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Variable ventilation induces endogenous surfactant release in normal guinea pigs

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Arold, Stephen P., Béla Suki, Adriano M. Alencar, Kenneth R. Lutchen, and Edward P. Ingenito. Variable ventilation induces endogenous surfactant release in normal guinea pigs. *Am J Physiol Lung Cell Mol Physiol* 285: L370–L375, 2003; 10.1152/ajplung.00036.2003.—Variable or noisy ventilation, which includes random breath-to-breath variations in tidal volume (VT) and frequency, has been shown to consistently improve blood oxygenation during mechanical ventilation in various models of acute lung injury. To further understand the effects of variable ventilation on lung physiology and biology, we mechanically ventilated 11 normal guinea pigs for 3 h using constant-VT ventilation (n = 6) or variable ventilation (n = 5). After 3 h of ventilation, each animal underwent whole lung lavage for determination of alveolar surfactant content and composition, while protein content was assayed as a possible marker of injury. Another group of animals underwent whole lung lavage in the absence of mechanical ventilation to serve as an unventilated control group (n = 5). Although lung mechanics did not vary significantly between groups, we found that variable ventilation improved oxygenation, increased surfactant levels nearly twofold, and attenuated alveolar protein content compared with animals ventilated with constant VT. These data demonstrate that random variations in VT promote endogenous release of biochemically intact surfactant, which improves alveolar stability, apparently reducing lung injury.

acute lung injury; pressure-volume curve; noise

MECHANICAL VENTILATION (MV) is a mainstay of supportive therapy among patients with respiratory failure. However, recent studies have shown that MV can injure the lung through a process known as ventilator-induced lung injury (VILI), leading to abnormalities indistinguishable from those that result from severe pneumonia or sepsis (4, 12, 19, 22). New ventilator strategies designed to minimize VILI combine small tidal volumes (VT) to reduce excessive stretching and tissue injury with continuous positive end-expiratory pressure (PEEP) to prevent alveolar collapse and optimize oxygenation (1). Although these strategies help limit further damage, they do not directly contribute to reversal of existing tissue damage or the biochemical abnormalities that exist in the injured lung.

It has recently been shown that when variability is introduced into MV on a cycle-by-cycle basis, such that VT and frequency are permitted to fluctuate randomly within a specific range, gas exchange and lung mechanics improve compared with conventional MV (2, 10, 13). This approach, known as noisy ventilation or variable ventilation (VV), enhances gas exchange through the process of stochastic resonance, in which intermittent larger breaths stretch the alveolar surface and recruit collapsed regions of the lung without causing damage (17). Although these physiological effects are noteworthy, it has also been established that different stretch patterns applied to the lung and its epithelial layer have significant effects on lung biology that may be of greater physiological consequence (23).

Surfactant, a lipoprotein mixture secreted by specialized cells that line the surface of the lung, lowers surface tension at the alveolar air-liquid interface, preventing collapse of airways and alveoli. Stretching that occurs during a tidal breath is thought to be essential for normal surfactant homeostasis (15); however, it has been demonstrated that ventilating the lung with high inflation volumes enhances surfactant secretion within the lung (5, 14) and increasing degrees of strain augment surfactant release in primary cell culture (23).

On the basis of these observations, we hypothesized that variable stretch patterns applied to the lung epithelium during noisy ventilation could amplify surfactant release without the deleterious effects known to be associated with high-VT ventilation. Furthermore, this may be achieved without the high inflation pressures or volumes that are accompanied by a deep inflation or sigh. To test whether VV is capable of promoting surfactant release in vivo, we measured surfactant levels and chemical composition in bronchoalveolar lavage (BAL) samples isolated from anesthetized, tracheostomized guinea pigs after 3 h of VV support. Responses were compared with those of animals that received conventional low-VT MV support, and we found that VV significantly increased alveolar surfactant content compared with conventional MV.

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METHODS

Animal model. The protocol was approved by Boston University and Harvard Medical School Animal Care and Use Committee. Normal male Hartley guinea pigs (500–600 g body wt) were studied under an institutionally approved animal protocol. All animals were cared for in a similar fashion and studied within 2 wk of arrival from the distributor. Guinea pigs were randomized into one of three groups: *1*) constant-volume ventilation (CVV) using small V_T of 5 ml/kg at a frequency of 60 cycles per minute ($n = 6$), *2*) VV described in detail below ($n = 5$), or *3*) unventilated control ($n = 5$). Both ventilated groups were supported with 3 cmH_2O PEEP and 0.21 fraction of inspired O_2 (room air).

