Rapid Imaging of Experimental Infection with Technetium-99m-DTPA After Anti-DTPA Monoclonal Antibody Priming

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Antibodies accumulate nonspecifically in infectious foci due to the locally increased vascular permeability. This study describes a method of infection imaging in which $^{99m}$Tc-DTPA (diethylentriaminepentaacetic acid) is trapped at the target by a previously administered anti-DTPA monoclonal antibody, DTInl. Methods: Rats with Staphylococcus aureus-infected calf muscle were injected intravenously with DTInl. Two to 24 hr after DTInl injection, $^{99m}$Tc-DTPA was injected intravenously. In separate experiments, excess DTInl was cleared from the circulation 2 hr after injection with bovine serum albumin (BSA)-DTPA-ln, galactosylated BSA-DTPA-ln, goat antimouse IgG or avidin. Additionally, the effect of DTInl (IgG2a), reacting with DTPA loaded with different ligands, was investigated.

Results: Priming with DTInl resulted in specific retention of $^{99m}$Tc-DTPA in the abscess. Such $^{99m}$Tc-DTPA abscess uptake was not dependent on the interval between the DTInl and the $^{99m}$Tc-DTPA injection: Optimal $^{99m}$Tc-DTPA abscess uptake was already achieved within a 2-hr time span between the DTInl and DTPA injections. However, relatively high $^{99m}$Tc-DTPA background was observed due to slow clearing of DTInl from vascular permeability. Background reduction with various agents had a prominent effect on DTInl as well as $^{99m}$Tc-DTPA biodistribution. The best reduction was obtained using BSA-DTPA-ln. Optimal $^{99m}$Tc-DTPA abscess uptake in the three-step protocol was obtained at higher DTInl doses (>100 $\mu$g).

Conclusion: Infectious foci in a rat model can be imaged earlier with extremely low background levels after priming with DTInl, followed by BSA-DTPA-ln and imaging with $^{99m}$Tc-DTPA, as compared with directly labeled IgG.

Key Words: technetium-99m-DTPA; monoclonal antibody priming; infection imaging; pretargeting protocols.

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Scintigraphic imaging of focal infection is currently performed with various agents, such as $^{67}$Ga-citrate, radiolabeled leukocytes or $^{111}$In-labeled human IgG (1,2). Large proteins such as IgG and human serum albumin localize nonspecifically in infectious and inflammatory foci due to the locally enhanced vascular permeability (3,4). Although labeled IgG is a convenient radiopharmaceutical, its relatively slow blood clearance, which causes persistently high background activity, interferes with the early diagnosis of infection and inflammation (5).

Reduction of background activity may be accomplished by pretargeting protocols. In these methods, the infectious focus is pretargeted and the radionuclide is administered afterwards as a low molecular weight ligand. The small ligand is rapidly excreted when not targeted to the infectious focus. Streptavidin and biotin have been used in such multistep approaches (6–8). Rusckowski et al. pretargeted mice with Escherichia coli infection with cold streptavidin and injected $^{111}$In-biotin 3 hr later (8). Higher absorb-to-background ratios were obtained compared with $^{111}$In-streptavidin or $^{111}$In-IgG. Similar results were observed in tumor pretargeting studies using antichelate antibodies and radiometal labeled chelates (9–12).

In this study, we investigated a multistep strategy for rapid infection imaging using an anti-DTPA (diethylentriaminepentaacetic acid) monoclonal antibody (MAb) as the pretargeting agent and $^{99m}$Tc-DTPA as the targeting radiopharmaceutical.

MATERIALS AND METHODS

Radiopharmaceuticals

Technetium-99m-IgG. Human nonspecific IgG in kit form (Technescan-HIG; Mallinckrodt Medical B.V., Petten, The Netherlands) was labeled with 750 MBq $^{99m}$Tc eluate according to the manufacturer's instructions.

