The following full text is a publisher’s version.

For additional information about this publication click this link.
http://hdl.handle.net/2066/24756

Please be advised that this information was generated on 2019-04-16 and may be subject to change.
Rapid Imaging of Experimental Infection with Technetium-99m-DTPA After Anti-DTPA Monoclonal Antibody Priming

Marion H.G.C. Kranenborg, Wim J.G. Oyen, Frans H.M. Corstens, Egbert Oosterwijk, Jos W.M. van der Meer and Otto C. Boerman

Departments of Nuclear Medicine, Urology and Internal Medicine, University Hospital Nijmegen, The Netherlands

Antibodies accumulate nonspecifically in infectious foci due to the locally increased vascular permeability. This study describes a method of infection imaging in which 99mTc-DTPA (diethylenetriaminepentaacetic 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, 99mTc-DTPA was injected intravenously. In separate experiments, excess DTInl was cleared from the circulation 2 hr after injection with bovine serum albumin (BSA)-DTPA-In, galactosylated BSA-DTPA-In, goat antiserum IgG or avidin. Additionally, the effect of DTInl dose on 99mTc-DTPA abscess uptake was determined in a three-step protocol. The distribution of the radioisotopes was studied by γ counting of dissected tissue and gamma camera imaging. Results: Priming with DTInl resulted in specific retention of 99mTc-DTPA in the abscesses. Such 99mTc-DTPA abscess uptake was not dependent on the interval between the DTInl and the 99mTc-DTPA injection: Optimal 99mTc-DTPA abscess uptake was already achieved within a 2-hr time span between the DTInl and DTPA injections. However, relatively high 99mTc-DTPA background was observed due to slowly clearing DTInl 99mTc-DTPA complexes. Background reduction with various agents had a prominent effect on DTInl as well as 99mTc-DTPA biodistribution. The best reduction was obtained using BSA-DTPA-In. Optimal 99mTc-DTPA abscess uptake in the three-step protocol was obtained at higher DTInl doses (>100 μg). Conclusion: Infectious foci in a rat model can be imaged earlier with extremely low background levels after priming with DTInl, followed by BSA-DTPA-In and imaging with 99mTc-DTPA, as compared with directly labeled IgG.

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

J Nucl Med 1997; 38:901–906

Scintigraphic imaging of focal infection is currently performed with various agents, such as 67Ga-citrate, radionabeled leukocytes or 111In-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 111In-biotin 3 hr later (8). Higher abscess-to-background ratios were obtained compared with 111In-streptavidin or 111In-IgG. Similar results were observed in tumor pretargeting studies using antichelate antibodies and radiosynthesized labeled chelates (9–12).

In this study, we investigated a multistep strategy for rapid infection imaging using an anti-DTPA (diethylenetriaminepentaacetic acid) monoclonal antibody (MAb) as the pretargeting agent and 99mTc-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 99mTc 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 99mTc-DTPA was approximately 0.2 nM−1, which is similar to that for 111In-DTPA (13). The IgG2a variant of MAB G250 (14) was used as a non-DTPA binding-control antibody. DTInl and G250 were labeled with 181t (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 470 μg 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...
bionts as determined by the method of Green (16). In vivo, the
\[ {^{99m}Tc-DTPA} \] binding capacity of biotinylated DTIn1 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 120 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
radiolabeled 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 intrave­
nously 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
inoculation. All radiopharmaceuticals were intrave­
nously 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
inoculation. All radiopharmaceuticals were intrave­
nously 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
inoculation. All radiopharmaceuticals were intrave­
nously 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
inoculation. All radiopharmaceuticals were intrave­
nously 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
inoculation. All radiopharmaceuticals were intrave­
nously 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
inoculation. All radiopharmaceuticals were intrave­
nously 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
inoculation. All radiopharmaceuticals were intrave­
nously 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
inoculation. All radiopharmaceuticals were intrave­
nously 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
inoculation. All radiopharmaceuticals were intrave­
nously 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
inoculation. All radiopharmaceuticals were intrave­
nously 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
inoculation. All radiopharmaceuticals were intrave­
nously 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
inoculation. All radiopharmaceuticals were intrave­
nously injected.
Three-Step Targeting of Infectious Foci

