or turpentine-induced inflammation. For comparison, Tc-99m-nanocolloid- and ^{99m}Tc-labeled nonspecific IgG were tested. In rats with *Pneumocystis carinii* pneumonia (PCP), Tc-99m-liposomes were directly compared to In-111 labeled nonspecific IgG. **Results:** Technetium-99m-liposomes accumulated in the infectious and inflammatory muscle foci over 24 hr (0.59% injected dose per gram tissue (%ID/g) for *E. coli;* 0.98 %ID/g for *S. aureus;* 1.18 %ID/g for turpentine). Abscess-to-muscle ratios increased to values as high as 24.0, 41.7 and 44.5 for the respective models at 24 hr postinjection. Technetium-99m-liposomes visualized the foci as early as 1 hr postinjection. Technetium-99m-IgG visualized *S. aureus* infection, but abscess-to-muscle ratios and abscess uptake at the later time points were significantly lower. Technetium-99m-nanocolloid failed to visualize any of the muscle foci. In PCP however, ^{99m}Tc-liposomes did not show preferential localization in the infection. The control agent ^{111}In-IgG showed a significant, two-fold increase in lung uptake. **Conclusion:** Technetium-99m-Stealth® liposomes preferentially accumulated in abscesses, leading to very high target-to-nontarget ratios. This property appears to be related to a process based on uptake of long-circulating particles. In a specific type of infection, i.c. PCP, ^{99m}Tc-liposomes did not accumulate in diseased lung tissue, thus mimicking the in vivo behavior of labeled leukocytes.

Key Words: polyethyleen glycol; immunoglobulin; technetium-99m-liposomes; indium-111-IgG; infection imaging; inflammation; *Pneumocystis carinii*

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Imaging infection and inflammation at early time points with convenient radiopharmaceuticals approaches the clinician's concept of optimal imaging of infectious and inflammatory disease (*1*). Since none of the currently available radiopharmaceuticals is ideal with regard to biodistribution, pharmacokinetics or accumulation in a focus, both preclinical and clinical efforts are made to develop an agent that meets these goals. One of the promising, new agents for scintigraphic detection of infection and inflammation is radiolabeled liposomes. Liposomes consist of lipid bilayer membranes, enclosing aqueous compartments in which aqueous labels can be entrapped. In the past liposomes have been widely studied for achieving controled drug delivery and for imaging purposes (*2–5*). However, these conventional liposomes are rapidly cleared from the circulation by phagocytic cells of the mononuclear phagocyte system (MPS) (*6*). Therefore, their use for diagnostic imaging is limited as the efficient MPS uptake competes with accumulation at the target site. Development of new liposome formulations characterized by prolonged circulation time was a significant step forward. The so-called sterically stabilized, or Stealth® (Liposome Technology, Inc., Menlo Park, CA), liposomes have been shown to preferentially localize at diseased sites (*7,8*). In Stealth® liposomes, polyethylene glycol (PEG) is incorporated in the phospholipid bilayer, thereby drastically reducing the recognition of the liposomes by the MPS and thus increasing circulatory half-life. Recently, we have demonstrated excellent targeting of experimental focal infection with ^{111}In-labeled Stealth® liposomes (*9*). When abscess accumulation and biodistribution are favorable, a ^{99m}Tc label is to be preferred over ^{111}In. In this study, we evaluated ^{99m}Tc-labeled Stealth® liposomes. Applicability of the ^{99m}Tc label and the performance in several infection and inflammation models were studied. The performance of ^{99m}Tc-liposomes was compared to other reagents used in clinical practice.

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MATERIALS AND METHODS

Animal Models

Muscle Infection/Inflammation. A calf muscle abscess was induced in young, male, randomly-bred Wistar rats (body weight 200–220 g). After ether anesthesia, approximately 2×10^8 colony-forming units (CFU of *Staphyloccoccus aureus* or 1×10^9 CFU of *Escherichia coli* in 0.1 ml 50:50% suspension of autologous blood and normal saline was injected in the left calf muscle (*10*). Sterile inflammation was induced by injection of 0.15 ml of turpentine in the left calf muscle of ether-