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Lysophospholipid and fatty acid inhibition of pulmonary surfactant: Non-enzymatic models of phospholipase A_2 surfactant hydrolysis

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Abstract

Secretory A_2 phospholipases ($sPLA_2$) hydrolyze surfactant phospholipids cause surfactant dysfunction and are elevated in lung inflammation. Phospholipase-mediated surfactant hydrolysis may disrupt surfactant function by generation of lysophospholipids and free fatty acids and/or depletion of native phospholipids. In this study, we quantitatively assessed multiple mechanisms of $sPLA_2$-mediated surfactant dysfunction using non-enzymatic models including supplementation of surfactants with exogenous lysophospholipids and free fatty acids. Our data demonstrated lysophospholipids at levels ≥10 mol% of total phospholipid (i.e., ≥10% hydrolysis) led to a significant increase in minimum surface tension and increased the time to achieve a normal minimum surface tension. Lysophospholipid inhibition of surfactant function was independent of the lysophospholipid head group or total phospholipid concentration. Free fatty acids (palmitic acid, oleic acid) alone had little effect on minimum surface tension, but did increase the maximum surface tension and the time to achieve normal minimum surface tension. The combined effect of equimolar free fatty acids and lysophospholipids was not different from the effect of lysophospholipids alone for any measurement of surfactant function. Surfactant proteins did not change the percent lysophospholipids required to increase minimum surface tension. As a mechanism that causes surfactant dysfunction, depletion of native phospholipids required much greater change (equivalent to >80% hydrolysis) than generation of lysophospholipids. In summary, generation of lysophospholipids is the principal mechanism of phospholipase-mediated surfactant injury in our non-enzymatic models. These models and findings will assist in understanding more complex in vitro and in vivo studies of phospholipase-mediated surfactant injury.

Keywords: Surfactant; Lysophospholipid; Secretory phospholipase A2; Surface tension; Free fatty acid

1. Introduction

Pulmonary surfactant facilitates the work of breathing by reducing surface tension in alveoli and small conducting airways [1–4]. This surface activity is due to the combined physical properties and complex interactions of phospholipids (~80%), neutral lipids (~10%) and proteins (~10%) [5]. The phospholipid and protein components of surfactant are depleted in lung diseases characterized by acute inflammation including the acute respiratory distress syndrome (ARDS) [6–8], burn injury [9], and asthma [10–13].

Approximately 80% of the phospholipid component in surfactant is phosphatidylcholine (PC), and 50% of the PC component is dipalmitoyl-PC, which is essential for maximal surface tension lowering activity [5,14]. The second most abundant surfactant phospholipid (~10%) is the anionic phosphatidylglycerol (PG) which plays an important role in phospholipid–protein interactions [15,16]. The fatty acid content of PG and the other surfactant-associated phospholipids are similar to that reported for PC.

Abbreviations: BAL, bronchoalveolar lavage; $sPLA_2$, secretory phospholipase A_2; ARDS, acute respiratory distress syndrome; PL, phospholipid; PC, phosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; PG, phosphatidylglycerol; LPC, lysophosphatidylcholine; LPG, lysophosphatidylglycerol

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We and others have demonstrated that $sPLA_2$'s hydrolyze surfactant in vitro and disrupt surfactant's surface tension lowering activity [17,18]. Secretory phospholipases hydrolyze the sn-2 fatty acid from a phospholipid glycerol backbone, producing a free fatty acid and a lysophospholipid, generally without preference for specific sn-2 fatty acids [19–21]. For example, $sPLA_2$-mediated hydrolysis of PC produces lysophosphatidylcholine (LPC) and free fatty acids including palmitic acid, oleic acid and linoleic acid [22,23]. The lack of fatty acid preference by $sPLA_2$'s is distinct from cytosolic PLA_2's ($cPLA_2$) which preferentially target intracellular arachidonic acid leading to subsequent production of leukotrienes and prostaglandins [24].

There are ten known human $sPLA_2$ genes [20,25–27]. Several $sPLA_2$ have been identified in lung tissue and in leukocytes that infiltrate the inflamed lung [28–32]. Elevated $sPLA_2$ activity has been demonstrated in bronchoalveolar lavage (BAL) fluid in animal models of lung inflammation [33,34] and human studies of ARDS and asthma [12,13,33,35–37].