Monoclonal Antibodies. The production of anti-DTPA MAb DTInl (IgG2a), reacting with DTPA loaded with different metals, has been described (13). The affinity constant for $^{99m}$Tc-DTPA was approximately 0.2 nM$^{-1}$, which is similar to that for $^{111}$In-DTPA (13). The IgG2a variant of MAB G250 (14) was used as a non-DTPA binding-control antibody. DTInl and G250 were labeled with $^{111}$In (Amersham International, Buckinghamshire, U.K.) using the Iodogen method (15).

Biotinylated DTInl. DTInl was conjugated with NHS-LC-biotin (Pierce, Rockford, IL). Briefly, 0.8 mg DTInl and 740 $\mu$g NHS-LC-biotin in 50 mM sodium phosphate (pH 7.5) were incubated for 16 hr at 4°C. Thereafter, unreacted biotin was removed by PD10 (Pharmacia LKB Technology, Uppsala, Sweden) chromatography. Each DTInl molecule contained 18
biotins as determined by the method of Green (16). In vivo, the 99mTc-DTPA binding capacity of biotinylated DTInl and DTIn1 were similar.

**Bovine Serum Albumin (BSA)-DTPA-In.** BSA (Sigma Chemical Co., St. Louis, MO) was conjugated with the cyclic anhydride of DTPA (Sigma) in a 1:20 molar ratio as described by Hnatowich et al. (17). After PD10 chromatography to remove unreacted DTPA, excess InCl3 (Merck, Darmstadt, Germany) was added. Five DTPA molecules were conjugated per BSA molecule as determined by the ITLC method described by Hnatowich et al. (17).

Galactosylated BSA-DTPA-In. BSA-DTPA-In was galactosylated essentially as described by Marshall et al. (18). To 36.5 mg dry activated galactose 10 mg BSA-DTPA-In (5 mg/ml in 25 mM sodium borate, pH 8.5) were added and allowed to react for 2 hr. PD10 chromatography was used to remove unreacted galactose. Thirty-two galactose molecules were conjugated per BSA-DTPA-In molecule as determined by the method of Dubois et al. (19).

**Technetium-99m-DTPA.** A kit containing 1 mg DTPA, 0.6 mg calcium nitrate and 0.05 mg stannous sulfate (pH 5.0) was radioiodinated with a fresh 99mTc eluate.

**Animal Studies.**

**Animal Model.** A Staphylococcus aureus calf muscle abscess was induced in young, male Wistar rats according to the method of Oyen et al. (3). Experiments were initiated 24 hr after the S. aureus inoculation. All radiopharmaceuticals were intravenously injected.

**Biodistribution Studies.** Rats were injected intraperitoneally with a phenobarbital overdose, bled by cardiac puncture and killed. Tissues were dissected and weighed. The activity in tissues and injection standards was measured in a shielded well scintillation counter and expressed as the percentage of injected dose per gram (%ID/g). From these data absorb-to-blood ratios (ABR) and absorb-to-contralateral muscle ratios (AMR) were calculated. In all experiments, groups of five rats were used.

**Immunoscintigraphy.** Groups of four rats were anesthesized (nitrous oxide/oxygen/halothane) and placed prone on a gamma camera (Orbiter, Siemens, Hoffman Estates, IL) equipped with a 256 × 256 matrix. The images were analyzed by drawing regions of interest over the whole animal, the abscess and the contralateral body blood level of 4%ID/g and a total blood volume of 12 ml (3). Thirty minutes later, 4 MBq 99mTc-DTPA (3.5 μg) were injected. The biodistribution was determined 30 min p.i.

**Antibody Dose Optimization.** Increasing amounts of 125I-DTIn1 (10–900 μg per rat, labeled with 370 kBq 125I) were injected. Two hours later, BSA-DTPA-In was injected into each rat at a calculated 10-fold molar excess. Thirty minutes later, each rat was injected with 4 MBq 99mTc-DTPA (3.3 μg), and 99mTc-DTPA biodistribution was determined 1 hr p.i.

**Statistical Analysis.** All mean values are ± s.d. Statistical analysis was performed using one-way analysis of variance, with Bonferroni post-test correction for multiple comparisons.

