A mathematical model to predict protein wash out kinetics during whole-lung lavage in autoimmune pulmonary alveolar proteinosis

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Submitted 4 September 2014; accepted in final form 10 November 2014


PULMONARY ALVEOLAR PROTEINOSIS (PAP) is a rare lung disorder in which surfactant-associated phospholipids and proteins abnormally accumulate within alveoli and terminal bronchioles, leading to impaired gas exchange and progressive respiratory failure (6, 33, 40). PAP is classified into three groups based on etiology: autoimmune PAP (aPAP), secondary PAP, and hereditary PAP (6, 17, 40). aPAP is caused by granulocyte/macrophage colony-stimulating factor (GM-CSF) autoantibodies, which prevent surfactant removal by alveolar macrophages (20, 41). aPAP is the most prevalent form of PAP, comprising 90% of all PAP cases (6, 17, 40). Currently, whole-lung lavage (WLL) remains the only standard therapy for aPAP (4, 7, 29). Although WLL improves PAP in about 85–95% of patients, around 15–66% of such patients may require multiple and repeated WLL therapy (1, 4, 37). Removal of the lipoprotein material by WLL immediately improves both lung volume and ventilation/perfusion ratio, leading to a marked increase in arterial oxygen gas pressure (5, 29, 36). In contrast, the diffusion capacity recovers gradually and incompletely over a 6-mo period (36). In addition, WLL decreases the area of ground-glass opacities but not reticular opacities and inter-
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Glossary

- $A_B$: The effective surface area from the blood
- $A_s$: The effective surface area from the surfactant
- $K_b$: The transmission coefficient from the blood
- $K_s$: The transmission coefficient from the surfactant
- $m_{b, n-out}$: The masses of protein in the blood
- $m_{l-in-out}$: The masses of protein in instilling saline and draining lavage fluid; actually, no protein exists in instilling saline
- $m_{l}$: The masses of protein in the lavage fluid
- $m_{out}$: The protein mass of drainage
- $m_{s}$: The masses of protein in the surfactant
- $R_{c, t}$: The absorption rate of fluid into the circulation
- $S_A$: The alveolar surface area
- $V_A$: The alveolar volume
- $V_b$: The volume of blood
- $V_{in}$: The fluid volume of instilled saline
- $V_{l}$: The volume of lavage
- $V_{l-b}$: The fluid volume absorbed into the circulation
- $V_{out}$: The fluid volume of drainage
- $V_s$: The volume of surfactant

Materials and Methods

Participants

Nine patients were enrolled in five hospitals in Japan. These hospitals included Tohoku University Hospital, Tokyo Medical University Hachioji Hospital, Aichi Medical University Hospital, Dokkyo Medical University Koshigaya Hospital, and Niigata University Medical and Dental Hospital. Diagnosis of aPAP was performed on the basis of cytological analysis of BALF, pulmonary histopathological findings, or both with high-resolution computed tomography appearance (40). All cases were confirmed to have elevated serum GM-CSF autoantibody levels (21, 41). The institutional review board of each hospital approved the study, and all subjects provided written informed consent. The study protocol was designed according to The Ethical Guideline of Clinical Research by The Japanese Ministry of Health, Labour, and Welfare in 2008.

Data of arterial blood gas analyses and serum markers were collected within 3 days, and pulmonary function tests were within 2 wk prior to WLL.

Procedure of WLL

Seventeen lungs from nine patients with aPAP underwent WLL. We allowed each participating hospital to conduct WLL in accordance with their own procedures. Generally, after administration of general anesthesia, patients were intubated with a double-lumen endotracheal tube to isolate the lungs, after which mechanical ventilation was initiated. After ventilation of the bilateral lungs with 100% oxygen for 5–15 min, saline was instilled into the lavage lung while ventilation of the other lung with 100% oxygen was continued. The instilled saline was then retained for a few minutes and then discharged by gravity into a container until a decrease in outflow was observed. These procedures were then repeated. In each lavage cycle, we prepared a timetable to record the exact time (to the second) of the start of instilling saline, the start of retaining, and the start and end of lavage fluid drainage. We measured the volume of drained lavage fluid and used a 10-ml aliquot for further analyses. All samples were stored at −80°C until use.

Measurement of Substance Concentration

The serum and BALF concentration of IgG, GM-CSF autoantibody, transferrin, albumin, β₂-microglobulin, urea, gastrin, and SP-D were measured; IgG were quantified by an ELISA system using Human IgG ELISA Quantitation Set (Bethyl Laboratories, Montgomery, AL) according to the manufacturer’s instructions. GM-CSF autoantibody concentrations were measured by an ELISA system as described previously (17). β₂-Microglobulin, gastrin, urea, and SP-D concentrations were measured by latex agglutination immunoassay (LA; LZ test Eiken β₂-M-II; Eiken, Tokyo Japan), radioimmunoassay (gastrin RIA kit II; Fujirebio, Tokyo, Japan), urease-indophenol method (urea nitrogen test; Wako, Tokyo, Japan), and enzyme immunoassay (SP-D kit Yamasa EIA II; Yamasa, Tokyo, Japan), respectively. Serum transferrin and albumin concentrations were measured by turbidimetric immunoassay (TIA; N-Assay TIA Tf-H Nittobo; Nittobo, Tokyo, Japan) and bromocresol purple dye-binding assay (PureAuto A ALB; Kainos, Toyko, Japan), respectively, and those in the BALF were analyzed by LA (N-Assay LA Micro Tf Nittobo) and TIA (AutoWako Microalbumin). These serum samples were collected just before the beginning of WLL.

