Surfactant components modulate fibroblast apoptosis and type I collagen and collagenase-1 expression

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Vázquez de Lara, Luis, Carina Becerril, Martha Montaño, Carlos Ramos, Vilma Maldonado, Jorge Meléndez, David S. Phelps, Annie Pardo, and Moisés Selman. Surfactant components modulate fibroblast apoptosis and type I collagen and collagenase-1 expression. Am J Physiol Lung Cell Mol Physiol 279: L950–L957, 2000.—During lung injury, fibroblasts migrate into the alveolar spaces where they can be exposed to pulmonary surfactant. We examined the effects of Survanta and surfactant protein A (SP-A) on fibroblast growth and apoptosis and on type I collagen, collagenase-1, and tissue inhibitor of metalloproteinase (TIMP)-1 expression. Lung fibroblasts were treated with 100, 500, and 1,000 μg/ml of Survanta; 10, 50, and 100 μg/ml of SP-A; and 500 μg/ml of Survanta plus 50 μg/ml of SP-A. Growth rate was evaluated by a formazan-based chromogenic assay, apoptosis was evaluated by DNA end labeling and ELISA, and collagen, collagenase-1, and TIMP-1 were evaluated by Northern blotting. Survanta provoked fibroblast apoptosis, induced collagenase-1 expression, and decreased type I collagen affecting mRNA stability ~10-fold as assessed with the use of actinomycin D. Collagen synthesis and collagenase activity paralleled the gene expression results. SP-A increased collagen expression ~2-fold and had no effect on collagenase-1, TIMP-1, or growth rate. When fibroblasts were exposed to a combination of Survanta plus SP-A, the effects of Survanta were partially reversed. These findings suggest that surfactant lipids may protect against intraluminal fibrogenesis by inducing fibroblast apoptosis and decreasing collagen accumulation.

Pulmonary fibrosis; surfactant protein A

Fibroblast and myofibroblast migration from the interstitium to the alveolar spaces is a common pathological feature in subacute and chronic interstitial lung disease (ILD), acute lung injury, and acute interstitial pneumonia (3, 14, 21, 37). In all these disorders, lung damage may result in plasma-derived fluid flows through the disrupted alveolar walls into the alveolar lumen. The fluid enhances the formation of an intra-alveolar fibrin exudate that may serve as a scaffold for fibroblastic invasion, proliferation, and active collagen synthesis (34). In the process of migration through partially disrupted and denuded epithelial basement membranes, fibroblasts are exposed to the components of alveolar spaces including surfactant components.

Pulmonary surfactant is a complex mixture of lipids and specific proteins. Approximately 90% of the lipid fraction consists of a mixture of phospholipids, with phosphatidylcholine comprising about 80% of total surfactant phospholipids and approximately two-thirds of whole surfactant (12). A major constituent of the protein fraction is surfactant protein (SP) A, a sialoglycoprotein with an amino-terminal collagenous domain that belongs to a group of proteins known as collectins (22).

Earlier works on surfactant have focused on its surface tension-lowering properties and on its role in pulmonary mechanics, but in recent years, surfactant functions other than those related to the mechanics of ventilation have been reported (12). Now it is known that surfactant plays a role in the modulation of immune cell function in the lung including proliferation (18), cytokine production (16), and the expression of cell surface markers by macrophages (17).

There is still little known about the role of surfactant on lung fibroblast activity. There is evidence that an exogenous surfactant preparation devoid of SP-A can downregulate the synthesis of DNA and decrease the release of inflammatory mediators in cultured normal human lung fibroblasts (38). However, no information is available about the effects of surfactant lipids or SP-A on gene expression of extracellular matrix-related molecules by fibroblasts.

Normally, parenchymal lung fibroblasts are confined to the interstitium, and they have no contact with...
pulmonary surfactant. As mentioned before, however, in acute lung injury and ILD, fibroblasts and myofibroblasts migrate to the air spaces, contributing to the formation of intra-alveolar granulation tissue, and in these pathological conditions, these cells could be exposed to surfactant components.