Animals were anesthetized with intraperitoneal xylazine (5 mg/kg) and pentobarbital sodium (40 mg/kg) and tracheally cannulated, and a carotid arterial line was placed for arterial blood gas measurements. Each animal underwent a small subxiphoid incision and transdiaphragmatic dissection to expose the lungs without causing significant trauma. This procedure eliminates the influence of the chest wall in lung elastance measurements. Previous measurements determined the resting pleural pressure of a guinea pig to be ~2.5 cmH_2O (unpublished observation), and thus a PEEP of 3 cmH_2O was chosen. The ventilated groups subsequently received MV for a total of 3 h while placed on a heating pad to prevent hypothermia. Lung impedance was measured at baseline and 30-min intervals using the optimal ventilator waveform technique described in detail elsewhere (11, 18) and fit to a mechanical impedance model to measure airway resistance, tissue damping, and lung elastance (8). Arterial blood gases were assessed at baseline and hourly thereafter. At the end of the study period, animals underwent whole lung lavage and were euthanized with a lethal dose of intracardiac pentobarbital sodium (100 mg/kg).

Lavage fluid collection and processing. After completion of in vivo studies, the lungs were lavaged twice with 10 ml of warmed saline with a consistent return of ~18 ml. BAL fluid was subjected to low-speed centrifugation for removal of cells, and surfactant was pelleted by high-speed centrifugation (30,000 *g*, 45 min at 4°C). Each pellet was assayed for total phospholipids (PLs) and total protein. Three surfactant pellets from each group underwent lipid extraction and thin-layer chromatography (TLC) for determination of PL subtype composition. Two pellets were used to determine relative amounts of surfactant proteins (SP) A and B by Western blot analysis.

PL and total protein assay. Total PL content of each sample was determined by the method of Stewart (16). The bicinchoninic acid spectrophotometric assay (Pierce, Rockford, IL) was used for protein determinations according to the manufacturer's specifications. Levels were referenced to a bovine serum albumin standard curve. Detection of protein content in surfactant pellets (at 1 mg/ml PL concentration) was determined by solubilizing samples in 0.5% SDS standards in a solution of 1 mg/ml aqueous phosphatidylcholine to account for any binding of bicinchoninic acid to the lipid component of surfactant.

TLC. Total PLs were extracted from 400-μg samples of surfactant (6). PL extracts were dried under vacuum, resuspended in chloroform, and spotted onto silica G/H TLC plates along with authentic PL standards. Separation was achieved using $CHCl_3$-MeOH-2-propanol-H_2O-triethylamine mobile phase (HPLC-grade solvents) (20). After separation, PL components were detected by staining with atomized molybdenum blue. Plates were scanned, and quantitative densitometry was performed (Gel-Pro software, Media Cybernetics, Silver Spring, MD).

Western blot assessment of SP content. From each sample, 100 μg of purified whole surfactant (equal volumes from all pellets for comparative purposes) were applied to 12% (for SP-A) or 15% (for SP-B) SDS-polyacrylamide gels. Proteins were separated under reducing conditions, blotted onto a polyvinylidene difluoride membrane, and analyzed by Western blot using primary antibodies raised to purified calf lung SP-A and SP-B. Cross-reactivity with guinea pig surfactant apoproteins was demonstrated during antibody preparation and testing. Blots were developed using a streptavidin-biotin peroxidase amplification system (Sigma, St. Louis, MO) and subjected to quantitative densitometry using the Gel-Pro Analyzer software package.