Increasing concentrations of group IB $sPLA_2$ caused parallel increases in both hydrolysis of surfactant PL and increased minimal surface tension. We have recently observed hydrolysis of surfactant PL and surfactant dysfunction by other human $sPLA_2$s, including groups IIA, IID, V and X [18,31]. Despite the consistency of each enzyme being able to cause surfactant dysfunction, the efficiency of hydrolysis against the principal phospholipids (PC, PG and phosphatidylethanolamine) varies substantially with each enzyme. This apparent discrepancy suggests that multiple mechanisms contribute to the ability of $sPLA_2$'s to lead to surfactant dysfunction.

The three most obvious mechanisms of $sPLA_2$-mediated surfactant dysfunction are: the release of lysophospholipids, which can disrupt the packing of the phospholipid film at the air–liquid interface as reported by Holm et al. [38], release of free fatty acids, which can also disrupt packing of the phospholipid film, or the depletion of phospholipid leading to an inability to maintain the phospholipid film. In this report, we quantitatively assess the impact of each of these mechanisms using in vitro non-enzymatic models. Our results demonstrate that lysophospholipids have a greater effect on disrupting surfactant surface tension than free fatty acids or total phospholipid depletion.

2. Materials and methods

2.1. Materials

All lysophospholipids and fatty acids were obtained from Avanti Lipids (Alabaster, AL) and include: 1-palmitoyl-glycerolphosphocholine, 1-palmitoyglycerolphosphoglycerol, palmitic acid, oleic acid and arachidonic acid. Survanta® is a commercially available extracted surfactant (Ross Laboratories, St. Louis, MO). Porcine surfactant was obtained from whole lung lavage of juvenile pigs post euthanasia (graciously provided by Dr. Jakob Vinten-Johanssen). To maintain high levels of hydrophobic and hydrophilic surfactant proteins, the porcine surfactant was washed repetitively with saline as described [17], but not organically extracted.

2.2. Surfactant function

A pulsating bubble surfactometer (Electronetics, Amherst, NY) was used to quantitate several measurements of surfactant surface tension (γ) lowering activity [39]. Unless otherwise stated, all measurements were made at a final concentration of 1.2 μmol phospholipid/ml (equal to 1 mg/ml), in buffered saline containing 5 mM $CaCl_2$ [17]. Pulsations were continued for a maximum of 10 min or until $\gamma \leq 1.0$ mN/m for at least 3 min. The minimum surface tension (γ_{min}) represents the minimum surface tension of at least 3 consecutive min achieved during each 10 min run [17]. The maximum surface tension (γ_{max}) represents the initial surface tension value obtained prior to the onset of pulsations. Time to achieve a normal γ_{min} represents the time to reach and maintain a value of $\gamma \leq 3$ mN/m, defined by the upper confidence limit (99.9%) for all γ_{min} measurements of native Survanta during these experiments.

2.3. Non-enzymatic models of hydrolysis

To model the impact of lysophospholipid and fatty acid generation on surfactant function, samples of surfactant were mixed daily by initially drying an aliquot of lysophospholipid or free fatty acid under N_2 in a glass mixing tube to which surfactant was added for a final total phospholipid concentration of 1.2 μmol/ml. Concentrations of supplemented lysophospholipids and fatty acids are reported as % of total phospholipids on a mole:mole basis, which equates to the % hydrolysis. The total concentration of phospholipids including native surfactant phospholipids (Survanta® or porcine surfactant), exogenous lysophospholipids, and exogenous free fatty acids was maintained at 1.2 μmol/ml for all experiments. This approach was utilized in order to accurately mimic and compare the effects of the individual hydrolysis products. Mixing of the supplemented lysophospholipid and fatty acid into the surfactant was achieved using repetitive vortexing at 37 °C for a minimum of 30 min [17]. Phospholipid concentrations were determined prior to mixing based on the measurement of lipid phosphorus [40].