A Mathematical Kinetic Model to Estimate the Concentration of Proteins in the Lavage Fluid

We postulated that proteins both in the accumulated surfactant material and in the pulmonary capillaries transfer into the lavage fluid. Under such circumstances, the rate of protein transfer to the lavage fluid is assumed to be as follows:

\[
\frac{dm_{l}}{dt} = \frac{dm_{b}}{dt} + \frac{dm_{s}}{dt} + \frac{dm_{l-b}}{dt} \tag{1}
\]

where the first term in the right-hand side is the transfer rate from surfactant, the second term is the transfer rate from blood, and the
third term is the rate of instilling and drainage. The transfer rate from surfactant \( \frac{dm_s}{dt} \) and the transfer rate from blood \( \frac{dm_b}{dt} \) are modeled by analogy to the heat transmission model as

\[
\frac{dm_s}{dt} = K_s \cdot A_s \left( \frac{m_s}{V_s} - \frac{m_1}{V_1} \right) \tag{2}
\]

\[
\frac{dm_b}{dt} = K_b \cdot A_b \left( \frac{m_b}{V_b} - \frac{m_{1,b}}{V_{1,b}} \right) \tag{3}
\]

where \( K_s \) and \( K_b \) are the transmission coefficient from the surfactant and the blood to the lavage fluid, respectively. \( A_s \) and \( A_b \) are the effective surface area from the surfactant and the blood to the lavage fluid, respectively. The parameters \( m_s, m_a, \) and \( m_b \) represent the masses of protein in the lavage fluid, surfactant, and blood, respectively. \( V_1 \) represents the fluid volume in lavage. \( V_s \) and \( V_b \) represent the fluid volume in surfactant and blood. We assumed that \( m_a, V_s, \) and \( V_b \) are constant during the WLL.

We calculated the temporal variation of the mass of protein and the volume of fluid in the stages of instilling, retaining, draining, and preparing in each lavage cycle, as described as follows.

**Instilling stage.** The volume change of protein and lavage fluid in the lung is expressed as:

\[
\frac{dm_{in-out}}{dt} = 0 \tag{4}
\]

\[
\frac{dV_1}{dt} = \frac{dV_{in}}{dt} - \frac{dV_{1-b}}{dt} \tag{5}
\]

where \( V_{in} \) is the fluid volume of instilled saline, and \( V_{1,b} \) is the fluid volume absorbed into the circulation expressed as

\[
\frac{dV_{1-b}}{dt} = A_b \cdot R_d \tag{6}
\]

where \( R_d \) is the absorption rate of fluid into the circulation. The concentration of protein was calculated as the ratio of the mass of protein to the fluid volume calculated from Eqs. 1–6 according to the procedures described in the following subsection.

**Retaining stage.** No saline is instilling in the retaining stage, which means

\[
\frac{dV_{in}}{dt} = 0 \tag{7}
\]

The variation of the mass of protein and the volume of fluid were calculated from Eqs. 1–7.

**Draining stage.** The lavage fluid is drained in this stage, which means

\[
\frac{dm_{in-out}}{dt} = -\frac{dm_{out}}{dt} \tag{8}
\]

\[
\frac{dV_1}{dt} = \frac{dV_{out}}{dt} - \frac{dV_{1-b}}{dt} \tag{9}
\]

where \( m_{out} \) and \( V_{out} \) are the protein mass and the fluid volume of drainage. The variation of the mass of protein and the volume of fluid were calculated from Eqs. 1–3 and 6, 8, and 9.

**Preparing stage.** Substance transfer in this stage may be considered to be similar to that in the retaining stage.

**Data Processing and Statistics**

Data including patient identity, protein concentrations in the serum or in the BALF of the right and left lungs, vital capacity, the number of cycles, the volume of instilled saline or drained lavage fluid, and time for each lavage stage were entered into a file (Microsoft Excel 2010). Using theoretical equations that solved protein concentrations in the drained lavage fluid (described in RESULTS), we wrote a program using Visual Basic Application to calculate the theoretical concentrations of proteins on the basis of specific variables.

Estimation of the protein concentration in the drained lavage fluid was carried out by numerically integrating differential equations using the following parameters: the volume of instilled saline, drained lavage fluid, time of each stage, the concentration of proteins in the first lavage cycle, effective alveolar and capillary surface area described below, and a given set of transmission coefficients, \( K_s \) and \( K_b \). The resulting concentration curve was optimized with actual measurements manually by changing transmission coefficients. The effective areas of alveolar surface and pulmonary capillaries were calculated according to the equations described in Appendix A (10).

Numerical data were evaluated for normal distribution by using Shapiro-Wilk tests. Nonparametric data were analyzed by using Kruskal-Wallis rank sum test. Multiple comparisons were performed through a Bonferroni-adjusted Wilcoxon rank-sum test. All tests were two-sided, and \( P \) values <0.05 were considered statistically significant. Data were analyzed by using R-version 2.15.2 (R Foundation for Statistical Computing, Vienna, Austria).

**RESULTS**

**Demographic and Clinical Findings for Study Subjects**

Nine patients with active aPAP were enrolled in this study. Demographic data are shown in Table 1.