**RESULTS**

**Two-Phase Targeting of Infections.**

Priming rats with DTIn1 for 24 hr had a profound effect on the 99mTc-DTPA biodistribution (Fig. 1). Whereas almost no differences in the biodistribution between DTIn1 and G250 were observed (data not shown), 99mTc-DTPA activity was significantly higher in DTIn1 primed rats in all examined tissues with the exception of the kidneys (p < 0.0001).

**Optimization of the Time Between DTIn1 and DTPA Injection.**

In Table 1, biodistribution of 125I-DTIn1 is shown 4, 8 and 26 hr after injection. The %ID/g 125I-DTIn1 in the blood decreased with time. More importantly, the 125I-DTIn1 uptake in the abscess was similar up to 26 hr after injection. Neither the ABR nor the AMR of 125I-DTIn1 improved with time.

The %ID/g 99mTc-DTPA in blood decreased significantly with increasing intervals between the DTIn1 and DTPA injections (Table 1). The 99mTc-DTPA uptake in other organs and in the abscess was not significantly affected by the interval between the DTIn1 and 99mTc-DTPA injections. The ABR slightly improved from 0.27 at the 6-hr interval to 0.46 at the 24-hr interval between the DTIn1 and 99mTc-DTPA injections. No significant differences were observed in 99mTc-DTPA AMRs. Optimal absorb uptake of 99mTc-DTPA had been achieved during the 2 hr between the DTIn1 and DTPA injections. However, due to the high DTIn1 blood levels, 99mTc-DTPA background levels remained high.

To clarify the role of the two-step strategy, biodistribution studies with 99mTc-IgG were performed (Table 2). High background levels were also observed with 99mTc-IgG. The AMR was significantly higher using 99mTc-IgG, but no significant differences were observed in ABRs when 99mTc-IgG 4 hr post-infection was compared with 99mTc-DTPA in the two-phase protocol with a 2-hr timespan. Early imaging of infectious foci, in terms of ABRs, was not improved using this two-step protocol.

The three-phase targeting protocol was also studied in a biodistribution experiment. Ten rats received 300 μg DTIn1 labeled with 370 kBq 125I. After 2 hr, 5 of the 10 rats received 650 μg BSA-DTPA-In. Rats in both groups received 4 MBq 99mTc-DTPA (4 μg) 2.5 hr after the first injection, and biodistribution was determined 1 hr later.
Three-Step Targeting of Infectious Foci

The effect of BSA-DTPA-In on ⁹⁹ᵐTc-DTPA abscess uptake and whole-body distribution was studied scintigraphically. Administration of BSA-DTPA-In resulted in a marked change in whole-body distribution of ⁹⁹ᵐTc-DTPA (Fig. 2). With the two- and three-step protocols, the abscesses were clearly visualized. However, a notable decrease of circulating ⁹⁹ᵐTc-DTPA was observed in BSA-DTPA-In-treated rats. With the three-step protocol, the abscess-to-background ratio increased to 14.8 ± 3.1 2 hr after ⁹⁹ᵐTc-DTPA injection. Due to the rapid excretion of the nontargeted ⁹⁹ᵐTc-DTPA, the abscess uptake as a percentage of residual activity increased up to 16.3% 2 hr after ⁹⁹ᵐTc-DTPA injection. In contrast, rats receiving ⁹⁹ᵐTc-DTPA only showed minimal abscess uptake (2.3% ± 0.3% of whole-body activity 20 min p.i.), and the abscess-to-background ratio did not exceed 2.

In the biodistribution experiment, striking differences between two- and three-step protocols were observed. A decrease in ⁹⁹ᵐTc-DTPA uptake was seen in blood (17-fold reduction), abscess (1.9-fold decrease) and other organs of rats treated with BSA-DTPA-In (Fig. 3). More importantly, the ⁹⁹ᵐTc-DTPA ABR was significantly higher in three-phase protocol rats (1.97 ± 0.42 versus 0.22 ± 0.03; p < 0.001), whereas the AMR was not different. The %ID/g ¹²⁵I-DTIn1 in blood, kidneys and lungs significantly decreased, whereas a significant increase was seen in the liver and spleen, indicating DTIn1-BSA-DTPA-In complexation and subsequent metabolization (Fig. 3 inset). The amount of ¹²⁵I-DTIn1 in the abscess was similar in both pretargeting protocols.