To model the impact of total phospholipid depletion in isolation from the effects of hydrolysis products, we diluted surfactant preparations with Tris-buffered saline (5 mM, pH 7.4) containing 5 mM $CaCl_2$ to achieve final phospholipid concentrations of 0.12–2.4 μmol/ml. Reduction of phospholipid concentration equates to the % loss of total phospholipids from 1.2 μmol/ml standard used in the surfactometer.

To assess the impact of surfactant-associated proteins in our model, two surfactant mixtures were used. Survanta® contains only small amounts of the hydrophobic surfactant proteins (SP-B, SP-C) and no hydrophilic surfactant proteins (SP-A, SP-D). Juvenile porcine surfactant, which was not organically extracted, contains all surfactant proteins. In separate studies, endogenous concentrations of LPC and LPG have been measured in Survanta® (1.0% and 0.4% mole/mole, respectively), and in porcine surfactant (<0.5% for both) using HPLC and evaporative light scatter [13].

2.4. Statistics

All data are presented as the mean±S.E.M. of at least 3 experiments. Significance was evaluated by unpaired t-test, assuming equal variance and using one-tailed (γ_{min} and time to achieve a normal γ_{min}) and two-tailed t-tests (γ_{max}) where appropriate.

3. Results

3.1. Accumulation of lysophospholipids

Hydrolysis of surfactant by $sPLA_2$ generates lysophospholipids that may have detergent effects, altering the surface lowering function of the surfactant film. To quantitatively assess the effect of lysophospholipids on surfactant function, LPC was substituted for Survanta® at increasing concentrations while maintaining a total phospholipid concentration of 1.2 μmol/ml. At LPC concentrations of ≥10 mol%, γ_{min} significantly increased in a dose dependent manner ($P<0.01$, Fig. 1). Remarkably similar results were obtained when LPG was supplemented into the surfactant instead of LPC which

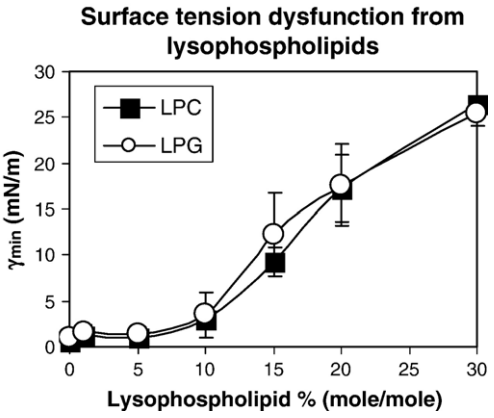

Fig. 1. Effect of lysophospholipid on minimum surface tension. Surfactant PL (Survanta®) was replaced with either lysophosphatidylcholine or lysophosphatidylglycerol on an equimolar basis. Final phospholipid concentration (including exogenous lysophospholipids) in all experiments was 1.2 μmol/ml. Minimum surface tension (γ_{min}) was measured in a pulsating bubble surfactometer. Significantly increased surface tension was seen at lysophospholipid levels >10 mol% LPC ($P<0.01$) and ≥15 mol% LPG ($P\leq 0.02$).

achieved statistical significance at concentrations ≥15 mol% ($P\leq 0.02$). These results suggest disruption of phospholipid packing is mediated more by loss of the acyl chains at the sn-2 position than the head group structure.

3.2. Accumulation of free fatty acids

The other products generated by $sPLA_2$-mediated hydrolysis of phospholipids are free fatty acids (FFA). Palmitic acid is the most abundant fatty acid in pulmonary surfactant. When palmitic acid was added to Survanta®, no significant increase in γ_{min} was seen even at the highest concentration (30 mol%), which equates to 30% hydrolysis (Fig. 2). Oleic acid, a monounsaturated fatty acid also present in surfactant, did significantly increase γ_{min} at concentrations of >15 mol% ($P<0.02$), but the magnitude of surfactant dysfunction was less than seen with the lysophospholipids. The highly unsaturated arachidonic acid significantly increased γ_{min} at concentrations ≥5 mol% ($P<0.01$). Although a potential role for arachidonic acid liberation from fetal surfactant has been described [41], in general, arachidonate levels in most pulmonary surfactants are extremely low a and therefore should not play a significant role in $sPLA_2$-mediated surfactant hydrolysis and dysfunction.