In this study, we examined the effects of the exogenous surfactant replacement preparation Survanta and of SP-A on human lung fibroblasts with respect to 1) proliferation and apoptosis and 2) expression of α1 type I (α1(I)) procollagen, interstitial collagenase [matrix metalloproteinase (MMP)-1] or collagenase-1, and tissue inhibitor of metalloproteinase (TIMP)-1. These three molecules were selected because they play a critical role in tissue fibrogenesis. Lung fibrotic response is primarily characterized by collagen accumulation and by an imbalance between MMP-1, which mainly degrades fibrillar collagens, and its tissue inhibitor TIMP-1 (30). Survanta was chosen because it is a natural bovine lung extract containing phospholipids, neutral lipids, fatty acids, and surfactant-associated hydrophobic proteins B and C and because it is in widespread clinical use. Survanta is devoid of SP-A and SP-D.

MATERIALS AND METHODS

Fibroblast isolation and culture. Primary human lung fibroblasts were obtained in our laboratory as previously described (5). Briefly, cells were harvested from individuals having lung resections for the removal of a primary lung tumor. No morphological evidence of disease was found in the tissue samples used for fibroblast isolation. Lung fibroblasts were isolated by trypsin dispersion, and cells were grown in Ham’s F-12 medium (GIBCO BRL, Life Technologies, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS; GIBCO BRL). Fibroblasts (passages 5–8) were cultured at 37°C in 5% CO2–95% air. After 24 h, FBS-containing Ham’s F-12 medium supplemented with 10% FBS, 100 U/ml of penicillin, and 100 μg/ml of streptomycin.

SP-A preparation. SP-A was prepared from alveolar proteinosis lavage material. The insoluble protein fraction of the lavage material, which contains SP-A, was dissolved in 3 M urea and 20% glycerol and subjected to preparatory isoelectric focusing. At the end of the focusing period, the SP-A-containing fractions were placed in a dialysis bag with 1 M NaCl and dialyzed against water. The salt is included in the initial dialysis to help dissociate the ampholines from the protein. Dialysis continued against multiple changes of water at 4°C for at least 4 days. After dialysis, the SP-A preparation was characterized by protein determination, electrophoretic analysis, SP-A ELISA, and lipopolysaccharide determination (and removal if necessary). We estimated the purity of the SP-A to be >98%.

Growth rate assay. Early confluent fibroblasts were trypsinized, harvested, resuspended in Ham’s F-12 medium supplemented with 10% FBS, counted, plated in 96-well culture plates at a concentration of 15 × 103 cells/well, and incubated at 37°C in 5% CO2–95% air. After 24 h, FBS-supplemented medium was replaced with serum-free medium (SFM) with either 100, 500, or 1,000 μg/ml of Survanta (Abbott Laboratories, Columbus, OH) or 10, 50, or 100 μg/ml of purified human SP-A and incubated for 48 h.

Cell number was determined with the cell proliferation reagent WST-1 (Boehringer Mannheim) as previously described (40). WST-1 is a tetrazolium salt cleaved by the mitochondria of viable cells to yield a soluble formazan chromophore. The medium of corresponding wells was replaced with fresh medium, and the dye solution was added to each well according to the manufacturer’s specifications (Boehringer Mannheim). Absorbance was read on an ELISA plate reader at a wavelength of 450 nm, with a reference wavelength of 620 nm. In a parallel experiment, growth rate was also evaluated in the presence of 1 mM of Z-Val-Ala-Asp fluoromethyl ketone, caspase-1 inhibitor (Calbiochem, La Jolla, CA). Cells were cultured for 48 h in the presence of 500 μg/ml of Survanta plus 0.1% sodium citrate for 2 min on ice. Apoptotic cells were detected with a commercial kit (in situ cell death detection kit, Boehringer Mannheim). Fifty microliters of TUNEL reaction mixture were added to the slides, and they were incubated in a humidified chamber at 37°C for 1 h. The slides were mounted with 50 μl of 50% glycerol in PBS and visualized with an Olympus microscope equipped with fluorescein filters. Negative controls included preparations without enzyme. The images were captured by a color charge-coupled device camera (JVC), imported to Adobe Photoshop software, and printed on a standard ASA 100 color film (Kodak).

