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Different Ventilation Strategies Affect Lung Function but Do Not Increase Tumor Necrosis Factor-α and Prostacyclin Production in Lavaged Rat Lungs In Vivo

Serge J. C. Verbrugge, Ph.D., Stefan Uhlig, Ph.D.,† Sebastian J. C. M. M. Neggers, M.Sc.,‡ Christian Martin, Ph.D.,§ Heinz-Dieter Held, Dipl.-Biol.,§ Jack J. Haitsma, M.D.,* Burkhard Lachmann, M.D., Ph.D.‖*

Background: **Using an *in vivo* animal model of surfactant deficiency, the authors compared the effect of different ventilation strategies on oxygenation and inflammatory mediator release from the lung parenchyma.**

Methods: **In adult rats that were mechanically ventilated with 100% oxygen, acute lung injury was induced by repeated lung lavage to obtain an arterial oxygen partial pressure < 85 mmHg (peak pressure/positive end-expiratory pressure [PEEP] = 26/6 cm H_2O). Animals were then randomly assigned to receive either exogenous surfactant therapy, partial liquid ventilation, ventilation with high PEEP (16 cm H_2O), ventilation with low PEEP (8 cm H_2O), or ventilation with an increase in peak inspiratory pressure (to 32 cm H_2O; PEEP = 6 cm H_2O). Two groups of healthy nonlavaged rats were ventilated at a peak pressure/PEEP of 32/6 and 32/0 cm H_2O, respectively. Blood gases were measured. Prostacyclin (PGI_2) and tumor necrosis factor-α (TNF-α) concentrations in serum and bronchoalveolar lavage fluid (BALF) as well as protein concentration in BALF were determined after 90 and 240 min and compared with mechanically ventilated and spontaneously breathing controls.**

Results: **Surfactant, partial liquid ventilation, and high PEEP improved oxygenation and reduced BALF protein levels. Ventilation with high PEEP at high mean airway pressure levels increased BALF PGI_2 levels, whereas there was no difference in BALF TNF-α levels between groups. Serum PGI_2 and TNF-α levels did not increase as a result of mechanical ventilation when compared with those of spontaneously breathing controls.**

Conclusions: **Although alveolar protein concentration and oxygenation markedly differed with different ventilation strategies in this model of acute lung injury, there were no indications of ventilation-induced systemic PGI_2 and TNF-α release, nor of pulmonary TNF-α release. Mechanical ventilation at high mean airway pressure levels increased PGI_2 levels in the bronchoalveolar lavage–accessible space. (Key words: Lung injury; mechanical ventilation; mediators; shear force.)**

RECENT studies in rodents have shown that mechanical ventilation can be sufficient to elicit production and release of proinflammatory mediators.[1–3] In isolated nonperfused rat lungs, Tremblay *et al.*[2] have shown that mechanical ventilation at tidal volumes of 40 ml/kg body weight without positive end-expiratory pressure (PEEP) induces inflammatory mediator expression after 2 h in the lung tissue and results in inflammatory mediator release into the bronchoalveolar lavage–accessible space. In the same study, the use of 10 cm H_2O PEEP was shown to reduce inflammatory mediator expression and release at the same degree of end-inspiratory overstretching.[2] These responses occurred in both healthy rat lungs and lungs of rats exposed for 50 min to lipopolysaccharide (LPS).[2] Although these studies provided evidence that injurious ventilation strategies may result in pulmonary mediator release, the studies by von Bethmann *et al.*[1,3] indicated that ventilation may also cause systemic mediator release. In isolated and perfused mouse lungs from healthy donors, a peak inspiratory pressure (PIP) of 25 cm H_2O with 2 cm H_2O of PEEP induced inflammatory mediator release into the perfusate.[1,3] These studies support the idea proposed by Kolobow *et al.*,[4] that detrimental modes of mechanical ventilation may not only induce local inflammatory reactions in the lung but

* Research Fellow, Department of Anesthesiology, Erasmus University Rotterdam.

† Research Coordinator, Division of Pulmonary Pharmacology, Research Center Borstel.

‡ Medical Student, Department of Anesthesiology, Erasmus University Rotterdam.

§ Research Fellow, Division of Pulmonary Pharmacology, Research Center Borstel.

‖ Professor, Department of Anesthesiology, Erasmus University Rotterdam.

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Address reprint requests to Prof. Dr. Lachmann: Department of Anesthesiology (Room Ee 2393), Erasmus University, PO Box 1738, 3000 DR Rotterdam, The Netherlands.

