Acute Effects of Liposome Aerosol Inhalation on Pulmonary Function in Healthy Human Volunteers*

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Administering liposome-encapsulated drugs by aerosol is a feasible way of targeting drugs to the lungs. Prior to clinical application of aerosolized liposomes as drug carriers, their relative safety must be established. We evaluated the effects of inhaling nondrug-containing liposomes (15 and 150 mg of lipid per milliliter) for 1 h on pulmonary function and on oximetry in ten healthy nonsmoking volunteers. Spirometry was performed prior to and at intervals after inhalation, and subjects were monitored with pulse oximetry. Liposome inhalation was well tolerated, and no oxygen desaturation, decrements in pulmonary function, or side effects were noted. We conclude that inhalation of small particle aerosols of SPC liposomes produces no acute deleterious effects on pulmonary function in healthy subjects.

*(Chest 1991; 99:1268-70)*

**SFC = soy phosphatidyl choline; PBS = phosphate-buffered saline**

The concept of targeted drug delivery is gaining increasing acceptance, particularly for treating pulmonary diseases.1 This mode of therapy could confine drug effects to affected tissues, minimize systemic spillover, and avoid potential toxic side effects common to systemic administration.

Administering liposome-encapsulated drugs by aerosol could target drugs to the lungs, and, specifically, to pulmonary alveolar macrophages. Liposomes are composed of naturally occurring or synthetic phospholipids into which a variety of drugs can be incorporated. Liposomes are ingested by phagocytes and, thus, afford a means to target antimicrobials and other drugs intracellularly to these cells. This an important consideration when treating pulmonary infections involving facultative intracellular organisms such as mycobacteria and fungi. Pulmonary delivery of liposome-encapsulated drugs has also been shown to confer attractive therapeutic advantages, including limiting systemic levels and confining drug effects to the lung,2 providing localized sustained-release therapy,3 and facilitating aerosolization of water-insoluble drugs or of drugs poorly tolerated by inhalation.4

Of several studies that have examined phospholipid aerosols as artificial surfactants in respiratory distress syndrome, none has found deleterious effects on gas exchange.5 A few authors have recently reported on the administration of drug-carrying liposome aerosols to small numbers of healthy volunteers,6,7 although no study performed to date has systematically examined any objective measures to document whether liposome aerosol inhalation might adversely affect pulmonary function. This is an important prerequisite, as it is known that individuals can have significant decreases in their measured forced expiratory volumes in the absence of symptomatic complaints.8 Therefore, before aerosolized liposomes can be confidently used in a clinical setting, their safety must be established. We elected to examine pulmonary physiologic parameters in healthy subjects during and after inhalation of nebulized liposomes.

**Materials and Methods**

We studied ten healthy nonsmoking adults who were prescreened by history, physical examination, and spirometry. Subjects with a known allergy to soybean products were excluded. After informed consent per our institutional review board for human studies, the study was carried out in our pulmonary function laboratory under the supervision of a pulmonologist.

On the morning of each study, baseline measurements of the FVC, FEV₁, FEV₁/FVC, ratio, FEF25-75%, and PEFR were obtained with a calibrated, ATS-approved pneumotachograph spirometer. Each subject's best measurements were compared to predicted values using the standards of Knudson et al.9
Sterile multilamellar liposomes were prepared under aseptic conditions from soy phosphatidylcholine (Phospholipon 90, American Lecithin Co.) by dispersion of a thin lipid film (15 or 150 mg of lipid per milliliter) in PBS via mechanical agitation. All preparations were tested for sterility, refrigerated at 4°C, and used within 48 hours of preparation.

Liposomes were warmed to room temperature prior to use, and a 30-ml volume was aerosolized via an air-driven slipstream nebulizer (Puritan-Bennett model 1917) (60-ml reservoir; flow rate of 13 to 15 L/min or 26 psi). The output and aerosol characteristics of liposomes with this nebulizer had been previously established as producing droplets with a mass median aerodynamic diameter of 2.4µ ± 0.2µ and a geometric standard deviation of 2.8µ ± 0.2µ, which are deposited in the alveolar region. Based on the apparent subjective safety of lipid aerosols containing 15 mg of lipid per milliliter used in a previous study, this starting concentration was selected for the first five volunteers. Since much higher lipid concentrations could eventually be required for certain therapeutic purposes, a tenfold increase in aerosol concentration (150 mg of lipid per milliliter of saline) was tested in the remaining five volunteers. During a 1-h inhalation via a mouthpiece with normal tidal breathing, oxygen saturation was continuously monitored noninvasively by pulse oximetry.