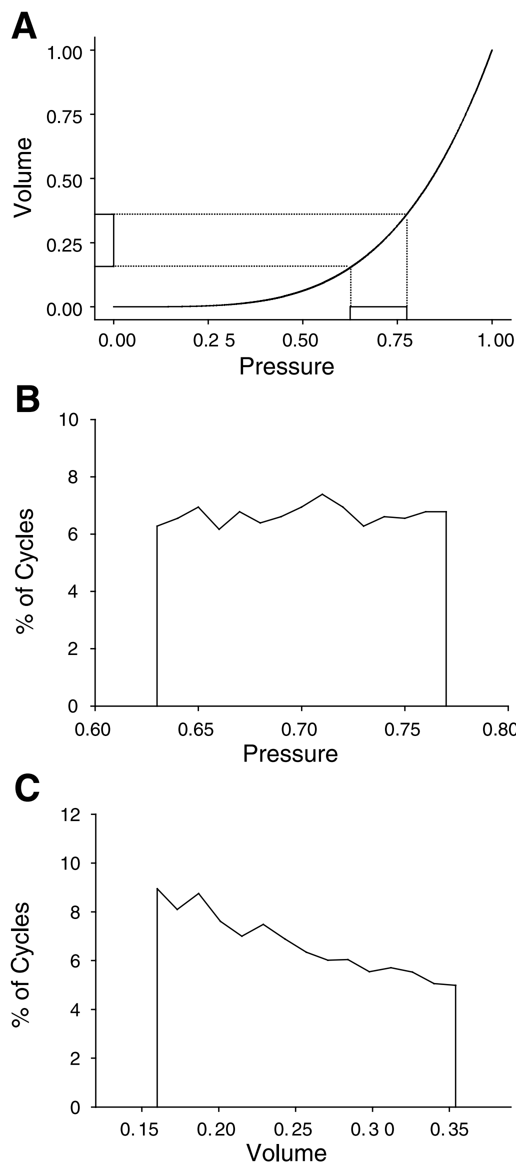

Fig. 1. Pressure-volume curve and distributions of peak pressures and tidal volumes in a lung model. *A*: normalized lung volume vs. normalized pressure during lung inflation. *B*: peak airway pressures. *C*: tidal volumes that generate a uniform distribution of peak airway pressures. In actual experiments, mechanical ventilator is programmed to deliver distribution of tidal volume (V_T) in *C*, resulting in a nearly uniform distribution of peak airway pressures similar to that in *B*.

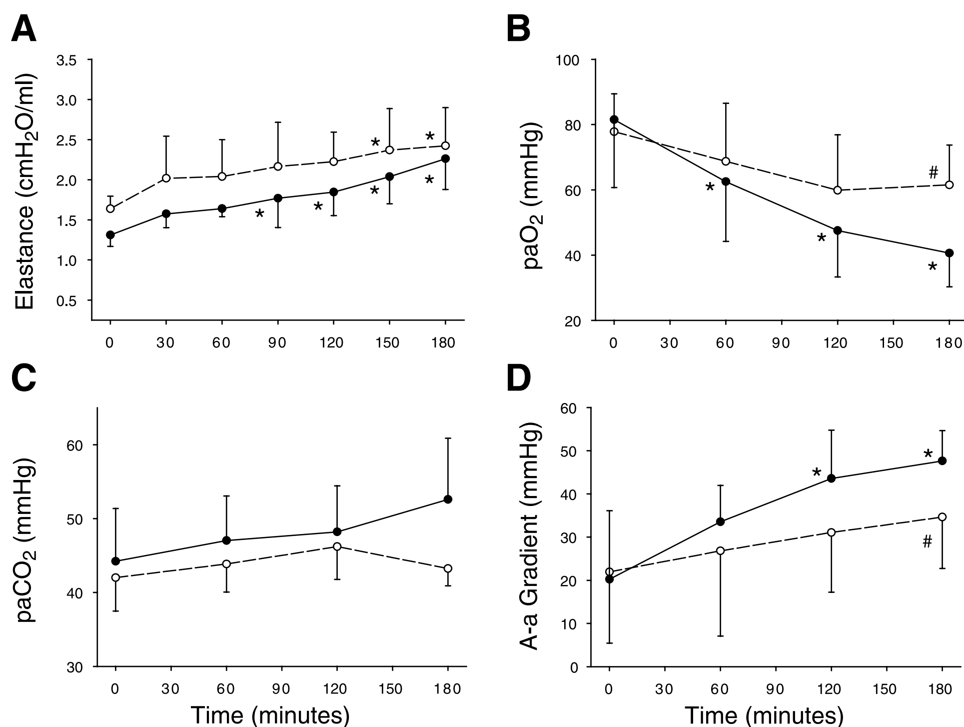

Fig. 2. Mechanical and physiological results. Tissue elastance, arterial P_{O_2} (PaO_2), arterial P_{CO_2} ($PaCO_2$), and alveolar-arterial (A-a) gradient are shown as a function of time for variable ventilation (○) and constant-volume ventilation (●). $*P < 0.05$.