Table 1. Overview of the Different Experimental Groups with Recovery Percentage of BALF ($F_{I_{O_2}}$ 1.0 in All Ventilated Groups)

Group	Time (min)	Additional	n	Recovery BALF (%)
Nonventilated, nonlavaged				
Control		Nontreated, spontaneously breathing	16	81 ± 2.6
LPS_{ip}	90		3	85 ± 5.0
	240	15 mg/kg LPS intraperitoneally	3	80 ± 5.7
LPS_{it}	90	5 ml/kg LPS in 2 ml saline intratracheally	3	80 ± 2.2
	240		3	81 ± 3.8
Sal_{it}	90	2 ml saline intratracheally	3	82 ± 9.7
	240		3	86 ± 8.0

Mechanical ventilation		PIP/PEEP (cmH_2O)	Frequency (bpm)	I/E	Additional		
Nonlavaged							
13/3H	90	13/3	30	1:2		13	80 ± 4.2
	240					13	80 ± 4.3
32/6H	90	32/6	30	1:2		10	82 ± 5.4
	240					10	83 ± 6.8
32/0H	90	32/0	30	1:2	Increase dead space	10	91 ± 3.2
	240					10	92 ± 7.0
Lavaged							
PLV	90	26/6	30	1:2	Perflubron 15 ml/kg	10	78 ± 4.8
	240					10	86 ± 5.5
Surf	90	26/6	30	1:2	Surfactant 100 mg/kg	10	83 ± 3.5
	240					10	86 ± 2.8
28/8	90	28/8	30	1:2		10	94 ± 4.6
	240					10	95 ± 2.8
32/6	90	32/6	30	1:2		10	102 ± 2.9
	240					10	100 ± 7.2
OLC	90	32/16	100	1:1		10	84 ± 2.9
	240					10	86 ± 3.6

LPS = lipopolysaccharide; H = healthy, nonlavaged; PIP = peak inspiratory pressure; PEEP = positive end-expiratory pressure; BALF = bronchoalveolar lavage fluid; Frequency = ventilatory frequency; I/E = inspiratory–expiratory ratio; PLV = partial liquid ventilation; Surf = exogenous surfactant administration; OLC = open lung concept.

may, *via* the spread of inflammatory mediators, also contribute to systemic multiple organ failure.

The mechanism of the ventilation-induced mediator release is unknown at present but may be a result of: (1) stimulus of stretch receptors present on endothelial cells,[5] macrophages,[6] or epithelial cells[7]; or (2) intrapulmonary neutrophil accumulation and activation.[8] It is becoming increasingly realized that next to peak inspiratory overstretching of the lung parenchyma,[9,10] impairment of the surfactant system (as a result of mechanical ventilation)[11,12] contributes to lung parenchymal stretch and ventilation-induced lung injury.[13]

The present study was designed to compare the effect of different ventilation strategies on alveolar protein infiltration, oxygenation, and inflammatory mediator release from the lung in an *in vivo* rat model of acute lung injury.

Materials and Methods

Preparation of Animals

The study was approved by the local animal committee of the Erasmus University (Rotterdam, The Netherlands). Care and handling of the animals were performed in accordance with the European Community Guidelines (86/609/EC). The studies were performed in male Sprague-Dawley rats (body weight, 250–330 g; Harlan CPB, Zeist, The Netherlands). An overview of the different experimental groups is presented in table 1.

Nonventilated Animals

One group of animals served as spontaneously breathing controls (control group, n = 10). Six additional animals served as nonventilated, serum tumor necrosis

factor-α (TNF-α)-positive controls. They were injected with 15 mg/kg LPS intraperitoneally (5 ml *Salmonella* abortus equi S. form; Metalon GmbH, Wusterhausen, Germany) and exposed for 90 (n = 3) and 240 (n = 3) min, respectively, to LPS (group LPS_{ip}). This group was used as a positive control for serum TNF-α.

Six animals served as nonventilated, bronchoalveolar lavage TNF-α–positive controls. They received 5 mg/kg LPS dissolved in 2 ml saline intratracheally as previously described[12] and were allowed to breathe spontaneously for 90 (n = 3) and 240 (n = 3) min, respectively (group LPS_{it}). This group was used as a positive control for bronchoalveolar lavage TNF-α. To exclude an effect of intratracheally administered saline on bronchoalveolar lavage TNF-α, six animals were given 2 ml saline intratracheally and were allowed to breathe spontaneously for 90 (n = 3) and 240 (n = 3) min, respectively (groups Sal_{it}).