Spirometry was repeated at 15 and 60 minutes after inhalation. A significant decrease in flows from the baseline (≥15 percent for FEV₁, or ≥25 percent for FEF25-75%) would have been considered indicative of an early-phase reactive airways response. In the absence of an early-phase response, subjects were trained to use a disposable peak flowmeter, to record PEFR at 3, 5, and 8 hours after inhalation, and instructed to note any symptoms such as dyspnea, cough, fatigue, pleuritic pain, chills, fever, or dizziness.

Table 1 — Pulmonary Physiologic Parameters in Normal Volunteers after One-Hour Inhalation of Small-Particle SPC Liposome Aerosol

<table>
<thead>
<tr>
<th>Lipid Concentration and Case, Sex, Age (yr)</th>
<th>FEV₁, L/FVC, L</th>
<th>Mean Oxygen Saturation, percent (During)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 mg/ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1, M, 36</td>
<td>5.03/5.86</td>
<td>5.18/5.90</td>
</tr>
<tr>
<td>2, F, 31</td>
<td>4.25/5.10</td>
<td>4.52/5.23</td>
</tr>
<tr>
<td>3, M, 30</td>
<td>4.11/4.83</td>
<td>4.10/4.78</td>
</tr>
<tr>
<td>4, F, 29</td>
<td>3.51/3.84</td>
<td>3.52/3.82</td>
</tr>
<tr>
<td>5, F, 38</td>
<td>3.27/3.84</td>
<td>3.17/3.79</td>
</tr>
<tr>
<td>150 mg/ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6, F, 29</td>
<td>3.55/4.01</td>
<td>3.72/4.08</td>
</tr>
<tr>
<td>7, M, 33</td>
<td>3.53/3.70</td>
<td>3.30/3.91</td>
</tr>
<tr>
<td>8, F, 25</td>
<td>3.09/3.70</td>
<td>3.15/3.77</td>
</tr>
<tr>
<td>9, F, 42</td>
<td>3.58/4.11</td>
<td>3.61/4.17</td>
</tr>
<tr>
<td>10, M, 42</td>
<td>4.12/5.02</td>
<td>4.12/5.02</td>
</tr>
</tbody>
</table>

RESULTS

Each subject’s baseline values served as their own control, and the reported values are the actual values of FVC and FEV₁ in liters at the various time points. The mean oxygen saturation is also included and did not vary more than 3 to 5 percentage points from baseline. The FEF25-75 percent and PEFR were unchanged over the course of the study. The subjects were asymptomatic throughout the study period, and none showed any objective evidence of oxygen desaturation or of early or late phase response (see Table 1). Data analysis by the paired Student’s t-test showed no statistical differences.

DISCUSSION

We objectively searched for possible acute adverse reactions associated with inhaling liposomes. In healthy subjects, we clearly demonstrated no deleterious acute effects of liposome aerosols on spirometric values or on oxygenation.

Several investigators have administered aerosolized liposome-encapsulated agents to small numbers of human volunteers⁴-⁹ without subjective evidence of side effects. We recently completed a study in mice and found no alterations in bronchoalveolar lavage cell profiles or in pulmonary histopathology after daily exposure to small particle SPC liposome aerosols five days per week for four weeks for one to two hours per day.¹²

Although several studies have addressed liposome toxicity in vitro and with parenteral administration,¹³-¹⁶ there is a paucity of information on the safety of pulmonary liposome delivery.¹ Liposomes should be nontoxic, since phosphatidylcholine comprises approximately 70 to 80 percent of the normal lung surfactant pool.¹⁷ We selected SPC because it is readily available in a pharmaceutically pure grade in quantities necessary for larger clinical use, and because it has been used in liposome drug formulations previously studied.⁴-⁷,⁹,¹⁸

The clinical potential for the application of this method of drug delivery is in its relative infancy.³ Our study supports observations by others regarding the subjective safety of inhaled liposomes⁴-⁹ and is the first study to systematically and objectively assess pulmonary function in humans after acute inhalation of liposome aerosols.

ACKNOWLEDGMENTS: We thank Ms. Barbara Wilson, R.B.T., and Mr. Fred Smolensky of the Puritan-Bennett Corp. for facilitating the use of the nebulizers in this study.

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