The mean age at WLL was 54.3 ± 11.4 yr old, with a male-to-female ratio of 2:1. The duration of the disease from onset was variable, ranging 10–96 mo. Patients showed no evidence of active pulmonary infection. Pulmonary functions and laboratory findings are described in Table 2.

The mean arterial oxygen pressure at room air was 64.0 ± 15.4 mmHg in seven patients and 55.2 and 67.4 mmHg for two patients under nasal oxygen supply. Percentage of vital capacity and percentage of carbon monoxide diffusing capacity were moderately to severely suppressed with 72.1 ± 17.7% and 51.0 ± 21.5%, respectively, whereas forced expiratory volume in 1 sforced vital capacity was relatively conserved with 85.8 ± 10.9%. The mean serum biomarker levels of Krebs von den Lungen-6, SP-D, and carcinoembryonic antigen were 20,720 ± 13,953 IU/ml, 471 ± 271 ng/ml, and 26.7 ± 22.9 ng/ml, respectively. The mean serum GM-CSF autoantibody levels were 45.8 ± 51.7 μg/ml. These patient characteristics were similar to a past large Japanese cohort with PAP (17).

**Timetables and Volume Balance for WLL**

As shown in Fig. 1, A and B, each lavage cycle consisted of four stages: instilling (from the beginning to the end of saline instillation), retaining (from the end of saline instillation until the beginning of drainage), draining (from the beginning until the end of drainage), and preparing (from the end of drainage until the beginning of the next saline instillation). Twelve lungs from seven patients underwent WLL with short-term cycles (210–285 s), whereas five lungs from three patients underwent WLL with long-term cycles (550–634 s) (Table 3). In eight patients, both lungs underwent WLL; however, for one patient, only the right lung underwent lavage. Data for instilled saline volume, discharged lavage fluid volume, and time for each of the stages as defined above are shown in Tables 3 and 4. Lavage was repeated 11 to 29 times (median of 20 cycles) until the lavage fluid appeared clearer (Fig. 1C).

Time required for total WLL time ranged from 5,200 to 11,796 s. Instilling, retaining, draining, and preparing time
L108 MATHEMATICAL MODEL FOR WHOLE-LUNG LAVAGE

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Table 1. Demographic data on study subjects who underwent WLL

<table>
<thead>
<tr>
<th>Case</th>
<th>Age, yr</th>
<th>Sex</th>
<th>Symptoms*</th>
<th>Onset to WLL†, mo</th>
<th>DSS‡</th>
<th>Smoking Status</th>
<th>Occupational Dust Exposure</th>
<th>Complications</th>
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<tbody>
<tr>
<td>1</td>
<td>45</td>
<td>M</td>
<td>DOE, Occasional</td>
<td>66</td>
<td>2</td>
<td>Ex-Smoker</td>
<td>No</td>
<td>Psoriasis, HT, DL</td>
</tr>
<tr>
<td>2</td>
<td>54</td>
<td>F</td>
<td>DOE</td>
<td>43</td>
<td>2</td>
<td>Never</td>
<td>No</td>
<td>HT, DL</td>
</tr>
<tr>
<td>3</td>
<td>67</td>
<td>M</td>
<td>Dyspnea, Cough, Hemopto</td>
<td>10</td>
<td>5</td>
<td>Ex-Smoker</td>
<td>No</td>
<td>Postcerebral Infarction</td>
</tr>
<tr>
<td>4</td>
<td>34</td>
<td>M</td>
<td>Dyspnea</td>
<td>42</td>
<td>5</td>
<td>Current Smoker</td>
<td>No</td>
<td>None</td>
</tr>
<tr>
<td>5</td>
<td>53</td>
<td>F</td>
<td>Dyspnea</td>
<td>96</td>
<td>5</td>
<td>Never</td>
<td>No</td>
<td>SSS</td>
</tr>
<tr>
<td>6</td>
<td>46</td>
<td>M</td>
<td>None</td>
<td>30</td>
<td>1</td>
<td>Current Smoker</td>
<td>Yes</td>
<td>None</td>
</tr>
<tr>
<td>7</td>
<td>66</td>
<td>M</td>
<td>DOE</td>
<td>25</td>
<td>4</td>
<td>Never</td>
<td>No</td>
<td>DM</td>
</tr>
<tr>
<td>8</td>
<td>67</td>
<td>F</td>
<td>Dyspnea</td>
<td>36</td>
<td>5</td>
<td>Never</td>
<td>No</td>
<td>None</td>
</tr>
<tr>
<td>9</td>
<td>57</td>
<td>M</td>
<td>DOE</td>
<td>28</td>
<td>2</td>
<td>Never</td>
<td>No</td>
<td>None</td>
</tr>
</tbody>
</table>

*Symptoms were recognized as respiratory symptoms. †Onset: time when the 1st respiratory symptom emerged or time of finding an abnormal image that was compatible with pulmonary alveolar proteinosis (PAP). ‡Disease severity score (DSS): defined based on respiratory symptoms and arterial oxygen tension (PaO2; see Ref. 3). DSS 1: no symptoms and PaO2 ≥70 mmHg. DSS 2: symptomatic and PaO2 ≥70 mmHg. DSS 3: 60 mmHg ≤ PaO2 <70 mmHg. DSS 4: 50 mmHg ≤ PaO2 <60 mmHg. DSS 5: PaO2 <50 mmHg. WLL, whole-lung lavage; DOE, dyspnea on exertion; HT, hypertension; DL, dyslipidemia; SSS, sick sinus syndrome; DM, diabetes mellitus.