In all animals, after induction of anesthesia with 2% enflurane in 65% nitrous oxide in oxygen, a sterile polyethylene catheter (outer diameter, 0.8 mm) was inserted into a carotid artery for drawing arterial blood samples. Before tracheotomy, the animals received 60 mg/kg pentobarbital sodium administered intraperitoneally (Nembutal; Algin, Maassluis, The Netherlands), and the enflurane concentration was decreased to 0.5–1.0%. Thereafter, a sterile metal cannula was inserted into the trachea.

In the nonventilated animals, a bronchoalveolar lavage was performed with saline (32 ml/kg heated to 37°C), and 4 ml heparinized blood was taken from the arterial line. The animals were then killed by an overdose of pentobarbital sodium through the penile vena.

Mechanical Ventilation

After cannulation of the trachea, muscle relaxation was induced in all other animals by intramuscular administration of 2 mg/kg pancuronium bromide (Pavulon; Organon Teknika, Boxtel, The Netherlands) followed by immediate connection to a ventilator. Body temperature was kept within normal range with a heating pad. The animals were mechanically ventilated in parallel (with more than one animal connected to the ventilator) with a Servo Ventilator 300 (Siemens Elema, Solna, Sweden) in a pressure-constant time-cycled mode with the following settings: frequency, 30 breaths/min; PIP, 13 cm H_2O; PEEP, 3 cm H_2O; inspiratory/expiratory ratio, 1:2; and 100% oxygen. Initially, PIP was increased to 20 cm H_2O for half a minute to recruit atelectatic areas (open-up procedure). Thereafter, the ventilator was returned to its previous settings, and blood gases were recorded (Instrumentation Laboratory, Synthesis 25, Milan, Italy). Anesthesia was maintained with hourly injections of pentobarbital sodium (60 mg/kg intraperitoneally), and muscle relaxation was maintained with hourly injections of pancuronium bromide (2 mg/kg intramuscularly).

Nonlavaged Animals

One group of nonlavaged (healthy [H]) ventilated animals served as controls, and ventilator settings were not changed. Control ventilation (PIP, 13 cm H_2O; PEEP, 3 cm H_2O; inspiratory/expiratory ratio, 1:2; frequency, 30 breaths/min; fraction of inspired oxygen [$F_{I_{O_2}}$], 1.0) was continued for 35 + 90 (n = 13) and 35 + 240 (n = 13) min (group 13/3H). The 35-min period was included to compensate for a period during which the animals in other ventilated groups were being lavaged (see following section).

To investigate whether washout of alveolar macrophages by the lavage procedure might have affected TNF-α release, 40 healthy animals were not lavaged (H) but underwent mechanical ventilation only. After the open-up procedure, they were ventilated for 35 min at control settings (PIP, 13 cm H_2O; PEEP, 3 cm H_2O; I/E ratio, 1:2; frequency, 30 breaths/min; $F_{I_{O_2}}$, 1.0) to compensate for the 35-min period in which the animals in the other experimental groups were being lavaged and ventilated. After this period, the animals were exposed to the following ventilator settings (frequency, I/E ratio, and $F_{I_{O_2}}$ were not changed): (1) a PIP of 32 cm H_2O with 6 cm H_2O of PEEP for 90 (n = 10) and 240 (n = 10) min (group 32/6H); (2) a PIP of 32 cm H_2O without PEEP for 90 (n = 10) and 240 (n = 10) min (group 32/0H). In this group, dead space was increased to keep arterial carbon dioxide partial pressure > 20 mmHg.

Lavaged Animals

In 100 animals that received mechanical ventilation, acute lung injury was induced after the open-up procedure by repeated bronchoalveolar lavage according to Lachmann *et al.*[14] Each lavage was performed with saline (32 ml/kg) heated to 37°C. Just before the first lavage, PIP and PEEP were elevated to 26 and 6 cm H_2O, respectively. Lung lavage was performed over a 35-min period and repeated four to five times to achieve an arterial oxygen partial pressure ≤ 85 mmHg. $F_{I_{O_2}}$, I/E ratio, and frequency were not changed unless stated otherwise. Immediately after lavage, these animals were randomized to be treated with

Partial liquid ventilation (PLV) at a dose of 15 ml perfluorocarbon per kilogram body weight (Perflubron;

Alliance Corporation, San Diego, CA) for 90 (n = 10) and 240 (n = 10) min (PIP, 26 cm H_2O; PEEP, 6 cm H_2O).