The effect of BSA-DTPA-In on $^{99m}$Tc-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 $^{99m}$Tc-DTPA (Fig. 2). With the two- and three-step protocols, the abscesses were clearly visualized. However, a notable decrease of circulating $^{99m}$Tc-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 $^{99m}$Tc-DTPA injection. Due to the rapid excretion of the nontargeted $^{99m}$Tc-DTPA, the abscess uptake as a percentage of residual activity increased up to 16.3% 2 hr after $^{99m}$Tc-DTPA injection. In contrast, rats receiving $^{99m}$Tc-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 $^{99m}$Tc-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 $^{99m}$Tc-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 $^{125}$I-DTN1 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 $^{125}$I-DTN1 in the abscess was similar in both pretargeting protocols.

The three-step approach resulted in significant improvement of the ABR for $^{99m}$Tc-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 $^{99m}$Tc-DTPA in the blood (Table 3). BSA-DTPA-In had the most prominent effect on $^{99m}$Tc-DTPA blood levels, with a 5.6-fold reduction compared with the two-step protocol. Only slightly (but significantly) decreased $^{99m}$Tc-DTPA abscess uptake was observed after injection of BSA-DTPA-In or gal-BSA-DTPA-In. Lower $^{99m}$Tc-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>24 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>0.26 ± 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>

p.i. = postinjection.
Higher $^{99m}$Tc-DTPA levels were observed in the spleen after GAM-IgG injection and in the liver and spleen after avidin injection. The $^{99m}$Tc-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 GAM-IgG, $^{125}$I-DTIn1 blood levels significantly decreased compared with the two-phase protocol (Table 3). Injection of BSA-DTPA-In did not reduce $^{125}$I-DTIn1 blood levels. Elevated levels of $^{125}$I-DTIn1 in liver and spleen were observed after BSA-DTPA-In, avidin or GAM-IgG injection, indicating removal of complexed DTIn1 by cells of the mononuclear phagocyte system. After gal-BSA-DTPA-In injection, $^{125}$I-DTIn1 was cleared through the liver, indicating that the galactose moiety directed the gal-BSA-DTPA-In-DTIn1 complexes to the liver.

**Antibody Dose Escalation Studies**

The amount of DTIn1 in all organs in terms of protein mass increased linearly with increasing amounts injected DTIn1 (data not shown), indicating that saturation was not reached.

Biodistribution data for $^{99m}$Tc-DTPA after priming with various doses of DTIn1 are shown in Figure 4. The %ID/g in the abscess was significantly higher at $\geq 300 \mu g$ compared with $100 \mu g$ ($0.31 \pm 0.05$ versus $0.19 \pm 0.01$; $p < 0.05$). Consequently, the ABR and AMR significantly increased when increasing the DTIn1 dose from 100-300 $\mu g$ (ABR: $0.88 \pm 0.03$ versus $1.79 \pm 0.30$ ($p < 0.05$); AMR: $5.63 \pm 1.71$ versus $8.79 \pm 2.25$ ($p < 0.05$)), indicating that 300 $\mu g$ 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 $^{111}$In-IgG and $^{99m}$Tc-IgG a relatively long time ($\geq 24$ 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 DTIn1, the abscess was visualized with $^{99m}$Tc-DTPA. DTPA abscess uptake was based on antibody-antigen interaction because priming with G250 did not result in any specific $^{99m}$Tc-DTPA uptake. Given the hyperemia and increased vascular permeability in acute infections, optimal abscess uptake of $^{99m}$Tc-DTPA was achieved within a 2-hr time interval between the DTIn1 and DTPA injections, since accumulation of DTIn1 in the abscess was very rapid.

