Aerosol Characteristics of $^{99m}$Tc-Pentetic Acid (DTPA) and Synthetic Surfactant (Exosurf)*

R. Edward Coleman, M.D.; Neil MacIntyre, M.D., F.C.C.P.; Gary Snyder, B.S.; Edward Pattishall, M.D., F.C.C.P.; and David Zaccardelli, Pharm.D.

This study evaluated the feasibility of using $^{99m}$Tc-pentetic acid (DTPA) as a radioactive tracer for aerosolized synthetic surfactant (DPPC, cetyl alcohol, tyloxapol). The $^{99m}$Tc-DTPA was admixed with surfactant and aerosolized using a nebulizer system interfaced to a ventilator with a cascade impactor attached to the endotracheal tube. Particle size distribution for DPPC, cetyl alcohol, and $^{99m}$Tc-DTPA were almost identical during the 0- to 15-, 15- to 30-, and 0- to 30-min collection periods. Tyloxapol exhibited a unique distribution pattern with increased deposition in large (>10 μm) and small (0.65 to 1.1 μm) particles. The mass median aerodynamic diameter for all aerosolized components was in the respirable range of 2.1 to 2.5 μm. A mixture of $^{99m}$Tc-DTPA with synthetic surfactant appears to be a reasonable method to evaluate surfactant deposition.

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| ALI = acute lung injury; MMAD = mass median aerodynamic diameter |

Aerosol administration of a drug offers several advantages, including rapid onset of action, delivery of the drug directly to the site of action, and often fewer side effects.1 Several factors are important in the aerosolization of drugs and their pulmonary deposition, including differences in nebulizer performance, pulmonary anatomy, airway disease, and ventilation mechanics. In spontaneously breathing adults, the deposited dose is usually a small percentage of the administered aerosol.2 Moreover, less radiolabeled aerosol is delivered to the lungs in intubated, mechanically ventilated patients, compared with nonintubated patients.3,4 Therefore, it is desirable to evaluate the pulmonary deposition and distribution of aerosolized pharmaceutical agents, especially those used in intubated patients.

Synthetic surfactant administered by nebulizer instillation is now used for the prophylaxis and treatment of premature neonates at high risk for or with established respiratory distress syndrome. Results from studies in animal models of acute lung injury (ALI) suggest that aerosolized surfactant improves lung function and may be more efficacious than nebulizer instillation.5 Aerosolization of surfactant for treatment of ALI has several advantages over bronchoscopic administration, including being less invasive, allowing for continuous administration, and potentially providing a more homogeneous distribution throughout the lung. Preliminary experience in humans with sepsis-induced ALI shows potential benefit from the administration of aerosolized exogenous surfactant (Exosurf, Burroughs Wellcome Co, Research Triangle Park, NC).6,7 Aerosolized Exosurf is currently being evaluated in large, placebo-controlled trials in intubated, mechanically ventilated patients with ALI.

To better understand the response to aerosolized Exosurf therapy, the deposition and distribution in intubated patients with ALI would be important to determine. One method of determining the distribution of an aerosolized drug is to use a radiolabel attached to the aerosolized drug. However, it is extremely difficult to radiolabel each of the three major components of Exosurf, dipalmitoylphosphatidylcholine (DPPC), cetyl alcohol, and tyloxapol, with a radiolabel safe for human use. Aerosolized $^{99m}$Tc-DTPA (diethylentriamine pentaacetate) has previously been used in several studies to demonstrate pulmonary deposition and clearance of aerosol in injured lungs.8,9 The purpose of this study was to determine and compare the distribution of the $^{99m}$Tc-DTPA when aerosolized with Exosurf. If the composition of aerosolized particles has a similar proportion of $^{99m}$Tc-DTPA and components of Exosurf, $^{99m}$Tc-DTPA could be used to study the acute distribution of Exosurf in the lungs of mechanically ventilated patients.

METHODS AND MATERIALS
The study used a ventilator (Siemens 900C, Siemens-Elema, Sweden) and nebulizer system (VISAN-9, Vortran Medical Tech-
technology Inc, Sacramento, Calif) as shown in Figure 1. The configuration is identical to that used in the current clinical trials evaluating Exosurf in sepsis-induced ALI, except that a six-stage viable cascade impactor (Graseby Andersen) was connected to the endotracheal tube (8 mm). The cascade impactor was factory calibrated to collect the following particle sizes: >10 μm (preseperator), 7 to 10 μm (stage 1), 4.7 to 7 μm (stage 2), 3.3 to 4.7 μm (stage 3), 2.1 to 3.3 μm (stage 4), 1.1 to 2.1 μm (stage 5), and 0.65 to 1.1 μm (stage 6). Triplicate determinations using two cascade impactors to collect the aerosol for 0 to 15 and 15 to 30 min, respectively, were performed. In addition, duplicate experiments using a single cascade impactor to collect the aerosol for 0 to 30 minutes were completed.