Exogenous surfactant at a dose of 120 mg/kg for 90 (n = 10) and 240 (n = 10) min. The surfactant used was isolated from minced pig lungs that were processed as previously described.[15] The freeze-dried material was suspended in warm saline to a concentration of 40 mg/ml and administered intratracheally, for which the animals were disconnected from the ventilator. The surfactant suspension was administered as a bolus dose followed by a bolus dose of air (12 ml/kg) directly into the endotracheal tube *via* a syringe, immediately followed by reconnection to the ventilator (PIP, 26 cm H_2O; PEEP, 6 cm H_2O).

An increase in PEEP and PIP of 2 cm H_2O for 90 (n = 10) and 240 (n = 10) min, resulting in a PEEP of 8 cm H_2O and a PIP of 28 cm H_2O (group 28/8).

An increase in PIP of 6 cm H_2O for 90 (n = 10) and 240 (n = 10) min, resulting in a PIP of 32 cm H_2O and a PEEP of 6 cm H_2O (group 32/6).

High PEEP for 90 (n = 10) and 240 (n = 10) min (open-lung concept [OLC]). In these groups, the lungs were opened by increasing PIP to 40 cm H_2O, PEEP to 20 cm H_2O, and I/E ratio to 1:1; frequency was set at 100 breaths/min after lung lavage. After 2–3 min, PIP was decreased to 32 cm H_2O, and PEEP was set at 16 cm H_2O. These settings were shown not to result in auto-PEEP because end-expiratory flow on a ServoScreen (Siemens) connected to the ventilator was zero. The high ventilatory frequency and I/E ratio were chosen to maintain normocapnia.

In all ventilated animals, blood gases were recorded at 5, 30, 60, and 90 min (in the animals ventilated for 90 min) and at 5, 30, 60, 120, 180, and 240 min (in the animals ventilated for 240 min) after starting the experimental mode of mechanical ventilation. At the end of the study period, bronchoalveolar lavage was performed in the ventilated animals with saline (32 ml/kg heated to 37°C), and 4 ml heparinized blood was taken from the arterial line, identical to the method described for nonventilated animals. The animals were then killed by an overdose of pentobarbital sodium through the penile vena.

Chemical Analysis

The blood and the bronchoalveolar lavage fluid (BALF) of all animals were centrifuged at 4°C at 400 × *g* for 10 min to remove cells and cellular debris. Supernatant of both blood and BALF were taken and snap-frozen on liquid nitrogen and stored at −80°C until further analysis.

The protein concentration of the BALF supernatant was determined with a photospectrometer (Beckman DU 7400; Beckman Instruments Inc., Fullerton, CA) at 595 nm using the Bradford method (Bio-Rad protein assay; Bio-Rad Laboratories, Munich, Germany) with bovine serum albumin (Sigma, St. Louis, MO) as a standard.[16]

Prostacyclin (PGI_2) was assessed as the stable metabolite 6-keto-prostaglandin (PG) F_{1a} and was measured by enzyme immunoassay (Cayman, Ann Arbor, MI). The cross-reactivity of the detecting antibody was 2,3-dinor-6-keto $PGF_{1\alpha}$ 8.7%; $PGF_{2\alpha}$ 2.1%; and PGE_2, $PGF1_\alpha$, PGD_2, and thromboxane B_2 all < 1%. Rat TNF-$_\alpha$ was assessed by rat-specific enzyme-linked immunosorbent assay (Genzyme, Cambridge, MA). The TNF test shows no cross-reactivity with any other rat (*i.e.*, interleukin [IL]-1β, IL-2, IL-4, interferon-γ, monocyte chemoattractant protein-1) or murine (*i.e.*, IL-1α, IL-3, IL-5, *etc.*) cytokine tested up to concentrations of 1 μg/ml. The lower limits of detection were 10 pg/ml for 6-keto-$PGF1_\alpha$ and 50 pg/ml for TNF. The intra-assay and interassay coefficient of variation was < 10% for both tests.

Statistical Analysis

Intragroup comparisons for arterial oxygen and carbon dioxide partial pressures at the time points before and after lavage and at 5 and 240 min were analyzed with a repeated-measures analysis of variance. Intergroup comparisons for protein, mediator levels, and arterial oxygen and carbon dioxide partial pressures were analyzed with analysis of variance. If analysis of variance resulted in a P value ≤ 0.05, a Tukey-Kramer post-test was performed. Statistical significance was accepted at $P \leq 0.05$. All data are reported as mean values ± SD. The statistical power of the cytokine measurements was calculated by assuming a two-sided t test with an α level that was adjusted by the Bonferroni method for the number of comparisons made.