The three-step approach resulted in significant improvement of the ABR for ⁹⁹ᵐTc-IgG 4 hr p.i. (1.97 ± 0.42 versus 0.35 ± 0.05; p < 0.0001).

Comparison of Different Background-Reducing Agents

All agents effectively reduced the %ID/g ⁹⁹ᵐTc-DTPA in the blood (Table 3). BSA-DTPA-In had the most prominent effect on ⁹⁹ᵐTc-DTPA blood levels, with a 5.6-fold reduction compared with the two-step protocol. Only slightly (but significantly) decreased ⁹⁹ᵐTc-DTPA abscess uptake was observed after injection of BSA-DTPA-In or gal-BSA-DTPA-In. Lower ⁹⁹ᵐTc-DTPA levels were observed in the liver and spleen using BSA-DTPA-In or in the liver using gal-BSA-DTPA-In.

### TABLE 1

Optimization of Time Between DTIn1 and DTPA Injection

<table>
<thead>
<tr>
<th>Organ</th>
<th>4 hr p.i.</th>
<th>8 hr p.i.</th>
<th>26 hr p.i.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>3.17 ± 0.17</td>
<td>2.45 ± 0.15</td>
<td>1.50 ± 0.21</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.10 ± 0.02</td>
<td>0.08 ± 0.01</td>
<td>0.10 ± 0.01</td>
</tr>
<tr>
<td>Abscess</td>
<td>0.83 ± 0.22</td>
<td>0.88 ± 0.32</td>
<td>0.66 ± 0.10</td>
</tr>
<tr>
<td>Liver</td>
<td>0.67 ± 0.05</td>
<td>0.55 ± 0.13</td>
<td>0.28 ± 0.03</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.82 ± 0.08</td>
<td>0.77 ± 0.12</td>
<td>0.43 ± 0.06</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.48 ± 0.04</td>
<td>0.47 ± 0.08</td>
<td>0.23 ± 0.04</td>
</tr>
<tr>
<td>ABR</td>
<td>0.28 ± 0.06</td>
<td>0.36 ± 0.15</td>
<td>0.44 ± 0.07</td>
</tr>
<tr>
<td>AMR</td>
<td>8.96 ± 3.07</td>
<td>10.26 ± 2.10</td>
<td>6.44 ± 0.17</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Organ</th>
<th>2-hr interval</th>
<th>6-hr interval</th>
<th>24-hr interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>1.72 ± 0.04</td>
<td>1.42 ± 0.08</td>
<td>0.97 ± 0.16</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.06 ± 0.01</td>
<td>0.08 ± 0.03</td>
<td>0.08 ± 0.01</td>
</tr>
<tr>
<td>Abscess</td>
<td>0.49 ± 0.12</td>
<td>0.48 ± 0.10</td>
<td>0.44 ± 0.06</td>
</tr>
<tr>
<td>Liver</td>
<td>0.39 ± 0.03</td>
<td>0.28 ± 0.12</td>
<td>0.22 ± 0.03</td>
</tr>
<tr>
<td>Kidney</td>
<td>1.04 ± 0.09</td>
<td>1.08 ± 0.23</td>
<td>0.88 ± 0.07</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.28 ± 0.02</td>
<td>0.20 ± 0.09</td>
<td>0.16 ± 0.02</td>
</tr>
<tr>
<td>ABR</td>
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<td>0.27 ± 0.14</td>
<td>0.46 ± 0.06</td>
</tr>
<tr>
<td>AMR</td>
<td>7.73 ± 2.21</td>
<td>7.43 ± 2.27</td>
<td>5.83 ± 0.16</td>
</tr>
</tbody>
</table>

p.i. = postinjection.
Higher \(^{99m}\text{Tc}\text{-DTPA}\) levels were observed in the spleen after \(\text{GAM-IgG}\) injection and in the liver and spleen after avidin injection. The \(^{99m}\text{Tc}\text{-DTPA}\) ABR significantly improved using avidin (2.7-fold), BSA-DTPA-In (3.6-fold) or GAM-IgG (3.7-fold).