However, relatively high background activity was seen due to slow clearance of $^{99m}$Tc-DTPA-DTIn1 complexes formed in the circulation. The two-phase protocol revealed no significant improvement in comparison to directly labeled $^{99m}$Tc-IgG at early time points.

To reduce the complexity of $^{99m}$Tc-DTPA with circulating DTIn1, BSA-DTPA-In was injected. This markedly changed the whole-body distribution of the subsequently injected $^{99m}$Tc-DTPA. The imaging studies showed only minor amounts of $^{99m}$Tc-DTPA in the circulation, whereas the abscess was clearly visualized.

Four different background-reducing agents were compared. Immune complexes formed between DTIn1 and avidin, GAM-IgG or BSA-DTPA-In should be cleared through the liver and spleen (9,21-25). DTIn1-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 DTIn1, whereas BSA-DTPA-In and gal-BSA-DTPA-In do. Each of the background-reducing agents significantly reduced the amount of $^{99m}$Tc-DTPA in the blood. The enhanced $^{99m}$Tc-DTPA liver and spleen uptake seen with avidin and GAM-IgG most likely represents $^{99m}$Tc-DTPA entrapment by DTIn1 complexes not yet metabolized. In contrast, reduced amounts of $^{99m}$Tc-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 $^{99m}$Tc-DTPA abscess uptake was observed with BSA-DTPA-In and gal-BSA-DTPA-In. This reduced abscess uptake resulted from blocking of DTIn1 antibody in the abscess or blockage of circulating DTIn1 (thereby reducing the number of circulating DTIn1-DTPA complexes contributing to $^{99m}$Tc-DTPA abscess uptake). These data suggest that $^{99m}$Tc-DTPA binding to prelocalized DTIn1 plays an important role in $^{99m}$Tc-DTPA abscess uptake, in view of the slight reduction in the amount of $^{99m}$Tc-DTPA in the abscess after BSA-DTPA-In injection.

**TABLE 2**

<table>
<thead>
<tr>
<th>Organ</th>
<th>4 hr p.i.</th>
<th>8 hr p.i.</th>
<th>24 hr p.i.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>2.32 ± 0.16</td>
<td>1.40 ± 0.11</td>
<td>0.48 ± 0.07</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.06 ± 0.01</td>
<td>0.06 ± 0.01</td>
<td>0.04 ± 0.01</td>
</tr>
<tr>
<td>Abscess</td>
<td>0.80 ± 0.08</td>
<td>0.77 ± 0.16</td>
<td>0.33 ± 0.05</td>
</tr>
<tr>
<td>Liver</td>
<td>0.96 ± 0.19</td>
<td>0.76 ± 0.08</td>
<td>0.28 ± 0.08</td>
</tr>
<tr>
<td>Kidney</td>
<td>8.21 ± 1.81</td>
<td>10.44 ± 0.87</td>
<td>6.96 ± 2.66</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.98 ± 0.08</td>
<td>0.75 ± 0.07</td>
<td>0.40 ± 0.06</td>
</tr>
<tr>
<td>ABR</td>
<td>0.35 ± 0.05</td>
<td>0.55 ± 0.08</td>
<td>0.69 ± 0.06</td>
</tr>
<tr>
<td>AMR</td>
<td>12.72 ± 2.54</td>
<td>13.56 ± 3.57</td>
<td>8.09 ± 0.55</td>
</tr>
</tbody>
</table>

p.i. = postinjection.
The best reduction of $^{99m}$Tc-DTPA background was obtained with BSA-DTPA-In: 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-In 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. Immuno-