Results

The recovery percentage of the BALF fluid in the different groups is presented in table 1. Arterial oxygenation and carbon dioxide levels over time with statistically significant differences are given in tables 2 and 3, respectively. In groups 28/8 and 32/6, oxygenation lev-

Table 2. Arterial Oxygenation (Pa_{O_2}) in the Different Ventilated Groups over Time

Time (min)	13/3 H	32/6 H	32/0 H	PLV	Surf	28/8	32/6	OLC
Before	548.5	520.6	532/8	539.4[9]	543.2	538.8[7]	530.6[7]	552.7
	(44.7)	(57.8)	(53/6)	(44.1)	(44.6)	(44.9)	(29.4)	(39.9)
After[2]	564.9	510.6	420.2	57.1	66.9[8]	68.8	67.4	66.9[8]
	(43.2)	(90.6)	(72.5)	(14.2)	(14.6)	(12.6)	(13.9)	(12.8)
5[1]	549.0	530.3	543.9	507.4[9]	534.1	98.9	105.6	556.3
	(39.9)	(71.5)	(50.2)	(55.6)	(43.8)	(29.4)	(45.0)	(58.3)
30[1]	563.3	518.9	340.0[4]	547.2	543.0	107.5	111.3	572
	(53.8)	(63.6)	(190.2)	(51.1)	(41.2)	(54.1)	(64.6)	(49.7)
60[5]	563.5	527.7	127.8	556.2	552.1	110.5	117.7	574.2
	(46.6)	(60.2)	(138.5)	(44.2)	(48.5)	(67.7)	(73.4)	(45.2)
90[5]	593.0	517.2	74.2	553.0	564.7	89.8	92.1	578.6
	(45.9)	(48.1)	(24.1)	(45.7)	(51.5)	(24.1)	(57.5)	(68.2)
120[5]	541.4	547.2	66.8	534.4	522.5	138.5	130.7	570.2
	(48.1)	(47.7)	(24.6)	(69.3)	(44.5)	(115.3)	(105.5)	(38.5)
180[5]	546.6	542.8	97.0	429.7[3]	508.6	133.3	125.2	579.0
	(56.5)	(65.8)	(77.6)	(142.5)	(60.7)	(120.2)	(110.7)	(44.7)
240[5]	554.0	516.2	67.3[6]	350.6[3,6]	494.0	128.0	124.4[9]	597.7
	(60.4)	(113.3)	(14.4)	(188.5)	(81.3)	(124.3)	(82.2)	(47.4)

Values are mean (SD). See table 1 for abbreviations. Intergroup differences: Significant differences, [1] 28/8 and 32/6 *versus* all others; [2] nonlavaged *versus* lavaged; [3] *versus* OLC; [4] *versus* OLC, PLV, Surf, 32/6 H; [5] 28/8, 32/6, and 32/0 H *versus* all others. Intragroup differences: Significant differences, [6] *versus* Before, After and 5 min; [7] *versus* After, 5 min, and 240 min; [8] *versus* Before, 5 min, and 240 min; [9] *versus* After.

els did not significantly recover from postlavage values. In the OLC, PLV, and surfactant groups, oxygenation levels were restored to prelavage values after 60 min, although in the PLV group they gradually decreased over the next 3 h. In group 32/0H, oxygenation levels decreased over time as a result of mechanical ventilation only, whereas they remained stable in group 32/6H.

The protein concentration was increased in the BALF of all lavaged animals after 90 and 240 min compared with controls (0.19 ± 0.05 mg/ml; fig. 1). However, treatment with OLC (90 min, 0.94 ± 0.34 mg/ml; 240 min, 2.38 ± 0.53 mg/ml), surfactant (90 min, 0.77 ± 0.38 mg/ml; 240 min, 1.96 ± 0.7 mg/ml), or PLV (90 min, 0.80 ± 0.33 mg/ml; 240 min, 1.83 ± 0.93 mg/ml) partially reduced the BALF protein concentrations compared with groups 28/8 (90 min, 1.80 ± 1.12 mg/ml; 240 min, 3.82 ± 1.54 mg/ml) and 32/6 (90 min, 3.66 ± 0.67 mg/ml; 240 min, 4.28 ± 1.42 mg/ml).