After injection of avidin, gal-BSA-DTPA-In or \(\text{GAM-IgG}\), \(^{125}\text{I}\text{-DTI1}\) blood levels significantly decreased compared with the two-phase protocol (Table 3). Injection of BSA-DTPA-In did not reduce \(^{125}\text{I}\text{-DTI1}\) blood levels. Elevated levels of \(^{125}\text{I}\text{-DTI1}\) in liver and spleen were observed after BSA-DTPA-In, avidin or GAM-IgG injection, indicating removal of complexed DTI1 by cells of the mononuclear phagocyte system. After gal-BSA-DTPA-In injection, \(^{125}\text{I}\text{-DTI1}\) was cleared through the liver, indicating that the galactose moiety directed the gal-BSA-DTPA-In-DTI1 complexes to the liver.

**Antibody Dose Escalation Studies**

The amount of DTI1 in all organs in terms of protein mass increased linearly with increasing amounts injected DTI1 (data not shown), indicating that saturation was not reached.

Biodistribution data for \(^{99m}\text{Tc}\text{-DTPA}\) after priming with various doses of DTI1 are shown in Figure 4. The %ID/g in the abscess was significantly higher at \(\geq 300\ \mu\text{g}\) compared with \(100\ \mu\text{g}\ (0.31 \pm 0.05\ \text{versus}\ 0.19 \pm 0.01;\ \text{p} < 0.05)\). Consequently, the ABR and AMR significantly increased when increasing the DTI1 dose from 100–300 \(\mu\text{g}\) [ABR: 0.88 ± 0.03 \text{versus}\ 1.79 ± 0.30 (p < 0.05); AMR: 5.63 ± 1.71 \text{versus}\ 8.79 ± 2.25 (p < 0.05)], indicating that 300 \(\mu\text{g}\) per rat was the optimal dose.

**DISCUSSION**

The development of an imaging technique to localize acute infection within a few hours is of great clinical importance (20). Using radiopharmaceuticals such as \(^{111}\text{In}\text{-IgG}\) and \(^{99m}\text{Tc}\text{-IgG}\) a relatively long time (\(\geq 24\ \text{hr}\)) is needed before a final diagnosis can be made (3). This is mainly related to slow blood clearance, resulting in slower increase of target-to-background ratios (3). We investigated whether a pretargeting protocol could overcome this drawback.

We evaluated the potential of an anti-DTPA MAb combined with radiolabeled DTPA for multistep targeting of infectious foci. After pretargeting with DTI1, the abscess was visualized with \(^{99m}\text{Tc}\text{-DTPA}\). DTPA abscess uptake was based on antibody–antigen interaction because priming with G250 did not result in any specific \(^{99m}\text{Tc}\text{-DTPA}\) uptake. Given the hyperemia and increased vascular permeability in acute infections, optimal abscess uptake of \(^{99m}\text{Tc}\text{-DTPA}\) was achieved within a 2-hr time interval between the DTI1 and DTPA injections, since accumulation of DTI1 in the abscess was very rapid.

However, relatively high background activity was seen due to slow clearance of \(^{99m}\text{Tc}\text{-DTPA}\) DTI1 complexes formed in the circulation. The two-phase protocol revealed no significant improvement in comparison to directly labeled \(^{99m}\text{Tc}\text{-IgG}\) at early time points.

To reduce the complexation of \(^{99m}\text{Tc}\text{-DTPA}\) with circulating DTI1, BSA-DTPA-In was injected. This markedly changed the whole-body distribution of the subsequently injected \(^{99m}\text{Tc}\text{-DTPA}\). The imaging studies showed only minor amounts of \(^{99m}\text{Tc}\text{-DTPA}\) in the circulation, whereas the abscess was clearly visualized.