BAL cytokines. Tumor necrosis factor-α, interleukin-6, and macrophage chemoattractant protein-1 were determined in BAL supernatants using an ELISA kit (Endogen, Woburn, MA) according to the manufacturer's instructions.

Design and application of VV. A computer model was used to predict that optimal gas exchange during VV occurs when peak inspiratory pressures (PIPs) are uniformly distributed around the mean PIP (17). To determine the pattern of V_T required to achieve this pressure distribution, we assumed that the pressure-volume curve of the lung follows a fourth-order polynomial (Fig. 1*A*). We generated a uniformly distributed sequence of PIPs (Fig. 1*B*) and applied the normalized pressure-volume curve to solve for a corresponding sequence of V_T values (Fig. 1*C*). This normalized V_T sequence was then scaled, such that the mean delivered volume was 5 ml/kg, with care taken to ensure that each animal received the same mean V_T. To maintain constant minute ventilation throughout the trial period and, thus, fair comparison for blood gas analysis, breath rate was adjusted on a cycle-by-cycle basis to match the minute ventilation in the CVV group of 300 ml/min. The sequence of V_T values at their corresponding frequencies was delivered by a computer-controlled ventilator (FlexiVent Rodent Ventilator, SCIREQ, Montreal, PQ, Canada).

Statistical analysis. Values are means ± SD. Data were analyzed by one-way ANOVA (SigmaStat, San Rafael, CA), and differences between groups were considered statistically significant for $P < 0.05$.

Table 1. *BAL protein and PL content*

	n	PL Content	Protein Content
Unvent	5	98 ± 19	10.0 ± 3.4
CVV	6	84 ± 29	17.2 ± 3.9*
VV	5	160 ± 28†	12.0 ± 1.6

Values are means ± SD in terms of mg return/kg body wt. BAL, bronchoalveolar lavage; PL, phospholipid; CVV, constant-volume ventilation; Unvent, unventilated; VV, variable ventilation. $*P < 0.05$ vs. Unvent; $†P < 0.05$ vs. CVV.

RESULTS

The physiological response to each mode of MV is summarized in Fig. 2. Lung elastance increased consistently in both groups over the ventilation period, with the changes with respect to baseline for the CVV group reaching significance after 90 min of MV and after 150 min of VV ($P < 0.05$). There were no significant differences in lung elastance between groups at any time point. Arterial P_{O_2} (PaO_2) also decreased in each group; however, the CVV group demonstrated a significant decline in oxygenation after 60 min of ventilation, whereas the decline in the VV group did not reach significance. PaO_2 was significantly higher in the VV group at 180 min (61 ± 12 mmHg) than in the CVV group (41 ± 10 mmHg, $P < 0.05$). Arterial P_{CO_2} values changed little over the course of MV, with the VV group generally demonstrating a slightly lower arterial P_{CO_2} throughout ventilation. Alveolar-arterial gradient before ventilation was ~21 mmHg in both groups and increased slightly in the VV group while increasing significantly in the CVV group from baseline values ($P < 0.05$). The CVV group concluded the experiment with a significantly higher alveolar-arterial gradient than the VV group (48 ± 7 vs. 34 ± 12 mmHg, $P < 0.05$).

As shown in Table 1, total BAL PL, a direct index of surfactant content, was increased nearly twofold in VV animals (160 ± 28 mg/kg) compared with unventilated controls (98 ± 19 mg/kg) and CVV animals (84 ± 29 mg/kg, $P < 0.05$). The CVV group was associated with a significant increase of >70% in BAL protein content

Table 2. *Surfactant PL comparison*

	PS, %	PE, %	SM, %	PC, %	PG, %	LCI
Unvent	5.7 ± 1.8	3.4 ± 0.9	1.0 ± 0.1	66.5 ± 2.6	15.1 ± 0.5	0.13 ± 0.04
CVV	10.6 ± 1.6	9.4 ± 1.7*	3.5 ± 1.0*	56.9 ± 6.5	9.3 ± 3.7*	0.36 ± 0.05*
VV	7.9 ± 2.1	5.0 ± 1.6†	1.7 ± 0.2†	62.1 ± 7.5	16.9 ± 2.2†	0.19 ± 0.06†

