Surfactant phospholipid catabolic rate is pool size dependent in mice

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Kramer, Boris W., Machiko Ikegami, and Alan H. Jobe. Surfactant phospholipid catabolic rate is pool size dependent in mice. Am J Physiol Lung Cell Mol Physiol 279: L842–L848, 2000.—We increased surfactant pool size by surfactant treatment in mice to test if the catabolism of the major component of surfactant, saturated phosphatidylcholine (Sat PC), was rate limited. By intratracheal instillation, we gave mice trace doses, doses of 45 or 110 μmol/kg, or three doses of 110 μmol/kg of Sat PC in surfactant that contained radiolabeled dipalmitoylphosphatidylcholine (DPPC) and a radiolabeled phospholipase A-resistant ether analog of DPPC. Two strains of mice with 2-fold differences in alveolar and total Sat PC pool sizes were used; the mice with the higher pool sizes had a 2.3-fold higher steady-state catabolic rate. Acute increases in alveolar surfactant given by intratracheal instillation increased catabolic rates ∼2-fold over the steady-state rates in both strains. There was minimal loss of the ether analog of DPPC from the lungs, and the alveolar macrophages did not accumulate more than 10% of the ether analog. In these two strains of mice, the catabolism of Sat PC was not rate limited because catabolic rate increased when alveolar pool sizes were increased.

Surfactant treatment; dipalmitoylphosphatidylcholine; alveolar macrophage

Although the alveolar pool size of surfactant differs between species, it is relatively constant at a steady state for each species (24, 32). The major component of surfactant, saturated phosphatidylcholine (Sat PC), is synthesized and translocated to lamellar bodies in alveolar type II cells for storage and secretion into the air space (8). Recycling and catabolism are primary functions of type II cells, and alveolar macrophages clear and catabolize ∼20% of Sat PC in the rabbit (37). Hyperinflation and exercise increase surfactant pool sizes by causing increased secretion, with a return to normal pool sizes within hours (26), although the mechanisms that regulate steady-state surfactant pool sizes are not known. In genetically altered mice, alveolar pool sizes can be changed as a result of different metabolic adaptations (13, 16, 21). For example, granulocyte-macrophage colony-stimulating factor (GM-CSF)-deficient mice have a decreased catabolism that results in alveolar proteinosis, which can be corrected by exogenous GM-CSF administration (13, 33). Mice that express interleukin-4 in Clara cells also have an alveolar proteinosis phenotype, but both net anabolism and catabolism increase to achieve a new steady state (16). Mice that lack surfactant protein (SP) D have increased alveolar and tissue pool sizes of surfactant lipids, but the surfactant proteins are not proportionately increased (21). Surfactant pool sizes can vary in different strains of mice. For example, beige mice have smaller alveolar SPs and larger lamellar body pools, with a higher percent recycling of Sat PC than wild-type mice, demonstrating another metabolic adaptation (10). In this study, we asked if surfactant pool size is regulated primarily by catabolic rate in two strains of mice, Black Swiss and C57BL/6, with different steady-state pool sizes (14, 15). Radiolabeled dipalmitoylphosphatidylcholine (DPPC) and a phospholipase A-resistant analog of DPPC were given together with large amounts of exogenous surfactant to acutely change the alveolar pool size in order to mimic surfactant treatment for acute respiratory distress syndrome. Catabolic rates were then measured to determine if the catabolic rate was rate limiting or if it varied with pool size. The contribution of alveolar macrophages to catabolism of surfactant was also evaluated.

MATERIALS AND METHODS

Surfactant. The surfactant used for intratracheal instillation was a mixture of lipid-extracted sheep surfactant and DPPC (Sigma, St. Louis, MO) in order to increase the concentration of DPPC and to minimize the volume of the injection solution. Of the total amount of Sat PC, two-thirds was from sheep surfactant and one-third was from supplemental DPPC. Trace amounts of radiolabeled phospholipids were also added. Alveolar wash fluid from adult sheep was centrifuged to recover surfactant, which was then extracted with chloroform-methanol (2:1) (28). Each radioactive surfactant preparation contained [3H]palmitoyl-labeled DPPC (American Radiolabeled Chemicals, St. Louis, MO) and the phospholipase A1- and A2-resistant [14C]choline-labeled 1,2-di-palmitoyl-sn-glycero-3-phosphocholine (DPC) ether (37). [14C]DPC-ether was synthesized by methyllating the diether-dimethylethanolamine precursor with [14C]methyl iodide (34). [3H]DPPC and [14C]DPC-ether were mixed with chloroform extracts of sheep surfactant. The extracts were dried by
rotary evaporation and resuspended in 0.9% NaCl with the use of glass beads (14) so that 45 μl contained 0.5 μCi of [3H]DPPC and 0.13 μCi of [14C]DPC-ether. Doses were calculated as micromoles of Sat PC per kilogram of body weight.