3.3. Combination of lysophospholipids and free fatty acids

Since $sPLA_2$-mediated hydrolysis of a phospholipid simultaneously produces a lysophospholipid and a free fatty acid (FFA) in equimolar amounts, we also simultaneously supplemented surfactant with LPC and a FFA (Fig. 3). Neither palmitic acid nor oleic acid added in equimolar amounts with LPC altered the threshold concentration of 10 mol% LPC required to produce significantly increased γ_{min} values. Only palmitic acid added at 15 mol% increased surface tension to a greater magnitude than LPC alone, although the mechanism of this is unknown. These results suggest that FFA's provide little synergistic effect to the disruption of minimum surface tension caused by lysophospholipids.

3.4. Depletion of total phospholipid

Secretory PLA_2s will not only produce lysophospholipid and free fatty acids by hydrolyzing PL, they will also deplete the concentration of intact PL in a surfactant film. The effect of PL depletion on γ_{min} was measured using dilution of the surfactant phospholipid concentration with buffered saline. Decreasing concentrations of surfactant from 2.4 to 0.12 μmol/

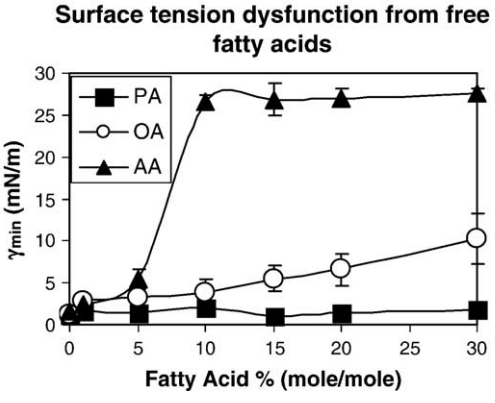

Fig. 2. Effect of free fatty acid content on minimum surface tension. Surfactant PL (Survanta®) was replaced with a free fatty acid (FFA) including palmitic acid (PA), oleic acid (OA) or arachidonic acid (AA) on an equimolar basis. Final phospholipid concentration in the sample without FFA was 1.2 μmol/ml, but decreased as increasing phospholipid was substituted by fatty acid. Minimum surface tension (γ_{min}) was measured in a pulsating bubble surfactometer. PA did not significantly increase γ_{min}, but significantly increased γ_{min} was seen with OA (>15 mol%, $P<0.02$) and AA (>5 mol%, $P<0.01$).

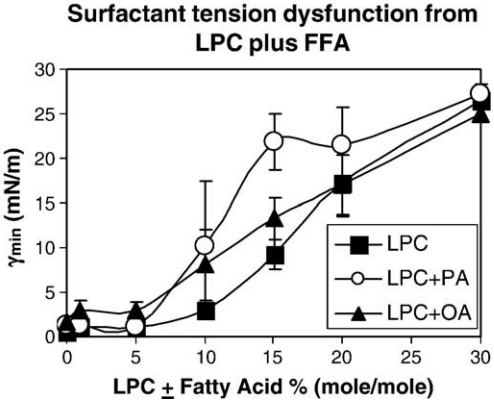

Fig. 3. Combined effects of lysophospholipid and free fatty acid on minimum surface tension. Surfactant PL (Survanta®) was replaced by increasing amounts of either LPC alone or in combination with either PA or OA, on an equimolar basis. Final phospholipid concentration (including exogenous LPC) in all experiments was 1.2 μmol/ml. Minimum surface tension (γ_{min}) was measured in a pulsating bubble surfactometer. Neither free fatty acid significantly altered the minimal threshold concentration of LPC which caused a significant increase in minimal surface tension (>10 mol%, $P<0.01$).