Simulation of Protein Concentrations in the Drained Lavage Fluid

The theoretical concentrations of IgG, transferrin, albumin, and β2-microglobulin in the drained lavage fluid were calculated at the end of the draining stage according to the equation described above and by using the procedures detailed in MATERIALS AND METHODS. The theoretical concentrations were plotted on a log scale against time after the beginning of WLL (Fig. 2). The plot for each patient was manually fitted with the protein concentration measured in the drained lavage fluid of each cycle by changing Ks and Kb. Plots for the theoretical concentrations of IgG, transferrin, albumin, and β2-microglobulin coincide with the measurements (Fig. 2, A–D). Data for Ks and Kb are shown in Fig. 3. Ks values for IgG, transferrin, albumin, and β2-microglobulin (2.03×10^-7±0.902, 1.95×10^-7±0.589, 1.84×10^-7±0.564, and 1.85×10^-7±0.658, respectively) did not vary among patients (Fig. 3A). Importantly, there was no significant difference in Ks values among these four proteins, suggesting that transfer from the surfactant to lavage fluid was independent of molecular weight. However, there was relative variability in Kb among proteins, especially with β2-microglobulin, which had a Kb that was two orders of magnitude higher than that of the other proteins. Kb values for IgG, transferrin, albumin, and β2-microglobulin were 4.97×10^-10±4.166, 5.61×10^-10±1.990, 3.82×10^-10±1.661, and 2.28×10^-8±0.773, respectively (Fig. 3B). No differences in Ks or Kb values of each protein were found between left and right lungs (data not

Table 2. Clinical parameters of patients

<table>
<thead>
<tr>
<th>Case</th>
<th>Arterial Blood Gas Analysis</th>
<th>Serum Biomarkers</th>
<th>Pulmonary Function Test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PaO2, mmHg</td>
<td>PaCO2, mmHg</td>
<td>A-aDO2, mmHg</td>
</tr>
<tr>
<td>1</td>
<td>81.3</td>
<td>35.8</td>
<td>24.0</td>
</tr>
<tr>
<td>2</td>
<td>75.6</td>
<td>39.4</td>
<td>25.1</td>
</tr>
<tr>
<td>3</td>
<td>67.4*</td>
<td>35.5*</td>
<td>269.0*</td>
</tr>
<tr>
<td>4</td>
<td>55.2*</td>
<td>38.4*</td>
<td>167.7*</td>
</tr>
<tr>
<td>5</td>
<td>46.4</td>
<td>34.8</td>
<td>60.1</td>
</tr>
<tr>
<td>6</td>
<td>72.3</td>
<td>38.8</td>
<td>29.2</td>
</tr>
<tr>
<td>7</td>
<td>51.2</td>
<td>36.7</td>
<td>52.9</td>
</tr>
<tr>
<td>8</td>
<td>46.2</td>
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</tr>
<tr>
<td>9</td>
<td>75.3</td>
<td>39.6</td>
<td>24.9</td>
</tr>
</tbody>
</table>

Normal Krebs von den Lungen-6 (KL-6), surfactant protein D (SP-D), carcinoembryonic antigen (CEA), and granulocyte/macrophage colony-stimulating factor autoantibody (GM-Ab) levels were within 500 IU/ml, 110 ng/ml, 5.0 ng/ml, 1.0 μg/ml, respectively. *Nasal oxygen supply. †Diffusing capacity of the lung for carbon monoxide (Dl,co) of case 5 was not detected because of low vital capacity (VC). ND, not done. A-aDO2, alveolar-arterial oxygen difference that was measured; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; FRC, functional residual capacity.
shown). Thus the simulation data confirm the appropriateness of our mathematical model and indicate that the transfer kinetics of proteins into the drained fluid was time dependent.

Durable Effects of the Time on the Lavage Efficiency

To determine the durable effect of each lavage cycle on the slope for the decreasing concentration of each protein in the drained lavage fluid, we evaluated the change in slope of the theoretical curves by varying the duration of the retaining stage in silico. For this purpose, we used the initial data settings in case 4, i.e., the instilling volume of saline; the durations (s) of instillation, retaining, draining, and preparing; and the volume of drained lavage fluid in the first lavage cycle. We found that decreasing curves for the albumin concentration became steeper upon substitution of the shorter time (Fig. 4A).

Next, we proceeded to confirm the effects observed in the simulation by using measurements in case 1. We evaluated the rate of declining albumin concentration in the lavage aliquots from a patient who occasionally underwent WLL for the left lung with short-term cycles (120 s, 1–20 cycles) and for the right lung with long-term cycles (540 s, 4–11 cycles). As shown in Fig. 4B, the slope of decline for the left lung appeared...
Table 3. Timetable of the stages in each cycle of WLL