**Study design.** Animals were injected intratracheally with different doses of radiolabeled surfactant to acutely change the alveolar pool size and to radiolabel that pool (Fig. 1). Eight-week-old female Black Swiss and C57BL/6 mice were anesthetized with methoxyflurane and orally intubated with a 25-gauge animal feeding needle. Groups of 5–8 animals/dosage group received 45 μl of surfactant containing sufficient Sat PC to deliver a trace dose or 45 μmol/kg (50 mg/kg) or 110 μmol/kg (120 mg/kg) of Sat PC. The trace dose was equivalent to approximately one-tenth of the alveolar pool size (14). In an independent experiment, C57BL/6 mice were given 110 μmol/kg of surfactant that was not radiolabeled 24 and 12 h before a third dose of 110 μmol/kg of radiolabeled surfactant. The surfactant pools were then measured 10 min and 6 and 24 h after administration of the radiolabeled surfactant.

**Clearance of [3H]DPPC from the lung.** The curves for [3H]DPPC clearance from the alveolar compartment and its recovery in alveolar macrophages and total lung are shown in Fig. 3. The clearance curves were different for the trace dose and the higher treatment doses. In C57BL/6 mice (Fig. 3, A–C), there were lower recoveries of [3H]DPPC after the trace dose than after the single doses of 45 and 110 μmol/kg or the three doses of 110 μmol/kg in both the alveolar wash fluid and total lung. Recoveries in alveolar wash fluid and the total lung were very similar for the 45 μmol/kg, the 110 μmol/kg, and the three-dose groups. In Black Swiss mice (Fig. 3, D–F), 4.9% of the [3H]DPPC was fluid, and alveolar macrophages was measured with liquid scintillation counting (Beckman LS 6500).

When more than two comparisons were made, ANOVA followed by Student-Newman-Keuls multiple comparison procedure was used. Curves were fit with the use of linear regression. The level of significance was set at $P < 0.05$.

**RESULTS**

**Sat PC pool sizes in Black Swiss and C57BL/6 mice.** Sat PC pool sizes in alveolar wash fluid and lung homogenates were ~2 times higher in C57BL/6 than in Black Swiss mice (Fig. 2). In Black Swiss mice, the average alveolar pool size was 10.7 μmol/kg compared with 22.3 μmol/kg in C57BL/6 mice. The total surfactant pool size in C57BL/6 mice was 59.8 μmol/kg compared with 31.8 μmol/kg in Black Swiss mice.

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recovered from alveolar wash fluid after the trace dose, and 26.5% of the 110 μmol/kg dose was recovered after 24 h (P < 0.01). For the total lung, 46.1% of the [3H]DPPC was recovered from the Black Swiss mice treated with 110 μmol/kg of Sat PC, and 15.3% was recovered from the animals given the trace dose after 24 h (P < 0.01). Percent recovery from alveolar macrophages did not change with dose for either strain of mice.

**Biological half-life of [3H]DPPC in total lung.** The biological half-life of [3H]DPPC in total lung of Black Swiss mice was 8.9 h after the trace dose; the half-life after the 110 μmol/kg dose increased to 21.4 h (Table 1). The biological half-life measured with the trace dose was 6.4 h in C57BL/6 mice. It increased with the 45 μmol/kg dose to 12.7 h and to 15.8 h after the 110 μmol/kg dose. The three-dose instillation of 110 μmol/kg yielded a half-life of 12.8 h. The half-life values increased ~2-fold for all high doses in both strains of mice.

**Net catabolism in total lung.** The averaged net Sat PC catabolism rate was approximated as micromoles per kilogram per hour for total lung over the intervals of 10 min to 6 h and 6–24 h after treatment, assuming the measured decline in phospholipids to be linear. Because the catabolic rate changes over time, the calculated catabolic rates represent an averaged rate over the time intervals of 10 min to 6 h and 6–24 h (Fig. 4). The steady-state catabolic rates measured with the trace dose were 1.4 ± 0.2 μmol/h for the Black Swiss mice and 3.2 ± 0.3 μmol/h for the C57BL/6 mice (P < 0.01). The C57BL/6 mice, with a steady-state total lung Sat PC pool size 1.9-fold higher than that for Black Swiss mice, had a catabolic rate that was 2.3-fold higher. The net catabolic rate increased when pool sizes were increased with surfactant treatment, and