Because previous studies had shown that ventilation alone may be sufficient to cause release of the eicosanoid $PGI^{2,3}$ as well as the important proinflammatory cytokine $TNF^{2,3}$ from isolated lungs *in vitro*, in the present study we focused on these two mediators. Figures 2 and 3 depict data on BALF and serum concentration of TNF-α and PGI_2, respectively. There was no significant increase in serum concentrations of these mediators caused by

Table 3. Arterial Carbon Dioxide Tension (Pa_{CO_2}) in the Different Ventilated Groups over Time

Time (min)	13/3 H	32/6 H	32/0 H	PLV	Surf	28/8	32/6	OLC
Before	33.6 (5.0)	33.1 (5.1)	35.6 (6.5)	35.1 (5.7)	32.9[10] (6.5)	32.9[8] (6.4)	33.3 (6.3)	33.5 (4.7)
After[2]	32.5 (6.3)	31.6 (5.5)	30.4 (5.6)	55.3 (10.0)	47.8[9] (14.6)	55.6 (7.8)	52.6[9] (10.1)	53.3[9] (8.2)
5[1]	30.2[3] (4.1)	31.9[3,4] (4.6)	26.5[3] (2.6)	35.2[4] (4.7)	34.5 (4.2)	53.7 (12.6)	40.8 (10.8)	36.6[4] (7.6)
30[1]	34.4 (5.7)	30.8 (5.5)	30.6 (6.6)	29.0 (5.1)	30.3 (3.5)	50.5 (11.6)	35.6 (8.8)	33.7 (7.9)
60[1]	31.1 (6.2)	30.2 (5.4)	35.8[5] (7.0)	25.2 (3.8)	28.6 (3.5)	50.6 (12.2)	33.9[5] (8.0)	34.2[5] (8.3)
90[1]	28.8 (5.4)	30.1 (3.9)	35.3[5] (6.0)	23.1[3] (3.9)	27.4[3] (4.0)	48.6 (10.9)	37.5 (8.1)	30.4 (3.6)
120	35.5[5] (8.1)	27.0[6] (7.0)	35.3[5,6] (7.5)	23.2[6] (3.5)	28.9[6] (4.3)	50.9 (12.1)	35.4[5] (11.0)	32.4 (10.7)
180[1]	29.0 (3.2)	27.0 (6.4)	33.5 (10.7)	23.9[3] (4.3)	28.8 (5.8)	49.8 (14.3)	35.4 (9.4)	33.3 (6.0)
240	33.0[5,6] (4.8)	29.6[6] (3.0)	35.3 (11.5)	26.3[6,7] (4.5)	30.8[6,10] (9.3)	50.4 (15.7)	38.0 (11.5)	33.9[6] (7.7)

Values are mean (SD). See table 1 for abbreviations. Intergroup differences: Significant differences, [1] 28/8 *versus* all others; [2] 13/3, 32/6 H, and 32/0 H *versus* all others; [3] *versus* 32/6; [4] *versus* 32/0 H; [5] *versus* PLV; [6] *versus* 28/8. Intragroup differences: Significant differences, [7] *versus* Before, After, 5 min; [8] *versus* After, 5 min, 240 min; [9] *versus* Before, 5 min, 240 min; [10] *versus* After.

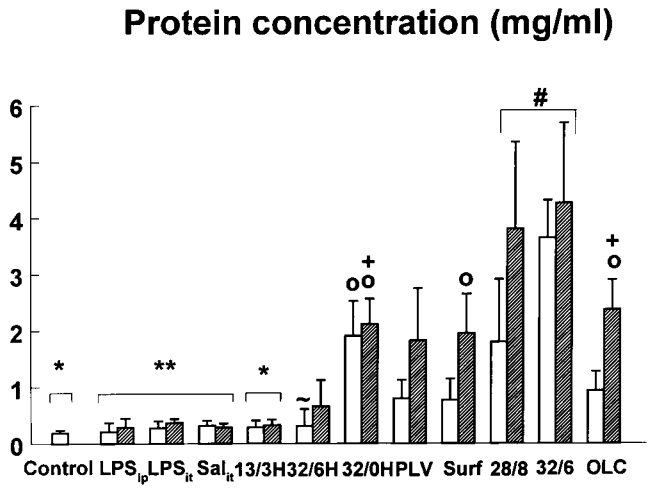
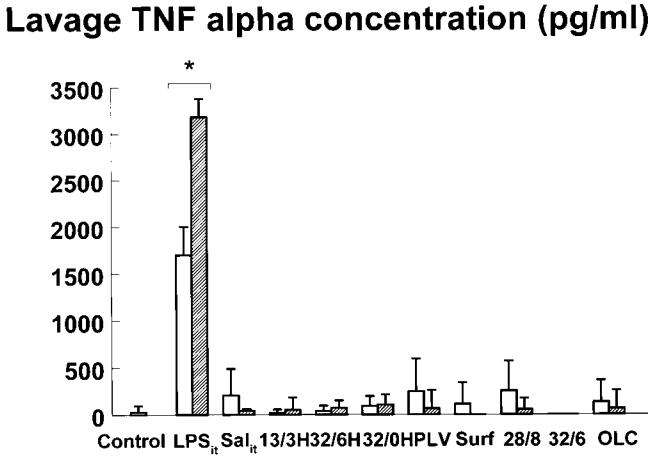