Values are means ± SD; $n = 3$. PC, phosphatidylcholine; PG, phosphatidylglycerol; PS, phosphatidylserine; PE, phosphatidylethanolamine; SM, sphingomyelin; LCI, lipid composition index. *$P < 0.05$ vs. Unvent; †$P < 0.05$ vs. CVV). Values do not add to 100% because other PLs not associated with surfactant or cell membranes were detected with thin-layer chromatography.

over the unventilated controls ($P < 0.05$), whereas no significant change in BAL protein content was observed in the VV group.

To assess whether the increase in PL after VV reflects surfactant, rather than an alternative PL source (i.e., cell membranes), PL subtype analysis was performed on three of the samples in each group and summarized in terms of a lipid composition index, defined as the ratio of membrane-associated PL (phosphatidylserine + phosphatidylethanolamine + sphingomyelin) to surfactant-associated PL (phosphatidylcholine + phosphatidylglycerol). Results are summarized in Table 2. In some cases (phosphatidylethanolamine and sphingomyelin), the CVV group demonstrated statistically significant increases in cell membrane-associated PL and, in one case (phosphatidylglycerol), a significant decrease in surfactant-associated PL compared with the unventilated and VV groups ($P < 0.05$). The CVV group had a significantly higher ratio of cell membrane PL to surfactant PL than both other groups ($P < 0.05$), suggesting the presence of some degree of injury that was noticeably absent in the VV group and the unventilated group. Additionally, the cytokine data in Table 3 demonstrate that CVV resulted in significant elevation in cytokine levels with respect to the unventilated group ($P < 0.05$) and VV resulted in a significant decline in cytokine content with respect to the CVV group in every case ($P < 0.05$).

Western blot analysis performed on two samples (Table 4) from each group shows that the composition of surfactant in the VV samples contained a normal complement of SP-A and SP-B. The use of equal volumes of purified lung surfactant for each sample indicates that secretion of lipid and protein components increased in physiologically appropriate amounts with VV.

DISCUSSION

The primary finding of this study is that VV promotes an increase in endogenous surfactant over 3 h of MV in healthy rodents. Additionally, animals ventilated with VV demonstrated improved oxygenation and diminished levels of protein, cell membrane-associated PL, and inflammatory mediators in the alveolar space compared with animals that received CVV during the same period. Biochemical analysis of surfactant PL and protein content indicates that significantly more surfactant was present during VV than during CVV and this additional surfactant is chemically intact. It has been previously demonstrated that VV, at a range of frequencies and V_T values, improves lung function and blood oxygenation in various models of acute lung injury (2, 10, 13). These improvements were primarily attributed to an increased capability of VV to recruit collapsed lung volume and, hence, increase blood oxygenation. The present study also suggests that because VV has a positive effect on lung biochemistry, it favorably influences lung function.

Animals that received CVV demonstrated significantly increased levels of protein, cell membrane-associated PL, and cytokines in BAL fluid, suggesting the presence of capillary leakage and cellular injury. These findings indicate development of VILI during CVV that is also reflected in increased lung elastance, decreased Pa_{O_2}, and increased alveolar-arterial gradient. It is possible that the ability of VV to prevent alveolar collapse and, hence, the development of capillary leakage in the alveolar compartment after VV is, at least in part, responsible for the increase of surfactant levels. Indeed, in a recent study, Boker and co-workers (3) reported that, in a porcine model of acute lung injury, a ventilation mode similar to VV reduced alveolar cytokine concentrations compared with animals ventilated with CVV, also suggesting that a ventilation mode incorporating variability reduces the development of VILI.

One confounding result in this study is that lung elastance generally increased in each of the ventilated groups; however, arterial oxygenation values and alveolar-arterial gradients diverged significantly, with the CVV group demonstrating a diminished capacity for

Table 3. *BAL cytokine concentrations*

	TNF-α	IL-6	MCP-1
Unvent	15 ± 1	50 ± 1	15 ± 5
CVV	160 ± 15*	1,400 ± 300*	90 ± 20*
VV	82 ± 10*†	250 ± 75*†	25 ± 10†

Values are means ± SD in pg/ml; $n = 3$. TNF-α, tumor necrosis factor-α; IL-6, interleukin-6; MCP-1, monocyte chemoattractant protein-1. *$P < 0.05$ vs. Unvent; †$P < 0.05$ vs. CVV.