A one-way valve was inserted into the ventilator circuitry just prior to the patient wye to allow diluting air for the 28 L/min cascade impactor sampling flow rate. Ventilator operating parameters were as follows: 750 ml tidal volume, 20 breaths per minute, and an I:E ratio of 1:3 with a square wave inspiratory flow pattern. Neither positive end-expiratory pressure nor inspiratory hold was used due to the restraints imposed by the sampling system. The nebulizer system was set to an operating temperature of 50°C and delivered 600 ml of aerosol-filled gas into the inspiratory limb of the ventilator circuit during exhalation.

Approximately 15 min prior to initiation of the experiment, 30 mCi of 99mTc-DTPA (volume <1 ml) was added to Exosurf (120 ml) in a nebulizer canister (TriNeb-400). The canister was warmed to the 50°C operating temperature in an auxiliary nebulizer system unit. The ventilator/nebulizer system was equilibrated for 5 min prior to the aerosol collection period with a nonradiolabeled Exosurf canister. A rubber test lung was inserted in place of the cascade impactor during this equilibration period. The Exosurf and 99mTc-DTPA canister was transferred to the experimental setup, the cascade impactor was attached to the end of the endotracheal tube, and the aerosol was collected. After completion of the sampling period, the cascade impactor was disassembled, the preseparator and impaction plates were rinsed with two 10-ml portions of methanol, and the rinse volumes were transferred to 30-ml vials.

The vials were counted on a gamma camera (Trionix Triad) with a control vial and the counts converted to millicuries per vial based on the known activity of the control. The vials were allowed to decay for 1 week, evaporated to dryness, and reconstituted with 4 ml of methanol. The vial contents were analyzed for DPPC, cetyl alcohol, and tyloxapol content by high-pressure liquid chromatography, gas chromatography, and ultraviolet detection procedures, respectively.

The particle size distribution differences between 99mTc-DTPA and the Exosurf components were compared by Pearson's χ² analysis. Log probability plots of the data were used to determine the mass median aerodynamic diameter (MMAD) and geometric standard deviation.

The radiochemical purity of the 99mTc-DTPA/Exosurf mixture was evaluated using silica gel instant-thin layer chromatography (ITLC-SG). Chromatography was performed on 99mTc-DTPA and the 99mTc-DTPA/Exosurf mixture, heated to 50°C for 30 min, at the time of preparation, 15, 30, 60, and 120 min after preparation. Two solvents, acetone and 0.9 percent sodium chloride, were used. With acetone, 99mTc-pertechnetate moves with the solvent front and 99mTc-DTPA and any reduced hydrolyzed species remain at the origin. With saline solution, 99mTc-pertechnetate and 99mTc-DTPA move with the solvent front and insoluble species remain at the origin.

RESULTS

The relative radioactivity on each cascade impactor stage was compared with the relative amount of

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FIGURE 1. Laboratory set-up depicting nebulizer unit (VISAN-9), ventilator (Siemens 900C), cascade impactor (Graseby Andersen), and circuit configuration.
DPPC, cetyl alcohol, and tyloxapol on each stage (Fig 2). There was no statistical difference between DPPC and cetyl alcohol compared with 99mTc-DTPA within each stage of the cascade impactor for the 0 to 15-min (DPPC; p=1.0, cetyl alcohol; p=0.94), 15 to 30-min (DPPC; p=1.0, cetyl alcohol; p=1.0), and 0 to 30 min (DPPC; p=0.99, cetyl alcohol; p=0.99) periods of aerosolization. The aerosol distribution patterns between 0 to 15-min and 15 to 30-min time periods were similar for DPPC (p=0.65), cetyl alcohol (p=0.24), tyloxapol (p=0.99), and 99mTc-DTPA (p=0.56).

Compared with 99mTc-DTPA, tyloxapol was distributed differently for 0 to 15 min (p<0.01), 15-to 30-min (p<0.01), and 0- to 30-min (p<0.01) min time periods, with generally a wider particle size distribution range than the other components.

The 0- to 30-min aerosolization resulted in slightly greater deposition on stage 3 (3.3 to 4.7 μm) for all components compared with the 0- 15-min and 15- to 30-min aerosolization. Of the total mass deposited in the cascade impactor, 60 to 80 percent of the DPPC, cetyl alcohol, and 99mTc-DTPA was on stages 4 (2.1 to 3.3 μm) and 5 (1.1 to 2.1 μm), whereas, 40 to 45 percent of the tyloxapol was deposited on these two stages.

The MMAD was similar for each component and for each period of aerosolization (Table 1). The range of MMAD was 2.1 to 2.5 μm with a geometric standard deviation of 1.5 to 2.5 μm.