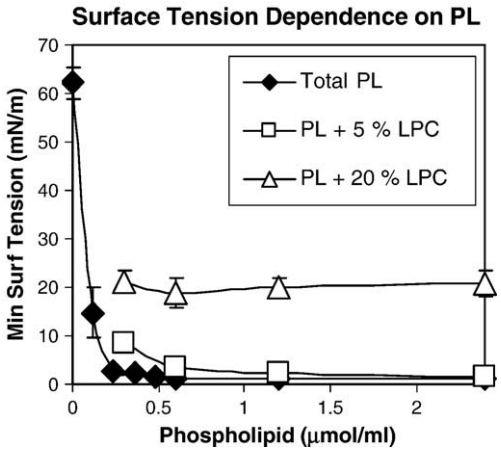

Fig. 4. Effect of total phospholipid on lysophospholipid-mediated inhibition of minimum surface tension. The γ_{min} of Survanta® with decreasing phospholipid (PL) concentrations (2.4.0 to 0.12 μmol/ml) was measured in a pulsating bubble surfactometer. The effect on γ_{min} of two LPC concentrations (5% and 20%, mole/mole) mixed with Survanta® over a similar range of total PL concentrations (0.3 to 2.4 μmol/ml) was also measured. Surface tension increased significantly at PL concentrations below 0.3 μmol/ml ($P<0.01$), and at all concentrations in the presence of 20 mol% LPC ($P<0.001$). The γ_{min} values with Survanta® containing either 5 mol% or 20 mol% LPC were not changed with different PL concentrations as compared to the γ_{min} at 1.2 μmol/ml.

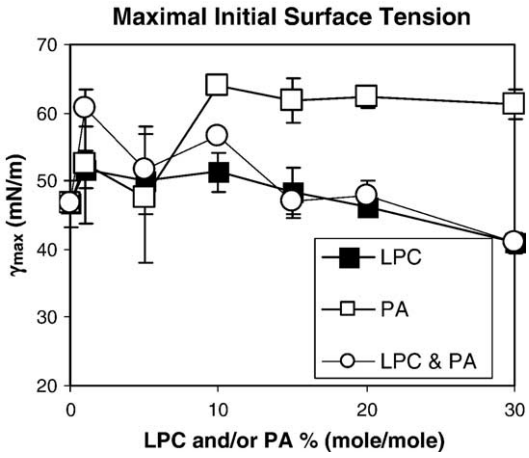

Fig. 5. Effect of surfactant lysophosphatidylcholine and palmitic acid on surfactant maximum surface tension. Surfactant PL (Survanta®) was replaced by increasing amounts of either LPC, PA or LPC in combination with PA as described in Figs. 1–3. Maximum surface tension (γ_{max}) was measured in the surfactometer using the initial surface tension prior to the onset of pulsations. PA increased γ_{max} at concentrations ≥10 mol% ($P<0.05$). LPC alone or in combination with PA did not alter γ_{max} at any concentration.

ml were analyzed (Fig. 4). At concentrations >0.3 μmol/ml, γ_{min} was consistently low, but at concentrations <0.3 μmol/ml, γ_{min} increased significantly in a dose dependent manner. Of note, the concentration of total PL in our surfactant models which mimicked the effects of hydrolysis through accumulation of lysophospholipids and fatty acids was 1.2 μmol/ml. Accordingly, these results indicate that >80% hydrolysis would be required for surfactant dysfunction to occur via a mechanism of total PL depletion.

To determine whether lysophospholipid generation and PL depletion had combined effects on minimal surface tension of surfactant, the PL depletion curve was repeated over a range of 0.3 to 1.2 μmol/ml with the addition of either 5 mol% or 20 mol% LPC (Fig. 4). At 5 mol% lysophospholipid, γ_{min} was not significantly altered except at the lowest phospholipid concentration (0.3 μmol/ml). At 20 mol% LPC, γ_{min} values were increased significantly as predicted by Fig. 1, but γ_{min} values were not otherwise altered by decreasing phospholipid concentrations.

3.5. Lysophospholipid and free fatty acid effects on γ_{max}

Changes in surfactant composition have been shown to affect maximum surface tension (γ_{max}) which can be used to assess initial adsorption of the phospholipids to the air–liquid interface [42]. The γ_{max} for Survanta alone was 46.7±3.4 mN/m, and mixtures containing LPC or LPC and PA together at concentrations up to 30 mol% had no significant effect (Fig. 5). Interestingly, PA alone at concentrations ≥10 mol% substitution significantly increased γ_{max} to >60 mN/m ($P<0.05$). Similarly, supplementation with oleic acid at concentrations ≥10 mol% increased γ_{max} (data not shown).