Table 4. *SP content*

	SP-A	SP-B
Unvent	0.49 ± 0.04	0.65 ± 0.20
CVV	0.43 ± 0.10	0.72 ± 0.20
VV	0.58 ± 0.02	0.66 ± 0.07

Values are means ± SD expressed as amount relative to control lane; $n = 2$. SP, surfactant protein. There was no significance between groups.

gas exchange. One possible explanation for this observation may lie in the fact that the VV group demonstrated a slightly less significant percent increase in lung elastance than the CVV group, and, toward the conclusion of the ventilation period, lung elastance increased at a diminished rate. Increased lung elastance is generally thought to be an indicator of atelectasis, flooding of alveolar units, or increased interfacial surface tensions resulting from surfactant deficiency and/or dysfunction. These differences in tissue elastance, however slight, may be a result of significant disparity in surface area for gas exchange due to edema or atelectasis. It is also conceivable that the lung injury we observed in the CVV group, in the form of increased protein content, cytokines, and cell membrane PLs, may have been sufficient to affect the diffusive properties of the lung, thereby compromising gas exchange. Finally, repetitive airway closure in CVV may have led to ventilation heterogeneities and, hence, mismatch between ventilation and diffusion.

The mechanism behind enhanced surfactant secretion after VV is likely due to the effects of the stretch pattern that result from its application on the lung epithelium. Wirtz and Dobbs (23) demonstrated that stretching cultured alveolar epithelial type II cells resulted in enhanced surfactant secretion and that the magnitude of secretion increased nearly exponentially with the change in surface area of the underlying membrane. It is conceivable that intermittent larger-volume excursions that occur during VV stretch the lung epithelial layer and promote surfactant secretion in a similar fashion. Tschumperlin and Margulies (21), also working with type II epithelial cells, established that, although epithelial cells tolerate moderate degrees of stretch, excessive strain resulted in cell membrane disruption and death. Therefore, it is likely that a specific threshold exists, such that distribution of V_T levels that generate stretch exceeding this threshold injures the lung, whereas distribution of V_T levels that do not exceed the threshold has beneficial effects on surfactant homeostasis. This suggests that there may be some optimal degree of variability in V_T that maximizes surfactant release; however, applying V_T levels that surpass this range would have a detrimental effect on cell viability, likely reducing any benefits that may have been realized from VV.

The surfactant isolation techniques utilized in this study isolated only the surface-active, large-aggregate subtype of pulmonary surfactant and did not address the small-aggregate concentrations in the BAL fluid. It is possible that the increases in surfactant content were due in part to VV inhibiting the conversion of large-aggregate surfactant to the less effective small-aggregate type. However, we do not believe this to be the primary mechanism. First, the larger volumes delivered during VV would likely promote small-aggregate conversion, rather than inhibition (9). Additionally, we found similar surfactant contents in the CVV group and the unventilated group. Because the latter group did not receive ventilation, these findings support the notion that enhanced secretion is likely the principal cause of increased surfactant in the VV group. Periodic recruitment maneuvers or sighs have often been used during treatment of patients during acute lung injury, and it may be suggested that this would have the same effect on surfactant secretion as VV. Unfortunately, the resulting lung volume recruitment and improvements in oxygenation from a sigh have been shown to be short-lived and may result in hemodynamic complications (7). Also, a recruitment maneuver may result in regional overdistension that may exceed the threshold for lung injury. Nonetheless, the maximum V_T delivered with VV during this study would correspond to ~9 ml/kg, considerably less than a vital capacity maneuver.

In summary, VV, by random stretching of the alveolar surface within a prescribed range, appears to promote release of chemically intact surfactant, enhancing pool size within the alveolar compartment. This surfactant lowers the forces at which collapsed airways and alveoli reopen, reducing lung injury and, ultimately, improving blood oxygenation. Thus VV is capable of promoting mechanical recruitment of collapsed alveoli, as well as increasing surfactant levels through stimulation of endogenous release from lung epithelial cells. The clinical application of VV could have a significant impact on morbidity and mortality of acute respiratory distress syndrome patients through both of these effects.

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DISCLOSURES

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