<table>
<thead>
<tr>
<th>Case</th>
<th>Left/Right</th>
<th>No. of Cycles</th>
<th>Total Time, s</th>
<th>Each Cycle, s†</th>
<th>Stage a,</th>
<th>Stage b,</th>
<th>Stage c,</th>
<th>Stage d,</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
<td></td>
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<td>Instilling, s</td>
<td>Retaining, s</td>
<td>Draining, s</td>
<td>Preparing, s</td>
</tr>
<tr>
<td>1</td>
<td>L</td>
<td>20</td>
<td>6260</td>
<td>258 ± 15.0</td>
<td>42 ± 3.0</td>
<td>120 ± 1.6</td>
<td>83 ± 4.2</td>
<td>13 ± 18.6</td>
</tr>
<tr>
<td></td>
<td>R‡</td>
<td>11</td>
<td>6895</td>
<td>561 ± 161.7</td>
<td>41 ± 8.1</td>
<td>425 ± 196</td>
<td>59 ± 16.4</td>
<td>8 ± 2.6</td>
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<tr>
<td>2</td>
<td>L</td>
<td>20</td>
<td>5200</td>
<td>210 ± 21.9</td>
<td>28 ± 3.0</td>
<td>120 ± 0</td>
<td>52 ± 23.2</td>
<td>13 ± 5.1</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>20</td>
<td>5450</td>
<td>220 ± 8.3</td>
<td>45 ± 5.9</td>
<td>120 ± 0</td>
<td>52 ± 16.1</td>
<td>8 ± 8.1</td>
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<tr>
<td>3</td>
<td>L</td>
<td>20</td>
<td>5583</td>
<td>237 ± 15.1</td>
<td>36 ± 7.0</td>
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<td>73 ± 18.4</td>
<td>10 ± 2.6</td>
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<td></td>
<td>R</td>
<td>20</td>
<td>6060</td>
<td>236 ± 38.8</td>
<td>35 ± 6.0</td>
<td>120 ± 0</td>
<td>64 ± 26.8</td>
<td>20 ± 16.8</td>
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<td>215 ± 19.4</td>
<td>35 ± 4.1</td>
<td>120 ± 0</td>
<td>54 ± 24.7</td>
<td>10 ± 3.3</td>
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<td></td>
<td>R</td>
<td>29</td>
<td>9018</td>
<td>285 ± 17.5</td>
<td>57 ± 3.9</td>
<td>120 ± 0</td>
<td>102 ± 16.8</td>
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<tr>
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<td>L</td>
<td>20</td>
<td>5230</td>
<td>227 ± 30.6</td>
<td>31 ± 11.9</td>
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<td>76 ± 31.9</td>
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<td>121 ± 20.2</td>
<td>19 ± 24.8</td>
</tr>
<tr>
<td>6</td>
<td>L</td>
<td>20</td>
<td>5395</td>
<td>266 ± 32.3</td>
<td>10 ± 0</td>
<td>120 ± 0</td>
<td>133 ± 32.0</td>
<td>5 ± 0</td>
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<tr>
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<td>R</td>
<td>20</td>
<td>5680</td>
<td>278 ± 42.2</td>
<td>28 ± 11.2</td>
<td>132 ± 24.6</td>
<td>112 ± 24.8</td>
<td>5 ± 0</td>
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<td>283 ± 31.6</td>
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<td>120 ± 0</td>
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<td>20</td>
<td>11796</td>
<td>634 ± 264.9</td>
<td>180 ± 32.1</td>
<td>199 ± 28.7</td>
<td>148 ± 50</td>
<td>96 ± 250.3</td>
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<td>8</td>
<td>L</td>
<td>16</td>
<td>10380</td>
<td>550 ± 49.5</td>
<td>188 ± 41.3</td>
<td>200 ± 6.2</td>
<td>153 ± 20.5</td>
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<td></td>
<td>R</td>
<td>20</td>
<td>11796</td>
<td>533 ± 185.8</td>
<td>146 ± 42.8</td>
<td>193 ± 24.2</td>
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<td>9</td>
<td>L</td>
<td>11</td>
<td>6180</td>
<td>627 ± 98.1</td>
<td>225 ± 45.2</td>
<td>231 ± 64.1</td>
<td>225 ± 63.3</td>
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<td>R</td>
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<td>225 ± 45.2</td>
<td>231 ± 64.1</td>
<td>225 ± 63.3</td>
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*Data are presented as a mean ± SD of time (s) required for 1 lavage cycle. Time for total on each stage of lavage cycle is expressed as a mean ± SD. Instilling time (stage a) is mean time (s) required for instilling saline into the lung. Retaining time (stage b) is mean time (s) applied for retaining saline in the lung. Draining time (stage c) is mean time (s) required for draining lavage fluid to the container. Preparing time (stage d) is mean time (s) required for preparation for the next saline instillation. †Each cycle time is the mean of stage a to d from 2nd to the last lavage. The 1st cycle required 120–1080 s. ‡Time (s) for the 1st 3 cycles ranged within 230–270 s, and that for the 4th to 11th cycles ranged within 625–680 s.

The instilling volume of the 1st cycle in case 1–7 was determined by the following equations: functional residual capacity (ml) × 0.45 or 0.55 + tidal volume for the left and right lung, respectively. In case 8 and 9, saline was allowed to be instilled into the lung as much as possible from a bottle at 30 cm height from the tracheal tube.
when the cumulative eliminated albumin in case 1 was estimated in silico with fixed lavage cycle time at 240 s, the eliminated albumin appeared to increase as the instilled volume increased during 0 to 3,200 s. After 3,200 s, the eliminated albumin gradually increased, but the volume effect seemed to be diminished.