Fig. 1. Protein concentration in the bronchoalveolar lavage fluid (BALF) in the different experimental groups after 90 (open) and 240 (dashed) min of mechanical ventilation. Data are mean ± SD from groups of 10 animals (except control, n = 16; 13/3H, n = 13; LPS_{ip}, LPS_{it}, and Sal_{it}, n = 3). Statistically significant (individual comparisons, $P < 0.05$) *vs. group 28/8 90 and 240, 32/6 90 and 240, open-lung content (OLC) 240, partial liquid ventilation (PLV) 240, surfactant 240, 32/0H 90 and 240; **vs. group 32/6 90, 28/8 240, 32/6 240, OLC 240, PLV 240, 32/0H 240; #vs. group 28/8 90, OLC 90 and 240, PLV 90 and 240, surfactant 90 and 240, 32/0H 90 and 240, 32/6H 90 and 240; +vs. groups OLC 90, PLV 90, and surfactant 90; °vs. groups 32/6 90 and 240; ~vs. groups PLV 240 and 28/8 90.

the ventilation procedures. Increased serum TNF levels were found only in the serum of the intraperitoneal LPS-treated animals (no serum TNF-α levels were detectable in Sal_{it} 90 and 240 and LPS_{it} 90 and 240). TNF levels in the BALF were significantly increased in groups LPS_{it} 90 and 240 when compared with all other groups (BALF TNF-α levels in groups LPS_{ip} 90 and 240 were 59 ± 55 pg/ml and 88 ± 78 pg/ml, respectively, and did not significantly vary from controls).

With respect to PGI_2, significantly increased concentrations of 6-keto-$PGF_{1\alpha}$ in BALF were observed in the OLC group after 240 min only (320.9 ± 179.4 pg/ml; controls, 105 ± 149 pg/ml). In serum, $PGF_{1\alpha}$ concentrations within each ventilated group were higher at 90 min than at 240 min. Within the lavaged-animal groups, all values at 240 min were lower than those at 90 min in all groups except for PLV 90 *versus* 32/6 240 and OLC 240. Because we found no evidence for a ventilation-induced release of cytokines into either the circulation or the alveolar space, we performed a statistical power analysis to calculate the difference in mediator levels that, under our conditions, would have been detected with a power of 80%. According to this analysis, we had an 80% chance to detect an increase in serum TNF by 170 pg/ml, in lavage TNF by 350 pg/ml, in serum PGI_2 by 170 pg/ml, and in lavage PGI_2 by 175 pg/ml.

No animal died during the experiment, nor did any develop pneumothorax.



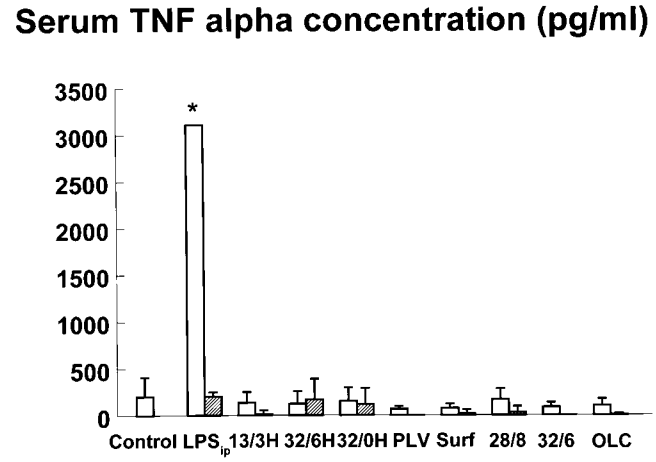


Fig. 2. Bronchoalveolar lavage fluid (BALF) and serum concentrations of tumor necrosis factor-α (TNF-α) in the different experimental groups after 90 (open) and 240 (dashed) min of mechanical ventilation. Data are mean ± SD from groups of 10 animals (except control, n = 16; Sal_{it} 90/240, LPS_{it} 90/240, and LPS_{ip} 90/240, n = 3; 13/3H, n = 13). BALF: groups LPS_{it} 90 and 240 had significantly higher TNF-α levels than all other groups, with levels at 90 min significantly higher than those at 240 min. *Statistically significant (individual comparisons, $P < 0.05$) *vs.* all other groups. Serum: statistically significant (individual comparisons, $P < 0.05$) *vs. all other groups.