3.6. Lysophospholipid and free fatty acid effects on time to γ_{min}

The time (or pulsations) required to reach a normal γ_{min} is an additional measure of surfactant function that is independent of the γ_{min} [42]. In Fig. 6, PA at concentrations ≥10 mol% significantly increased the time required to achieve a normal γ_{min} of <3 mN/m ($P<0.05$), even though PA did not increase the final γ_{min} achieved at any concentration. Interestingly, these findings correlate closely with the effects of PA on γ_{max} at the same concentrations in Fig. 5. For all experiments using supplementation of surfactant with LPC or LPC with PA at concentrations ≥10 mol% a γ_{min} <3 mN/m was not achieved

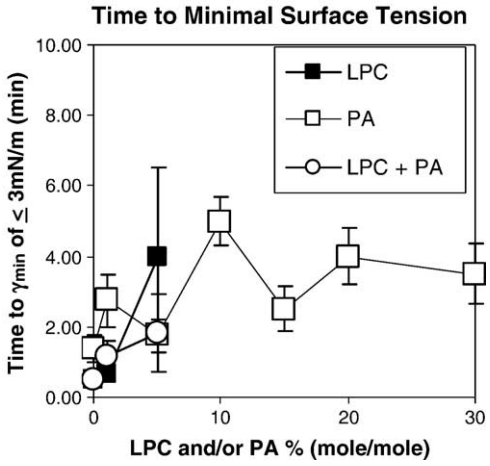

Fig. 6. Effect of lysophosphatidylcholine and palmitic acid on time required to achieve normal minimum surface tension. Surfactant PL (Survanta®) was replaced by increasing amounts of LPC, PA or LPC in combination with PA as described in Figs. 1–3. The time required to achieve a normal γ_{min} (<3 mN/m) was measured in the surfactometer. PA increased the time to normal γ_{min} at concentrations ≥10 mol% ($P<0.05$). Time to γ_{min} could not be calculated for all samples in which γ_{min} remained >3 mN/m for the entire 10 min of pulsations.

during the 10 min interval. Therefore, an absolute time to γ_{min} could not be calculated, although the results obviously exceeded the time required by Survanta alone. At a concentration of 5 mol% LPC and 5 mol% LPC with PA, the time to γ_{min} increased but did not achieve statistical significance. Supplementation with OA also increased time to γ_{min}, but since concentrations of OA $\geq$10 mol% increased γ_{min} to >3 mN/m, absolute values could not be calculated (data not shown).

3.7. Effect of surfactant proteins on lysophospholipids and free fatty acids

Surfactant proteins, especially SP-A, may modulate the activity of $sPLA_2$ [43]. To test the effect of surfactant protein content on the lysophospholipid-mediated increase in γ_{min}, we compared our results obtained with Survanta®, an extracted surfactant containing only low levels of the hydrophobic proteins (SP-B, SP-C), and the non-extracted porcine surfactant with a full complement of surfactant proteins including hydrophilic (SP-A, SP-D) and hydrophobic proteins. The effects of equimolar amounts of either LPC, PA, or both, on the γ_{min} of porcine surfactant are presented in Fig. 7. Increasing replacement of surfactant phospholipid with >15 mol% LPC significantly increased γmin ($P<0.05$). While PA alone had no effect, the addition of PA with LPC tended to decrease the amount of LPC required to increase γ_{min}. These results with porcine surfactant match those seen in Fig. 3 with protein-depleted Survanta®, suggesting the impact of surfactant proteins on the effects of lysophospholipids to increase γ_{min} in this non-enzymatic model are minimal. Similarly, values of γ_{max} and time to achieve a normal γ_{min} with supplementation of LPC were not different between the porcine surfactant and Survanta. Interestingly, the increase in γ_{max} and time to achieve a normal γ_{min} caused by PA with Survanta was not seen in porcine surfactant (data not shown). This difference suggests the surfactant proteins which facilitate adsorption of the phospholipids to the air–liquid interface might prevent or overcome the effects of the free fatty acid.