Exceptional Substances That Fail to Follow the Mathematical Model

Although we applied our mathematical model to the transfer of various substances during WLL, we found that the following substances did not follow the model.

**Gastrin and urea.** Measured levels of gastrin and urea did not exhibit an exponential decreasing phase but instead reached a plateau in the early stage of WLL (Fig. 5, A and B). Thus calculation of $K_s$ was difficult. Permeation of gastrin and urea from the blood to the lavage fluid occurred so quickly that the theoretical curves were hardly matched with the actual measurements, which themselves fluctuated markedly during the plateau phase.

**SP-D.** The SP-D concentration in the drained lavage fluid decreased consistently to a minor extent in the four lungs in the absence of an exponential phase and quickly reached a plateau in the early phase (Fig. 5C). As alveolar type II cells and nonciliated Clara cells abundantly release SP-D into the lower respiratory tract, this early plateau phase reflects its active release in situ.

**GM-CSF autoantibody.** Although the quantified GM-CSF autoantibody belongs to an IgG isotype, theoretical curves of the concentration in the drained lavage fluid did not fit with the measured autoantibody concentration even upon substitution of various sets of coefficients with $K_s$ and $K_b$ in all 17 lungs (Fig. 5D).

**DISCUSSION**

By using a mathematical model based on measured concentrations of proteins, this study investigated the transfer of proteins from the surfactant and blood into the lavage fluid during WLL. We confirmed that the transfer followed a time-dependent differential equation, which assumes that the rate of transfer is proportional to the transmission coefficient, the effective surface area, and the protein gradient between the body compartment and lavage fluid (44).

By using various methods (e.g., comparisons of the protein concentrations between the plasma, sputum, and BALF) and by proving that the IgG1/IgG2 ratio between the BALF and serum are comparable, previous studies demonstrated the transfer of circulating proteins into the alveolar spaces (2, 14, 18, 28, 39).

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**Fig. 2.** Theoretical concentrations (lines) and measured concentration (plots) of IgG (A), transferrin (B), albumin (C), and β2-microglobulin (D) in the drained aliquot of lavage fluid for each cycle. The vertical axis is the concentration of the protein on a log scale, and the horizontal axis indicates the time after the beginning of WLL.
More recently, intravenously injected GM-CSF autoantibodies were detected in the BALF of nonhuman primates and were observed to reproduce PAP (35). These results indicate that the antibody can cross the air-blood barrier (35). The kinetics of transfer from the blood to the air space and vice versa was studied both in vitro and in vivo (3, 23, 26, 27, 34). In one study, the transmission coefficient (10⁻¹⁰ cm/s) of various proteins across a monolayer of A549 cells was shown to indicate bidirectional transfer. These coefficients appear to be inversely correlated with the molecular weight of proteins (22). In another study, the transmission coefficient for proteins in a monolayer of rat alveolar epithelial cells in vitro was within 10⁻⁹⁻¹⁰⁻⁷ cm/s, whereas that for albumin in sheep lung in vivo was 5 x 10⁻¹⁰ cm/s (11, 17). Thus mass transfer from the blood to the air spaces may be continuously taking place even at steady state.

In previous studies by Ikegami et al. (15), surface tension maintained by surfactant materials covering the alveolar surface was found to have a probable role in interfering with mass transfer and subsequent accumulation of circulating proteins in the air spaces. Interference with the transfer is known to be disrupted by the elimination or deficiency of SP-B (15, 16). Lung lavage may remove surface-active materials in the alveoli and thus temporally disrupt the mechanisms that interfere with the influx of circulating proteins. It is for this reason that we focused on WLL to clarify the mechanism of protein transfer in the lung during WLL.

To postulate a mathematical model, we assumed that the transfer of proteins from each body compartment to the lavage fluid consists of two pathways, namely transfer from the accumulated surfactant to the lavage fluid and transfer from the blood to the lavage fluid. The latter may be further divided into two pathways, namely transfer from the blood through the surfactant and direct transfer to the lavage fluid. However, we did not distinguish between these two latter pathways in this study because the transfer of a protein across the air-blood barrier seemed to be rate limiting. We found that protein transfer from the surfactant to the lavage fluid appeared to have \( K_s \) values independent of the molecular weight and other properties. It is notable that the \( K_s \) values did not differ among patients, indicating the reproducibility of the model. However, mass transfer from the blood to the lavage fluid with variable \( K_b \) values did appear to be affected by the molecular weight of the protein because the protein was transferred through a semipermeable membrane consisting of endothelial cells, basement membrane, and type I pneumocytes. Transcytosis was proposed as the primary mechanism of protein transfer for large molecules and of partial paracellular diffusion of small molecules (7, 23). However, the true mechanism remains controversial. As indicated in this study, transfer of \( \beta_2 \)-microglobulin (molecular weight of 11 kDa) from the blood to the lavage fluid had \( K_b \) values that were two orders of magnitude higher than those of albumin, transferrin, and IgG, which had molecular weights of 66, 80, and 150 kDa, respectively. This difference suggests that \( \beta_2 \)-microglobulin diffusion possesses a mechanism that is different from that of other proteins, i.e., it is supposed to be mainly transcytosis for albumin, transferrin, and IgG but mainly paracellular diffusion for \( \beta_2 \)-microglobulin. Further analyses will be required to clarify the mechanisms by measuring the permeability of various substances with molecular weight of 10–60 kDa to confirm a "gap" in permeability coefficient \( K_b \) among substances with molecular weights in this range.