RNA isolation and Northern blot analysis. Total cellular RNA from lung fibroblasts was isolated by TRIzol reagent according to the manufacturer’s specifications (GIBCO BRL, Life Technologies). Total RNA (15 μg/lane) was fractionated on a 1% agarose gel containing 0.66 M formaldehyde. rRNA was visualized with ethidium bromide, and the fractionated RNA was electrophoretically transferred for 6 h at 16 V onto a Nytran transfer membrane. RNA was immobilized by bak-
were radiolabeled with a [32P]deoxycytidine triphosphate to monitored by assessing the level of 18S rRNA. The cDNA probes were exposed to Kodak BIOMAX MS film at 5 min, followed by 0.25 M SSC-0.1% SDS at 55°C and 0.1 M 33 saline-33 sodium citrate (SSC), 50% formamide, 50°C, with 10 mM CaCl2. Collagenolytic activity was measured at 80°C for 2 h and then prehybridized at 42°C for 18 h in hybridization buffer containing 5% dextran sulfate plus heat-denatured 32P-labeled cDNA probes. Membranes were washed in 2× SSC-0.1% SDS at 42°C for 25 min, followed by 0.25× SSC-0.1% SDS at 55°C and 0.1× SSC-0.1% SDS at 65°C. After they were dried, membranes were exposed to Kodak BIOMAX MS film at −70°C with an intensifying screen. Equal loading of RNA samples was monitored by assessing the level of 18S rRNA. The cDNA probes were radiolabeled with a [32P]deoxycytidine triphosphate to a specific activity of 200 × 106 dpm/µg with a multiprime DNA labeling kit (GIBCO BRL, Life Technologies).

Complementary DNA probes: cDNA clones for human collagenase-1 (MMP-1), TIMP-1, α1(I) procollagen, and 18S rRNA were obtained from the American Type Culture Collection (Manassas, VA).

RNA stability experiments. In parallel experiments, actinomycin D (at a final concentration of 12.5 µg/ml) was added to serum-free subconfluent cultures to stop gene transcription (5). Fibroblasts were removed before actinomycin addition as a time 0 control. Actinomycin-treated cells with and without exposure to 500 µg/ml of Survanta were removed for total RNA extraction 6, 12, and 24 h after the beginning of treatment. Total RNA from fibroblasts exposed to 500 µg/ml of Survanta without actinomycin was isolated at the same time points. Northern blot analysis was carried out with the use of α1(I) procollagen cDNA. After quantitation by densitometry, differences in RNA loading were normalized with a cDNA probe for 18S rRNA. The normalized collagen signal is expressed as a percentage of the 0-h control.

Collagenase activity assay. Conditioned media derived from T-75 flasks of control and Survanta-treated fibroblasts (500 µg/ml) were dialyzed against distilled water, lyophilized, and resuspended in 50 mM Tris-HCl buffer, pH 7.5, with 10 mM CaCl2. Collagenolytic activity was measured as reported elsewhere (29), and native guinea pig skin collagen labeled with [3H]acetic anhydride was used as a substrate. One hundred microliters of a 1.2 mg/ml collagen solution with a specific activity of 9.8 × 105 counts·min⁻¹ (cpm)·mg⁻¹ were used for each assay. Activation of latent collagenase was performed by preincubating the samples with trypsin (1–4 µg) for 10 min at 37°C, followed by a fivefold molar excess of soybean trypsin inhibitor (Sigma, St. Louis, MO). Incubations were carried out for 18 h at 30°C. Collagenolytic activity was calculated after background subtraction and is expressed as micrograms of collagen degraded at 30°C for 18 h.

Collagen measurement. To analyze collagen production by lung fibroblasts, hydroxyproline content was measured in three independent experiments by a colorimetric assay. Conditioned media derived from T-75 flasks of control and 500 µg/ml Survanta-treated fibroblasts were lyophilized and resuspended in 1 ml of distilled water. After acid hydrolysis, hydroxyproline was measured as described by Woessner (41), and results are expressed as micrograms of OH-proline per 106 cells per 48 h.

Statistical analysis. Results are expressed as means ± SD. Comparisons were made with Student’s t-test for paired observations. When more than two groups were compared, the Bonferroni correction for multiple comparisons was applied. Values of P < 0.05 were considered significant.