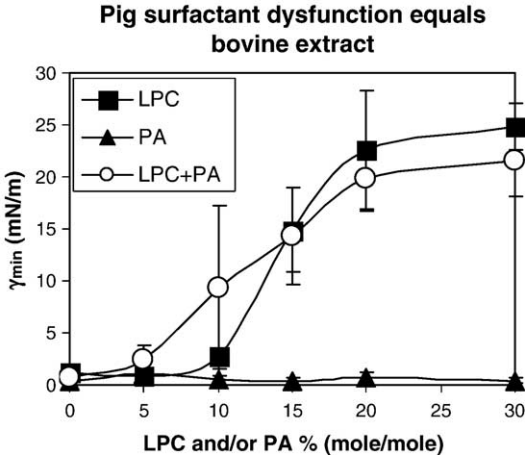

Fig. 7. Effect of surfactant protein on increased minimum surface tension mediated by lysophospholipids and free fatty acids. Surfactant PL (washed porcine surfactant isolated without organic extraction preserving surfactant proteins) was replaced by increasing amounts of LPC, PA or LPC in combination with PA as described in Figs. 1–3. The γ_{min} was determined using a surfactometer. Significant increases in γ_{min} were recorded at concentrations of $\geq$15 mol% LPC ($P<0.01$). PA alone had no effect and did not alter the effects of LPC when combined.

4. Discussion

We have previously reported that $sPLA_2$'s in vitro hydrolyze surfactant phospholipids and disrupt surface tension lowering activity [17,38]. However, those studies did not directly test the mechanisms by which $sPLA_2$-mediated changes in surfactant phospholipid composition influence surfactant function. Hydrolysis of surfactant by $sPLA_2$'s produces equimolar amounts of lysophospholipids and free fatty acids, and depletes intact phospholipids. Each of these enzymatic effects represent potential mechanisms for surfactant dysfunction.

Holm and colleagues first demonstrated that lysophospholipid accumulation disrupts surfactant's surface tension lowering function [38]. The same author subsequently demonstrated that the mechanism by which lysophospholipids disrupt surface tension results principally from altered phospholipid packing in the surface film, particularly during dynamic compression [42]. We used the non-enzymatic models detailed in this report to more precisely quantitate the impact of lysophospholipid-mediated surfactant disruption, and to compare the effects of lysophospholipids to other potential mechanisms.

Our results demonstrate that accumulation of lysophospholipids to a level >10% enzymatic hydrolysis was sufficient to statistically increase the γ_{min} of surfactants. The low levels of endogenous lysophospholipids that are present in Survanta® and porcine surfactant ($\leq$1 mol%) do not significantly impact these observations. The effect of lysophospholipids was independent of the lysophospholipid head group, total phospholipid concentration, or surfactant apoprotein content. Accumulation of saturated or monounsaturated free fatty acids typical of surfactant had little impact γ_{min}, but may provide some additional effect in the presence of lysophospholipids.

In contrast, depletion of total phospholipids as a contributing mechanism to $sPLA_2$-mediated increase in γ_{min} required the equivalent of >80% enzymatic hydrolysis. Therefore, lower hydrolysis would be required for significant surfactant dysfunction due to lysophospholipid accumulation than for phospholipid depletion suggesting that lysophospholipid accumulation is a more effective mechanism.

The addition of LPC, PA, or LPC with PA to surfactant also significantly altered the time required for surfactant to reach a normal γ_{min} value at concentrations at or below those required to increase γ_{min}. The increased time to normal γ_{min} caused by PA occurred despite the inability of PA to significantly increase γ_{min}, even at concentrations as high as 30 mol%. Notably, the only significant effect on γ_{max} seen with addition of LPC, PA or LPC with PA was caused by PA alone at similar concentrations. These unique results with PA suggest that it does have important effects on surfactant function and may alter the adsorption properties of surfactant. Thus, under in vitro conditions that mimic respiratory cycles of surfactant expansion and collapse, both lysophospholipids and free fatty

acids, the products of $sPLA_2$ hydrolysis of surfactant, may independently disrupt different aspects of surfactant function.