**Table 1. Biological half-life for [3H]DPPC in total lung**

<table>
<thead>
<tr>
<th>Sat PC</th>
<th>C57BL/6 Mice</th>
<th>Black Swiss Mice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trace dose</td>
<td>6.4</td>
<td>8.9</td>
</tr>
<tr>
<td>45 μmol/kg</td>
<td>12.7*</td>
<td></td>
</tr>
<tr>
<td>110 μmol/kg</td>
<td>15.8*</td>
<td>21.4*</td>
</tr>
<tr>
<td>110 μmol/kg, 3 doses</td>
<td>12.8*</td>
<td></td>
</tr>
</tbody>
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Values are means ± SE in h. Mice received saturated phosphatidylcholine (Sat PC) in surfactant radiolabeled with [3H]dipalmitoylphosphatidylcholine ([3H]DPPC). *P < 0.01 vs. trace dose.
the increases were dose dependent in C57BL/6 mice ($P < 0.05$).

Catabolic rate relative to Sat PC pool size and treatment dose. Because total lung surfactant pool sizes varied between the two strains of mice, a treatment dose of surfactant containing 110 μmol/kg of Sat PC resulted in an ~4.8-fold increase in pool size in Black Swiss mice compared with a 3-fold increase in C57BL/6 mice. After the catabolic rates calculated for the animals given the trace doses (assumed to be at steady state) were normalized to a value of 1.0, the Sat PC catabolized per hour was compared for the 10-min to 6-h and 6- to 24-h intervals (Fig. 5). The catabolic rates increased ~2-fold for the 10-min to 6-h interval independent of dose or strain of mice. The catabolic rates were somewhat lower over the 6- to 24-h interval for C57BL/6 mice treated with 45 or 110 μmol/kg of Sat PC. In contrast, catabolic rates increased ~40% for the Black Swiss mice and ~25% for the C57BL/6 mice that received 110 μmol/kg of Sat PC repeatedly.

Clearance of $^{14}$C/DPC-ether. The recovery of $^{14}$C/DPC-ether from alveolar wash fluid, alveolar macrophages, and total lung is shown in Fig. 6. DPC-ether was lost from the alveolar compartment, but there was minimal accumulation in alveolar macrophages. This lack of accumulation in alveolar macrophages occurred independent of strain or surfactant dose. The highest level of accumulation (10%) was observed in C57BL/6 mice after three doses of 110 μmol/kg. Differences between the two mouse strains and between the different dosage groups were not significant. Recoveries from total lungs remained almost unchanged over 24 h, indicating no measurable catabolism of the ether analog.

DISCUSSION

We instilled large doses of surfactant to acutely increase the alveolar and total lung Sat PC pools of mice to evaluate catabolic rates for Sat PC. We performed this physiological manipulation of the Sat PC pool sizes to explore if steady-state pool sizes of surfactant are regulated primarily by limitation of catabolism in mice. We measured steady-state catabolic rates using trace doses of surfactant containing radiolabeled Sat PC. We found that Sat PC catabolic rates differed by a factor of approximately two in the two strains of mice that had a 2-fold difference in alveolar pool size. However, catabolic rates doubled when the amount of Sat PC was increased acutely in both strains of mice, demonstrating that catabolism was not rate limiting in determining Sat PC pool sizes in mouse lungs.

The general understanding of surfactant catabolism is that both macrophages and type II cells contribute to the net catabolic rate and that there is very little loss of intact surfactant phospholipids from the lung across the epithelium or by clearance via the upper airways (28, 29). In our experiment, we assumed that trace doses, which are equivalent to one-tenth of normal pool size, do not change the catabolic rate. We assumed a homogeneous distribution of exogenous surfactant that mixed with the endogenous pool. We also assumed there would be no differences between the mouse strains in catabolism of the DPPC associated with species-heterologous surfactant (31).

Catabolism of surfactant has been studied primarily in rabbits and rats (6, 20), but newly described genetically based abnormalities in surfactant homeostasis in rabbits and rats have been described more recently (31).
mice make the mouse the model of choice for future studies of surfactant homeostasis (7). In the rabbit, Sat PC, SP-A, SP-B, and SP-C are catabolized by both macrophages and type II cells and recycling of all components by type II cells occurs to variable extents (12, 28, 39, 40). Estimates of the catabolic contribution of alveolar macrophages to Sat PC catabolism have been made with the use of the same phospholipase A1- and A2-resistant diether analog of DPPC that was used for this study (34, 35). In the rabbit, ~20% of Sat PC was catabolized by macrophages, and most of the radiolabel that was associated with lung tissue after alveolar wash was localized to type II cells. The ether analog accumulated in a lysosome-like subcellular fraction isolated from the type II cells (36). In the rat, some breakdown of surfactant phospholipids may occur at the surface of the alveolar epithelium, and both type II cells and macrophages have been shown to be involved in surfactant lipid and protein catabolism (3, 25).