**Results**

**Effect of Survanta and SP-A on fibroblast growth rate.** To investigate whether Survanta or SP-A affect cell growth, fibroblasts were exposed to 100, 500, or 1,000 µg/ml of Survanta or 10, 50, or 100 µg/ml of SP-A in SFM for 48 h. As shown in Fig. 1A, Survanta induced a dose-dependent loss of cell density as mea-

![Fig. 1](http://ajplung.physiology.org/)

Fig. 1. Effect of Survanta and surfactant protein A (SP-A) on fibroblast growth rate. Human lung fibroblasts were cultured in 96-well culture plates at a concentration of 15 × 10⁴ cells/well. At 48 h, cell number was estimated by WST-1 assay. Each point represents mean ± SD of 3 independent experiments performed in triplicate. A: fibroblasts incubated with 100 (○), 500 (▴), and 1,000 µg/ml (○) of Survanta; control cells in the absence of Survanta, (●). B: fibroblasts stimulated with 10 (○), 50 (▴), and 100 µg/ml (○) of SP-A; control cells, (●). C: cells exposed to 500 µg/ml of Survanta (○), 50 µg/ml of SP-A (○), and 500 µg/ml of Survanta plus 50 µg/ml of SP-A (●); control cells, (●).
sured by the WST-1 assay. The decrease in cell number was statistically significant when fibroblasts were exposed to 500 and 1,000 μg/ml of Survanta (P < 0.0002 and P < 0.0001, respectively), suggesting that Survanta provoked fibroblast death. To determine whether apoptosis was playing a role in this effect, growth rate was additionally examined in the presence of a highly specific, irreversible inhibitor of caspase-1-like proteases. At 40 h of culture, 1 nM inhibitor reduced by ~70% the decrease of growth rate induced by 500 μg/ml of Survanta [control cells, 1.735 ± 0.13; Survanta-treated fibroblasts, 1.128 ± 0.08; and Survanta plus caspase inhibitor-treated fibroblasts, 1.552 ± 0.1 optical density (OD); P < 0.01].

On the other hand, when cells were incubated for 48 h in the presence of SP-A, the growth rate was similar to that observed in control cells (Fig. 1B). In another set of experiments, fibroblasts were incubated with either 500 μg/ml of Survanta, 50 μg/ml of SP-A, or both. Figure 1C shows reversal of the Survanta effect when SP-A was present in the same culture.

Effect of Survanta and SP-A on fibroblast apoptosis. To further evaluate whether Survanta induced apoptosis, nuclear morphology was assessed by fluorescence images of fibroblasts stained with ethidium bromide and by DNA end labeling. Figure 2 depicts ethidium bromide-stained cultured fibroblasts incubated with 500 μg/ml of Survanta for 36 h. Microscopy of adherent cells revealed that exposure to Survanta increased the number of typical apoptotic nuclei, (i.e., nuclei with condensed and fragmented morphology; Fig. 2, B and C). By TUNEL, cleaved DNA within discrete nuclear fragments in a variety of sizes and numbers was also clearly detected (Fig. 3).

To quantify these changes, the fragmented DNA was measured with an ELISA assay that evaluates BrdU-labeled DNA fragments only in the cytoplasm of the adherent cells (Fig. 4). Exposure of human lung fibro-
blasts to 500 μg/ml of Survanta induced a significant increase in DNA fragmentation compared with that in control cells \((P < 0.01)\). Incubation of fibroblasts with 50 μg/ml of SP-A almost completely neutralized this effect.

**Effect of Survanta and SP-A on α1(I) procollagen expression.** Human normal lung fibroblasts grown at early confluence were incubated for 48 h in SFM containing either 50 μg/ml of SP-A, 500 μg/ml of Survanta, or both. As shown in Fig. 5, incubation of fibroblasts with 500 μg/ml of Survanta produced a marked down-regulation of α1(I) procollagen gene expression, whereas SP-A raised the levels of this transcript. When the signal for α1(I) procollagen mRNA was normalized to the level of 18S rRNA and quantified by densitometry, an ~10-fold decrease was noticed with Survanta; SP-A caused an ~2-fold increase. Similar results were obtained with 100 μg/ml of SP-A, whereas a dose of 10 μg/ml of SP-A had no effect (data not shown). When fibroblasts were exposed to both Survanta and SP-A, α1(I) procollagen mRNA expression also revealed a significant decrease, but an attenuation of the effect produced by Survanta alone was observed.