Four different background-reducing agents were compared. Immune complexes formed between DTI1 and avidin, GAM-IgG or BSA-DTPA-In should be cleared through the liver and spleen (9,21–25). DTI1-Gal-BSA-DTPA-In complexes should be cleared through the hepatic asialoglycoprotein receptor (26). Avidin and GAM-IgG do not interfere with the antigen-binding site of DTI1, whereas BSA-DTPA-In and gal-BSA-DTPA-In do. Each of the background-reducing agents significantly reduced the amount of \(^{99m}\text{Tc}\text{-DTPA}\) in the blood. The enhanced \(^{99m}\text{Tc}\text{-DTPA}\) liver and spleen uptake seen with avidin and GAM-IgG most likely represents \(^{99m}\text{Tc}\text{-DTPA}\) entrapment by DTI1 complexes not yet metabolized. In contrast, reduced amounts of \(^{99m}\text{Tc}\text{-DTPA}\) were observed in the spleen and/or liver with gal-BSA-DTPA-In and BSA-DTPA-In, indicating efficient blocking of the DTPA-binding site. Significantly decreased \(^{99m}\text{Tc}\text{-DTPA}\) abscess uptake was observed with BSA-DTPA-In and gal-BSA-DTPA-In. This reduced abscess uptake resulted from blocking of DTI1 antibody in the abscess or blockage of circulating DTI1 (thereby reducing the number of circulating DTI1-DTPA complexes contributing to \(^{99m}\text{Tc}\text{-DTPA}\) abscess uptake). These data suggest that \(^{99m}\text{Tc}\text{-DTPA}\) binding to prelocalized DTI1 plays an important role in \(^{99m}\text{Tc}\text{-DTPA}\) abscess uptake, in view of the slight reduction in the amount of \(^{99m}\text{Tc}\text{-DTPA}\) in the abscess after BSA-DTPA-In injection.
The best reduction of $^{99m}$Tc-DTPA background was obtained with BSA-DTPA-ln: a 5.6-fold reduction of $^{99m}$Tc-DTPA blood level was achieved with a concomitant 3.5-fold increase in the ABR. The use of a background-reducing agent that can block the DTIn1 antigen-binding site does not hamper the targeting of infectious foci with $^{99m}$Tc-DTPA. Therefore, the behavior of BSA-DTPA-ln in these studies was superior to the other background-reducing agents.

Goodwin et al. used a similar background reduction approach to image tumors in mice (9). A three-step protocol was designed using an anti-BLEDTA IV antibody, a human transferrin-chelate conjugate and $^{111}$In-BLEDTA IV. Due to the background reduction step, decreased $^{111}$In-BLEDTA IV tumor uptake and increased tumor-to-blood ratios were observed similar to our observations. In their study of mice with E. coli infection, Rusckowski et al. demonstrated that infection imaging could be improved, in terms of ABR and AMR, using streptavidin pretargeting and radiolabeled biotin (8).

With our three-step strategy, rapid imaging of infectious foci was achieved: high abscess-to-background ratios were obtained within 30 min p.i. of DTPA and 3 hr after the first injection. The three-phase targeting protocol may potentially improve the infection imaging at earlier times after tracer injection in humans. A limitation to this approach might be the development of a HAMA response after administration of DTIn1. For clinical studies, a humanized DTIn1 antibody is preferable.
multivalent chelates compared to monovalent chelates (77). The use of avidin might hamper the use of the avidin-biotin system for infection imaging in humans, especially since a human equivalent of avidin is not available. A potential drawback to the three-step method is the relatively large DTIn1 protein dose needed in humans. The use of multivalent DTPA might facilitate the use of lower DTIn1 doses in view of the higher affinity of antichelate antibodies for multivalent chelates compared to monovalent chelates (11).

CONCLUSION

Three-phase targeting of infectious foci results in early imaging with low background levels as compared with two-phase targeting protocols or directly labeled nonspecific IgG.

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