A number of unique factors that alter surfactant catabolism have been identified. Preterm and term newborn lungs catabolize surfactant components very slowly and recycle Sat PC more efficiently than the adult lung (11, 18). The newborn lung has very few macrophages, which may contribute to the slow catabolism (17). The rabbit lung achieved alveolar Sat PC pool sizes and catabolic rates comparable to the adult lung by ~2 wk of age (19). GM-CSF-deficient mice have large increases in alveolar and lung tissue surfactant components, with normal synthetic and secretory rates. Catabolism of Sat PC, SP-A, and SP-B is very slow, and macrophages from GM-CSF-deficient mice degrade Sat PC and SP-A more slowly than do macrophages from wild-type mice (42). Abnormal GM-CSF signaling may explain a number of cases of idiopathic alveolar proteinosis in humans (5). Alberti et al. (1) used alveolar lavage fluid from alveolar proteinosis patients to conclude that the major abnormality was slow catabolism. Alveolar proteinosis in humans and large increases in surfactant lipids in animals can also be caused by exposure of the lung to chronic inflammatory stimuli such as silica and heavy metal dusts (4, 41). In mice, expression of interleukin-4 by Clara cells causes an alveolar proteinosis phenotype that results from increases in the number of type II cells and increases in both surfactant component synthesis and catabolism that achieve a new steady state (16).

In lung injury, clearance rates for surfactant can be increased (22). Adult rabbits given single or repeated doses of surfactant rapidly clear the excess Sat PC from the lungs (30). Therefore, a low dose of surfactant for treatment of the adult lung may be of short-term
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benefit if the healthy lung can rapidly clear surfactant. Furthermore, the clearance rate may be increased with injury. Treatment of patients with acute respiratory distress syndrome with instilled surfactant in large doses given repeatedly may improve the outcome (9). The effects of large doses are illustrated with our results in mice. Only 26% of the treatment dose of 110 μmol/kg (120 mg/kg) was recovered by alveolar wash at 24 h. However, the initial dose given by airway instillation was 15 times the steady-state alveolar pool size, resulting in a pool size at 24 h that was five times larger than the steady-state pool size of ~10 μmol/kg. Therefore, although net catabolic rate increased with large doses of surfactant, the alveolar pool was increased by large surfactant doses. The alveolar Sat PC pool size in the human was estimated to be ~4 μmol/kg, and a surfactant dose of 100 mg/kg will contain ~60 μmol of Sat PC. If alveolar clearance in humans is similar to that in mice and no data are available, then a treatment dose will acutely increase the alveolar pool 15-fold, and the pool size would still be increased ~4-fold after 24 h.

We gave the mice the DPC-ether analog to evaluate the contribution of alveolar macrophages to the catabolism of Sat PC in mice based on the usefulness of this probe for quantifying the contributions of different cells to catabolism in rabbits (35). We anticipated an accumulation of DPC-ether in the macrophages recovered by alveolar wash, but no large amount of accumulation occurred. However, the trend of the curves for macrophage labeling with DPC-ether was toward increasing (Fig. 6) versus decreasing curves for DPPC (Fig. 3). One possibility is that macrophages from mice can catabolize DPC-ether, perhaps by phospholipases other than phospholipases A1, and A2. However, there was very little loss of the DPC-ether label from the lungs over 24 h, indicating minimal net catabolism. These results indicate that macrophages in mice may not be a major factor in catabolism. This conclusion is at odds with in vitro data that demonstrate active catabolism of DPPC by alveolar macrophages in mice (38) and with correction of the alveolar proteinosis in GM-CSF-deficient mice by bone marrow transplantation of GM-CSF-positive cells (27). The localization of the DPC-ether in the lung will need to be better characterized by evaluation of which cell types in the lungs contain the label.

The mouse lung can adapt to an acute increase in the surfactant pool size by increasing catabolic rate ~2-fold. If the amount of Sat PC given to the lung is large, the alveolar and tissue pools can remain elevated for >24 h. Catabolic rate is not rate limiting for the regulation of steady-state pool sizes of Sat PC in the lungs of mice.

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