![Fig. 3. DNA end labeling of fibroblasts through terminal deoxynucleotidyltransferase-mediated fluorescein-dUTP nick end labeling (TUNEL). A: control cells. C: fibroblasts exposed to 500 μg/ml of Survanta. E: fibroblasts exposed to 500 μg/ml of Survanta plus 50 μg/ml of SP-A. Arrows, apoptotic cells. B, D, and F: phase contrast of the same fields.](image)

![Fig. 4. ELISA DNA fragmentation assay in human normal lung fibroblasts. Results are means ± SD of absorbance of cells exposed to SFM (control), 500 μg/ml of Survanta, and 500 μg/ml of Survanta plus 50 μg/ml of SP-A for 36 h. O.D., optical density. Procedures were performed as described in METHODS.* P < 0.01.](image)

![Fig. 5. Effects of Survanta, SP-A, and Survanta plus SP-A on α1(I) procollagen, matrix metalloproteinase (MMP)-1, and tissue inhibitor of metalloproteinase (TIMP)-1 gene expression. Representative Northern blot of 15 μg total cellular RNA per lane extracted from control (lane 1) and stimulated fibroblasts. At early confluence, human lung fibroblasts were cultured in SFM and treated with 50 μg/ml of SP-A (lane 2), 500 μg/ml of Survanta (lane 3), or 50 μg/ml of SP-A plus 500 μg/ml of Survanta for 48 h.](image)
Under basal conditions, normal human lung fibroblasts expressed a 0.9-kb TIMP-1 transcript. No significant effect on its expression was found when cells were stimulated with Survanta, SP-A, or both.

**DISCUSSION**

Pulmonary structural remodeling in ILD has been regarded as primarily consisting of thickening of the alveolar walls by interstitial fibrosis. However, in both chronic ILD and acute lung injury, intra-alveolar fibrosis, characterized by fibroblast proliferation and connective tissue accumulation that can obliterate the air spaces, plays a major pathogenic role (3, 7, 8, 14, 21, 37).

A number of cytokines that promote the fibroproliferative response have been identified, including transforming growth factor-β, platelet-derived growth factor, insulin-like growth factor molecules, and tumor necrosis factor-α. These fibrogenic cytokines enhance fibroblast migration and proliferation and collagen accumulation (1, 2, 15, 25, 27, 36). Conversely, some cytokines, such as interferon-γ and fibroblast growth factor-1, are able to induce an antifibrogenic phenotype in fibroblasts (5, 13) and may reduce the fibrotic response (11).

However, other factors besides cytokines may be involved in the regulation of matrix remodeling in the lung, and our results, at least in vitro, suggest that pulmonary surfactant may be one of them. Although normally compartmentalized to the lung interstitium, fibroblasts may come into contact with the components of surfactant when these cells migrate and invade the air spaces.

Pulmonary surfactant is a complex comprised primarily of phospholipids (mainly dipalmitoylphosphatidylcholine and specific apoproteins) that is distributed as a layer along the surface of the alveolar hypophase at the air-fluid interface. However, the composition of surfactant is altered in a number of disease states, and changes in the relative amounts of the various surfactant lipids or proteins, particularly SP-A, have been reported (12). For example, decreased amounts of surfactant lipids are found in the bronchoalveolar lavage fluid of patients with acute lung injury or idiopathic pulmonary fibrosis (12).

It has previously been shown (38) that exogenous preparations of surfactant, either synthetic or from natural derivatives, inhibit DNA synthesis and the secretion of some inflammatory mediators by fibroblasts. These observations led us to hypothesize that surfactant had a possible antifibrogenic role. To address this issue, we used Survanta, a natural bovine extract enriched with tripalmitin and fatty acids, that has a proven clinical efficacy.

Our results showed that Survanta strongly upregulates collagenase gene expression, downregulates α1(I) procollagen gene expression, and induces programmed cell death in fibroblasts. Synthesis of collagen and collagenase activity paralleled gene expression results. These findings suggest that surfactant induces an an-

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Fig. 6. Effects of Survanta on α1(I) procollagen mRNA stability. Subconfluent cultures of human lung fibroblasts were maintained in SFM and treated with actinomycin D (A), Survanta (B), and Survanta plus actinomycin D (C). Results were derived from densitometric analysis of blots subsequently exposed to film. The α1(I) procollagen-to-18S ratio in cells harvested before actinomycin D treatment (time 0) is expressed as 100%, and subsequent time points after treatment are expressed as percentages of that value. Each point represents the mean ± SD of three different experiments.

To examine whether the effect of Survanta on α1(I) procollagen mRNA was reflected in collagen synthesis, total hydroxyproline content was measured in three different experiments. Serum-free conditioned media derived from fibroblasts treated with 500 μg/ml of Survanta showed a reduction of ~50% compared with control cultures (1.2 ± 0.1 vs. 0.63 ± 0.07 μg OH-proline/10⁶ fibroblasts; P < 0.01).