Surfactant proteins modulate the enzymatic activity of $sPLA_2$. SP-A inhibits in vitro surfactant PL hydrolysis by recombinant group IIA and group X $sPLA_2$s [43]. In our non-enzymatic model, there was no difference in the effects of lysophospholipid accumulation on washed porcine surfactant, isolated without organic extraction to preserve surfactant proteins, versus Survanta®, an extracted surfactant containing only low levels of the hydrophobic proteins (SP-B, SP-C). Consequently, the ability of surfactant proteins to modulate $sPLA_2$-mediated surfactant function is primarily through direct effects on enzymatic activity, not effects on $sPLA_2$ hydrolysis products within the phospholipid film at the air–liquid interface.

Application of the results from these non-enzymatic models to our previous reports of enzymatic hydrolysis of surfactant by $sPLA_2$ leads to similar conclusions regarding the importance of lysophospholipid accumulation [17]. In that report, concentrations of group IB $sPLA_2$ resulting in <10% hydrolysis of DPPC, created little or no surfactant dysfunction, whereas concentrations of group IB $sPLA_2$ resulting in $\geq$10% hydrolysis of PC caused minimum surface tension to increase significantly. In this report we showed that $\geq$10 mol% LPC significantly disrupted surface activity of surfactant. Hydrolysis of >80% surfactant PL was not required for surfactant dysfunction, suggesting PL (DPPC) depletion was not the major mechanism.

Application of the results from these non-enzymatic models to in vitro enzymatic studies of surfactant hydrolysis by $sPLA_2$'s other than group IB reveals some inconsistencies regarding the importance of lysophospholipid accumulation. Our group and others have reported that each $sPLA_2$ has a unique profile for hydrolysis of phospholipids with different head groups including the most abundant phospholipids in surfactant: PC, PG and PE [18–21]. The group IB, V and X enzymes generate significant hydrolysis of PC, and cause surfactant dysfunction, consistent with the generation of lysophosphatidylcholine [18]. However, the group IIA and group IID $sPLA_2$'s cause significant surfactant dysfunction, but do not readily hydrolyze PC. Although these enzymes do hydrolyze other surfactant phospholipids, particularly PG, they do not result in sufficient accumulation of lysophospholipids to explain the surfactant dysfunction based on the results from the non-enzymatic models reported here [18]. The recognition of these variable responses with different enzymes requires consideration that other potential $sPLA_2$-mediated mechanisms, such as the specific loss of individual phospholipids, may lead to surfactant dysfunction. The hydrolysis of PG, which could not be addressed in these models, may serve as a unique mechanism for $sPLA_2$-mediated surfactant dysfunction [13,18], through its important interaction with the surfactant proteins [44].

Our results from these models cannot fully address the correlation between lysophospholipids and surfactant function in more complex in vitro models or the in vivo milieu of an inflamed lung. In studies of human inflammatory airways diseases, including asthma and ARDS, in which increased phospholipase activity and surfactant dysfunction are present, only low levels of lysophospholipids have been reported [34,45]. The levels measured were far less than the 10 mol% levels required to increase γ_{min} in our non-enzymatic models. The most likely explanation for this discrepancy between in vitro and in vivo studies of phospholipases is that extracellular lysophospholipids undergo rapid reuptake by intact cells [46]. After cellular reuptake, the lysophospholipids undergo reacylation producing new phospholipids or triacylglycerols [34,47]. Extracellular lysophospholipids can also be cleared by further metabolism by lysophospholipases [48]. Although low, the levels of lysophospholipids that are present in the inflamed lung may still play important roles in surfactant dysfunction. The inhibition of surfactant function by proteins like fibrinogen which are present in the inflamed lung, is substantially increased in the presence of LPC [49]. The discrepancies between our current in vitro and in vivo observations should be reduced as more complex in vitro models are developed.

In summary, non-enzymatic models can be used to compare the relative contributions of various mechanisms to $sPLA_2$-mediated hydrolysis of surfactant phospholipid in vitro. These models demonstrate that accumulation of lysophospholipids have greater overall effects than accumulation of fatty acids or depletion of total phospholipid. The precise, quantitative nature of the results helps to more clearly define the biophysical relationships between $sPLA_2$-mediated changes in surfactant PL composition and surface activity. Establishing these relationships provides tools that enhance our analysis of surfactant injury mediated by the large $sPLA_2$ family.

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