Exosurf did not alter the chromatographic analysis of 99mTc-DTPA (Table 2). Both 99mTc-DTPA alone and the 99mTc-DTPA/Exosurf mixture at 50°C demonstrated radiochemical stability for 30 min. At 60 min, the radiochemical purity of the 99mTc-DTPA alone decreased with 36.4 percent of the radioactivity being 99mTc-pertechnetate. A further decrease in radiochemical purity of 99mTc-DTPA was noted at 120 min. Conversely, the 99mTc-DTPA/Exosurf mixture did not demonstrate any significant change in radiochemical purity during the 120-min study.

**DISCUSSION**

This study demonstrated that the two predominant components of Exosurf (DPPC, cetyl alcohol) are aerosolized in a similar fashion to 99mTc-DTPA when admixed with Exosurf and measured by cascade impactor techniques. The aerosol was collected over two separate time periods to determine if aerosol created at the beginning was similar in content and size to the aerosol generated at the end of a 30-min period. Similar aerosol size and content distribution for Exosurf and 99mTc-DTPA were evident during the 0- to 15-min and 15- to 30-min collection periods.

**Table 1—Particle Size Distribution: Mass Median Aerodynamic Diameter (Microns)**

<table>
<thead>
<tr>
<th>Component</th>
<th>0-15</th>
<th>15-30</th>
<th>0-30</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPPC</td>
<td>2.1±0.06</td>
<td>2.1±0.06</td>
<td>2.3±0.21</td>
</tr>
<tr>
<td>Cetyl alcohol</td>
<td>2.1±0.06</td>
<td>2.2±0.12</td>
<td>2.3±0.21</td>
</tr>
<tr>
<td>Tyloxapol</td>
<td>2.3±0.15</td>
<td>2.4±0.12</td>
<td>2.5±0.00</td>
</tr>
<tr>
<td>99mTc-DTPA</td>
<td>2.1±0.00</td>
<td>2.2±0.10</td>
<td>2.4±0.14</td>
</tr>
</tbody>
</table>

**FIGURE 2.** Distribution pattern of DPPC, cetyl alcohol, 99mTc-DTPA, and tyloxapol on each cascade impactor stage. Distribution of tyloxapol was different compared with 99mTc-DTPA for 0 to 15 (p<0.01), 15 to 30 (p<0.01), and 0 to 30 (p<0.01) min. However, all four components were primarily deposited on stages 4 and 5 for all three time periods.

Similar relative proportions of Exosurf components (DPPC, cetyl alcohol) and 99mTc-DTPA were contained within each particle size range during all collection times. However, while tyloxapol was aero-
sorized in a similar fashion as the other Exosurf components and 99mTc-DTPA, with most of tyloxapol depositing on cascade impactor stages 3, 4, and 5. Tyloxapol did deposit to a greater extent on the preseparator and stages 1, 2, and 6. Tyloxapol is a relatively minor component of Exosurf. Tyloxapol is formulated with DPPC and cetyl alcohol primarily to provide a uniform suspension and prevent agglomeration during storage. Reasons for the deposition pattern observed for tyloxapol are unclear and require further investigations.

In addition to similar aerosol distribution patterns, Exosurf and 99mTc-DTPA, when aerosolized using the nebulizer system (VISAN-9) and nebulizer canister (TriNeb 400), were produced as respirable particles. Previous studies have demonstrated that particles between 1 to 5 μm are associated with good pulmonary deposition. Smaller particles (<0.5 μm) are usually exhaled and larger particles (>5 μm) are deposited in the upper airways.

Thin layer chromatographic analysis demonstrated that Exosurf did not alter the radiochemical purity of 99mTc-DTPA for the initial 30 min after preparation. At 1 h after preparation, Exosurf provided stability to the radiochemical purity of the 99mTc-DTPA by decreasing the amount of dissociation of 99mTc from DTPA into pertechnetate. This radiochemical analysis demonstrates that the radioactivity (99mTc) remains bound to the DTPA under heated conditions. Thus, the distribution of radioactivity should reflect 99mTc-DTPA and not 99mTc-pertechnetate.

Due to lack of direct radiolabeling of Exosurf components, the exact distribution of aerosolized Exosurf in the lung cannot be unequivocally determined using cascade impactor methods. Specific differences in inspiratory flow, inspiratory hold, and humidity that exist in the human airways may alter the actual deposition pattern observed in the lung compared with the cascade impactor. In these experiments, however, we used typical ventilatory parameters (i.e., tidal volume, respiratory rate) and humidified conditions to simulate a clinical set-up through the endotracheal tube. The humidified environment in combination with particle exposure time within the experimental set-up should ensure all particles are equally hydrated prior to entry into the cascade impactor. Therefore, Exosurf and 99mTc-DTPA particles should not have significantly different distribution patterns in the lung due to in vivo humidification.

CONCLUSIONS

Synthetic surfactant (Exosurf) and 99mTc-DTPA are aerosolized in a similar fashion when using the VISAN-9 and TriNeb nebulizer system. The 99mTc-DTPA added to Exosurf could be used as a radiolabel for determining acute pulmonary deposition and distribution of aerosolized Exosurf using gamma camera imaging in intubated patients.

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