It is notable that the decrease in concentrations of low-molecular-weight substances in the lavage fluid, namely urea (molecular weight of 60 kDa) and gastrin (molecular weight of 2.1 kDa), was inconsistent with our mathematical model. The measured concentrations appeared to fluctuate and appeared to be independent of time. Moreover, the phase of exponential

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**Fig. 3. Coefficients of transfer of IgG, transferrin, albumin, and \( \beta_2 \)-microglobulin from surfactant \((K_s) (A) \) and blood \((K_b) (B) \) to the lavage fluid. The vertical axis indicates the transmission coefficients \((\text{cm/s})\) on a log scale. Statistical significance of coefficients between 2 proteins are shown in the figure.**
decrease was hardly defined in six out of ten lungs examined; when there was any decrease, the phase lasted within 1,000 s after the start of WLL (data not shown). This characteristic was likely due to the high permeability of the air-blood barrier to the molecules. Similarly, Rennard et al. (32) reported that urea was more able than glucose and albumin to permeate into the lavage fluid, as observed in normal volunteers with saline instilled into their lung segments.

SP-D is produced by alveolar type II cells and nonciliated Clara cells in the lower respiratory tracts and is secreted into the air space (43). Although SP-D is detectable in the sera of patients with aPAP, its levels are much lower than those of BAL (12). Thus SP-D transfer from the blood to the air space is negligible. The high concentration of SP-D in the lavage fluid was likely due to its continuous production in the lung. The rate of its production was estimated to be 6–13 mg/h on the basis of evaluation of four lungs (data not shown).

The lung is the organ that most abundantly produces GM-CSF, a factor that is critical for terminal differentiation of alveolar macrophages, as it promotes the expression of the transcription factor, PU.1 (38). It is suggested that IgG-type GM-CSF autoantibody is pathogenic and is known to be transferred from the lung capillaries into the air spaces immediately formed by GM-CSF autoantibody complex to become undetectable by our GM-CSF autoantibody ELISA system (30).

Furthermore, we had better to reconsider the adequacy of the present mathematical model when it was applied to substances

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**Fig. 4.** A: durable effect of the retaining stage in each lavage cycle on the theoretical decreasing curve of albumin concentration in the drained lavage fluid. The time assumed for the retaining stage was variable: a, 540 s; b, 360 s; c, 240 s; d, 120 s; e, 60 s; and f, 30 s. The vertical axis indicates the albumin concentration in the lavage fluid (mg/ml). The horizontal axis indicates the time after the beginning of WLL. B: theoretical (lines; black, left; gray, right) and measured (plots; ○, left, ▲, right) concentrations of albumin in the drained lavage fluid in each cycle. The vertical axis indicates the albumin concentration in the lavage fluid (mg/ml). The horizontal axis indicates the time after the beginning of WLL. C: simulation curves of cumulative amount of albumin drained in the drained lavage fluid when the retaining time varied with 90 (solid line), 210 (small dashed line), 450 (dotted line), or 570 (large dashed line) s. D: cumulative amount of eliminated albumin in the drained lavage fluid. An in silico evaluation by changing instilled saline volume varied with 600 (black solid line), 1,400 (dotted line), or 2,400 (gray solid line) ml.
with lower molecular weights by assuming two permeation coefficients, such as $K_{b1}$ (coefficients from the blood to the lavage fluid through surfactant) and $K_{b2}$ (from the blood directly to the lavage fluid).

In the present study, the recovery rate in the first draining lavage fluid was lower than those after the second lavage. Although the first instilled saline remained in the lower respiratory tracts, we did not mind the remaining volume at the first draining because we thought that the remaining lavage fluid could be recovered after the second draining. Therefore, we did not intentionally extend the first draining time longer than those of other cycles. Although we usually perform percussion or vibration on the patient’s chest, the recovery rate at the first draining was not improved by these procedures. It is likely that the low recovery rate and its variability of the first lavage shown in Table 4 were due to the early cessation of the first draining.

To date, methods of WLL for the treatment of PAP have not been standardized (25). Michaud et al. (29) recommended instilling 1 l of saline into the lavage lung and then to clamp the draining tube for 4–5 min (29). Bonella et al. (4) and Paschen et al. (31) determined the number of lavage cycles by measuring the optical density of each lavage fluid. They applied statistical evaluation to data from a number of WLLs to find the relationship between instilled saline volume and eliminated proteins. Although their approach is fundamentally different from ours, their finding that instilling volume is an important element for determining the amount of eliminated protein was confirmed in this study (Fig. 4D). The protocol for WLL used in this study were variable among participating hospitals, and thus time of each cycle varied between 213–630 s, including 120–540 s for the retaining time. As for our mathematical model, the number of cycles and the retaining times did not influence the efficiency of WLL. Based on Eq. 1, the amount of proteins eliminated by WLL was dependent on time after the beginning. According to the volume effect demonstrated by in silico simulation in this study (Fig. 4D), larger instilled volume appeared to improve the efficiency of lavage. However, the simulation also suggested that the effect is limited within some range of time. Previous studies, however, demonstrated the volume effect (4). In this regard, total eliminated albumin concentration significantly correlated with instilling saline volume in actually measured values in 17 WLLs of the present study with Rho value at 0.69. However, we have to consider the possibility that it also prolonged the duration of instilling and draining time, and thus longer time for each lavage cycle increases the elimi-
nated protein(s). Thus our mathematical model may be useful to predict the amount of eliminated proteins at a certain time point after the beginning of WLL.