**Table 3**

<table>
<thead>
<tr>
<th>Organ</th>
<th>DTIn1</th>
<th>BSA-DTPA-In</th>
<th>Galactosylated BSA-DTPA-In</th>
<th>Goat anti-mouse (gG)</th>
<th>Avidin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>3.61 ± 0.27</td>
<td>3.77 ± 0.32</td>
<td>1.90 ± 0.10*</td>
<td>0.57 ± 0.06*</td>
<td>0.82 ± 0.09*</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.08 ± 0.004</td>
<td>0.08 ± 0.01</td>
<td>0.07 ± 0.005</td>
<td>0.11 ± 0.01*</td>
<td>0.11 ± 0.01*</td>
</tr>
<tr>
<td>Abscess</td>
<td>1.00 ± 0.21</td>
<td>0.93 ± 0.22</td>
<td>0.54 ± 0.14*</td>
<td>0.56 ± 0.05*</td>
<td>0.59 ± 0.07*</td>
</tr>
<tr>
<td>Liver</td>
<td>0.68 ± 0.10</td>
<td>1.07 ± 0.12*</td>
<td>3.28 ± 0.19*</td>
<td>2.67 ± 0.08*</td>
<td>1.99 ± 0.19*</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.65 ± 0.08</td>
<td>1.25 ± 0.11*</td>
<td>0.46 ± 0.03*</td>
<td>2.63 ± 0.42*</td>
<td>2.71 ± 0.54*</td>
</tr>
</tbody>
</table>

| Blood  | 2.25 ± 0.20 | 0.40 ± 0.04* | 1.16 ± 0.08* | 0.51 ± 0.04* | 0.72 ± 0.09* |
| Muscle | 0.08 ± 0.003 | 0.07 ± 0.01 | 0.08 ± 0.01 | 0.08 ± 0.01 | 0.08 ± 0.01 |
| Abscess| 0.56 ± 0.11 | 0.36 ± 0.09* | 0.39 ± 0.09* | 0.47 ± 0.05 | 0.48 ± 0.08 |
| Liver  | 0.45 ± 0.06 | 0.15 ± 0.02* | 0.33 ± 0.03* | 0.50 ± 0.04 | 0.57 ± 0.06* |
| Spleen | 0.29 ± 0.17 | 0.11 ± 0.01* | 0.22 ± 0.01 | 1.78 ± 0.25* | 1.34 ± 0.30* |
| ABR    | 0.25 ± 0.04 | 0.90 ± 0.28* | 0.34 ± 0.08 | 0.92 ± 0.09* | 0.67 ± 0.07* |
| AMR    | 7.29 ± 1.49 | 5.17 ± 0.86* | 5.34 ± 1.57 | 6.06 ± 0.75 | 5.89 ± 1.01 |

*Significant difference as compared with two-phase protocol (i.e., DTIn1) at p < 0.001.
†Significant difference as compared with two-phase protocol at p < 0.01.
‡Significant difference as compared with two-phase protocol at p < 0.001.

No significant differences were observed between DTIn1 and biotinylated DTIn1; only DTIn1 results are shown.

FIGURE 3. Comparison between the three-phase protocol (DTIn1, BSA-DTPA-In, DTPA; solid bars) and the two-phase protocol (DTIn1, DTPA; hatched bars). Biodistribution of $^{99m}$Tc-DTPA 1 hr after injection is shown. Biodistribution of $^{125}$I-DTIn1 3.5 hr after injection is shown in the inset.
multivalent chelates compared to monovalent chelates (77). The 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.

ACKNOWLEDGMENTS

We thank Mrs. J.C. Oosterwijk-Wakka and Mrs. M.C.A. de Weijert (University of Nijmegen, Department of Urology), Mr. E. Koenders (University of Nijmegen, Department of Nuclear Medicine) and Mr. G. Grutters and Mr. H. Eijkholt (University of Nijmegen, Central Animal Laboratory) for technical assistance. This study was partially supported by Research Grant 93–539 from the Dutch Cancer Society.

REFERENCES

24. Shumay RM, Primus PJ, GOLDENBERG DM. Second antibody clearance of radiolabeled antibody for cancer radi@Table: 3 row 3 column.