To determine whether Survanta could influence α1(I) procollagen mRNA stability in human lung fibroblasts, the half-life of the mRNA was assessed by measuring its decay after the addition of actinomycin D. As shown in Fig. 6, the α1(I) procollagen mRNA half-life was reduced from ~21 h in control fibroblasts to 11 h in the presence of Survanta, suggesting that Survanta decreased the stability of the collagen mRNA (P < 0.05).

**Effect of Survanta and SP-A on MMP-1 and TIMP-1 gene expression.** By Northern hybridization, MMP-1 transcript was not detected in untreated fibroblasts incubated in SFM. As shown in Fig. 5, Survanta strongly induced MMP-1 mRNA expression, whereas SP-A had no appreciable effect on the expression of this transcript. When both substances were present in the same culture, MMP-1 mRNA expression showed an increase but to a lesser extent than when treated with Survanta alone.

Collagenolytic activity of labeled type I collagen was measured in control and 24-h Survanta-treated cells. After trypsin activation, collagen digestion by conditioned media derived from fibroblasts exposed to Survanta showed a significant increase compared with that in control cells, which had no significant activity over background (8.8 ± 0.92 vs. 2.4 ± 0.23 μg of collagen degraded at 30°C for 18 h; P < 0.01). Collagenolytic activity was inhibited by 80 mM 1,10-phenanthroline.
tifibrogenic phenotype in human lung fibroblasts and, consequently, may play a role in preventing the accumulation of collagen-rich extracellular matrix proteins in the peripheral air spaces.

On the other hand, SP-A induced a moderate increase in the expression of \( \alpha_1(I) \)-procollagen mRNA and showed no apparent effect on collagenase and TIMP-1 expression as seen by Northern hybridization.

Nevertheless, when Survanta and SP-A were mixed in the same culture in a ratio similar to that found in normal surfactant, it seemed that the presence of the protein partially attenuated the effects exerted by Survanta on \( \alpha_1(I) \)-procollagen expression as well as on the induction of apoptosis. This balance of opposing actions when both substances are present has also been noted in other cell systems (18).

The mechanisms behind the observed changes induced by Survanta in collagen and collagenase gene expression remain to be elucidated. Regarding collagen, the results obtained after inhibiting new transcription with actinomycin D suggest that Survanta downregulates the \( \alpha_1(I) \)-procollagen gene at least partially through posttranscriptional mechanisms, destabilizing the mRNA. Thus an increased turnover of the preexisting \( \alpha_1(I) \)-procollagen mRNA in the cytoplasm appears to affect the steady-state level of the message, decreasing its expression.

There are a number of factors that influence collagen production by modulating the stability and efficiency of the utilization of mRNA. Among these, ascorbic acid induces a greater increase in collagen synthesis than can be attributed to the change in mRNA levels alone (9). On the other hand, some cytokines and growth factors can affect collagen production by altering mRNA stability. Thus, for example, similar results to those obtained with Survanta have been found with fibroblast growth factor-1, which also induces an antifibrogenic phenotype in lung fibroblasts (5). The steady-state level of \( \alpha_1(I) \)-procollagen mRNA is also strongly decreased by retinoic acid (19) and by alterations in the amount of intracellular nutrients, especially amino acid deprivation (20).

Regarding MMP-1, several inflammatory and growth-promoting cytokines have been shown to stimulate its expression, as observed with Survanta. These include, among others, interleukin-1\( \alpha \) and -1\( \beta \), tumor necrosis factor-\( \alpha \), platelet-derived growth factor, epidermal growth factor (6), and fibroblast growth factor-1 (5). Likewise, lipid mediators of inflammation such as leukotriene C\( \text{4} \) and platelet-activating factor are able to induce collagenase expression in different cell systems (4, 26).

It is possible that Survanta may exert its effect by several mechanisms. Lipids often act by influencing the fluidity of cell membranes. Actually, the effect of some lipids on the expression and/or synthesis of collagen and MMP-1 has been reported in other cell systems. Thus it has been demonstrated that dilinooleoylphosphatidylcholine decreases hepatic stellate cell activation as judged by the decrease in proliferative activity and procollagen I expression (32). Likewise, in

REFERENCES