In conclusion, we demonstrated that protein transfer in the lung during WLL followed a relatively simple, mathematical model based on diffusion and that this model could be expressed in terms of a number of differential equations. As an exception of the present mathematical model, substances with low molecular weight do not follow the theory. Our study, not only contributes to the design of an efficient regimen for WLL, but also reveals the mechanism of delivery of specific large drug molecules across the air-blood barrier, such as antibody drugs.

APPENDIX

The Effective Alveolar Surface Area

The effective alveolar surface area was calculated from the data for the alveolar volume, $V_A$, according to the following equations: $A_s = 6.4 \times 10^3 V_A^{2/3}$. For a person with 74 kg body wt, both $A_s$ and $V_A$ were reported to be 143 m$^2$ and 3,338 ml, respectively (10). The effective surface area of the pulmonary capillaries, $A_b$, was estimated from the following formula (10): $A_b = 0.89 A_s$. The relationship between alveolar surface area, $S_A$, and alveolar volume, $V_A$, depends on the number of alveoli. $S_A$ increases as the number of alveoli increases at a fixed value of $V_A$. According to Ref. 10, the average lung volume is 4,300 ml, and the average alveolar surface is $(143 \pm 12) \times 10^4$ cm$^2$ in normal subjects with an average body weight of 74 kg at 19–40 yr of age. Under these conditions, air-space volume density is 0.865 ± 0.013 cm$^3$/cm$^3$, and alveolar surface density is $370.6 \pm 28.9$ cm$^2$/cm$^3$.

We set

$$\beta = \frac{S_A}{V A} \quad \text{(A1)}$$

where, the right side of the equation is an expression for the constant shape parameter, $\beta$.

According to the report described above ($V_A$ and $S_A$ in the space $V$)

$$\frac{S_A}{V} = 370.6 \text{ cm}^2/\text{cm}^3 \quad \text{(A2)}$$

$$S_A = 143 \times 10^2 \text{ cm}^2 \quad \text{(A3)}$$

$$\frac{V_A}{V} = 0.865 \text{ cm}^3/\text{cm}^3 \quad \text{(A4)}$$

From Eqs. A2 and A3,

$$V = 3859 \text{ ml} \quad \text{(A5)}$$

and from Eqs. A4 and A5

$$V_A = 3338 \text{ ml} \quad \text{(A6)}$$


$$\beta = \frac{\sqrt{\frac{143 \times 10^2}{3338}}}{143 \times 10^2} = 0.02 \quad \text{(A7)}$$

On the basis of Eq. A1 (note that $S_A$ is in m$^2$ and $V_A$ is in ml),

$$S_A = \beta^2 \cdot \frac{V A}{2} = 6.403 \times 10^3 \cdot \frac{V A}{2} \quad \text{(A8)}$$

The value of $\beta$ may be considered as constant even with a change in $V_A$ in the same subject, as the number of alveoli and the shape do not change, particularly in the supine position.

Method for Optimizing the Transmission Coefficients

A program was written in Visual Basic Application using various coefficients to calculate the theoretical substance concentrations in the lavage aliquots. For explanation, we show an example of simulation used to obtain the best fitting curve shown in Fig. 2C. As shown in Appendix Fig. A1A, the value for $K_b$ could be determined to be $1.8 \times 10^{-7}$ cm/s by the least-square method until 3,000 s when $K_b$ was assumed to be 0 cm/s. Next, $K_b$ value was determined to be $5.2 \times 10^{-10}$ cm/s again by the least-square method by 9.018 s. As shown in Appendix Fig. A1B, the theoretical curve appeared closer to the dotted actual measurements. Then $K_b$ was changed to $6.1 \times 10^{-10}$ cm/s manually, as shown in Appendix Fig. A1C; the theoretical curve is in VA in the same subject, as the number of alveoli and the shape do not change, particularly in the supine position.
Innovative Methodology

L116  MATHEMATICAL MODEL FOR WHOLE-LUNG LAVAGE

I. Methods:

1. The mathematical model for whole-lung lavage (WLL) is derived from the pulmonary hemodynamics and the physiological processes involved in the procedure.

2. The model incorporates the following key processes:
   a. Blood flow and ventilation distribution
   b. Alveolar fluid clearance
   c. Epithelial barrier function
   d. Chemical and vascular responses

3. The model is designed to simulate the dynamics of fluid and solute transport in the lungs during WLL.

II. Results:

1. The model predicts that WLL can effectively reduce the burden of pulmonary edema and alveolar proteinosis.

2. The simulation results demonstrate the impact of different lavage techniques on the pulmonary mechanics and the recovery of lung function.

III. Discussion:

1. The model provides insights into the mechanisms underlying WLL and suggests potential improvements in the technique.

2. The findings have implications for the clinical application of WLL in various pulmonary diseases.

IV. Conclusion:

1. The mathematical model for WLL offers a valuable tool for understanding the physiological and therapeutic aspects of the procedure.

2. Future work should focus on validating the model against clinical data and integrating it into decision-making frameworks for WLL.

References:


sorption from the lung versus retention within the lung is highly size-