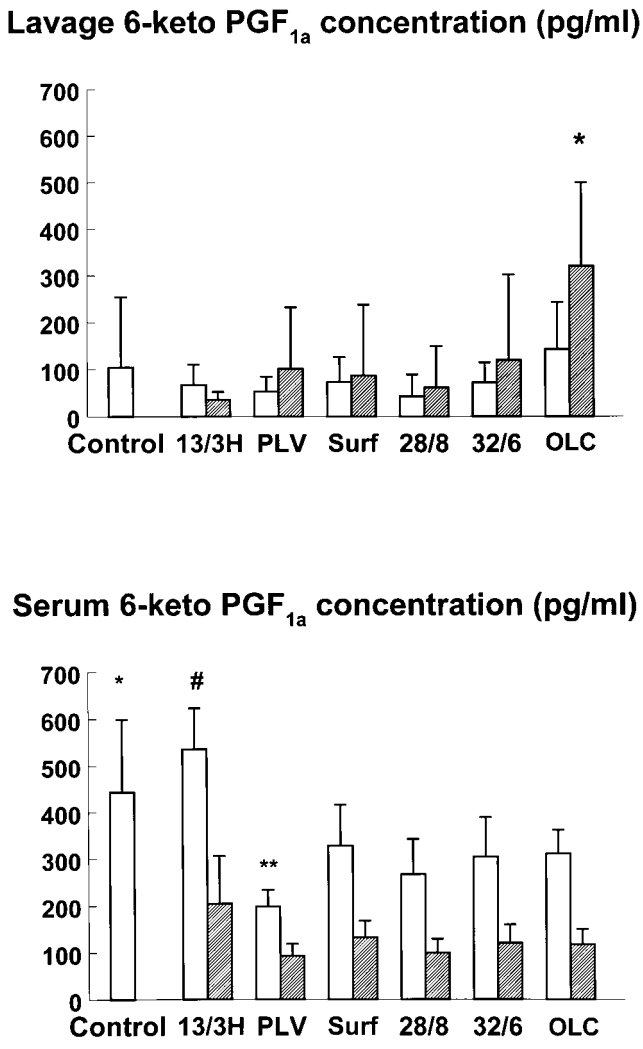


Fig. 3. Bronchoalveolar lavage fluid (BALF) and serum concentrations of 6-keto–prostaglandin (PG) F_{1a} in the different experimental groups after 90 (open) and 240 (dashed) min of mechanical ventilation. Data are mean ± SD from groups of 10 animals (except control, n = 16; 13/3H, n = 13). Prostacyclin (PGI_2) was assessed as the stable metabolite 6-keto-PGF_{1a}. BALF: statistically significant (individual comparisons, $P < 0.05$) *vs.* *all other groups. Serum: in all ventilated groups, the values at 90 min within one group were higher than those at 240 min. Within the lavaged-animal groups, all values at 240 min were lower than those at 90 min in all groups except for PLV 90 *vs.* 32/6 240 and OLC 240. Statistically significant (individual comparisons, $P < 0.05$) **vs.* all other groups except surfactant 90 and 13/390; #*vs.* all other groups except control; ***vs.* 32/6 90, OLC 90, and surfactant 90.

Discussion

This study demonstrates that surfactant therapy, PLV, and ventilation with high PEEP reduce protein accumulation in the bronchoalveolar lavage–accessible spaces in this rat lung lavage model of acute lung injury. TNF-α concentrations of BALF were increased only in the groups treated with LPS intratracheally and showed no differences between all other groups. Thus, our *in vivo* mediator results did not confirm the results of previous studies in isolated healthy and LPS-exposed lungs subjected to different modes of hyperinflation,[1–3] which showed ventilation-induced inflammatory mediator release and reduced release with ventilation strategies using higher levels of end-expiratory pressure. Moreover, of note, the large increase in serum TNF induced by LPS intraperitoneally shows that under our conditions, changes in serum TNF would have been noticed. The power analysis showed that we had a power of 80% to detect an increase of $\geq$ 170 pg/ml. The reasons for the lack of agreement between our *in vivo* studies and the aforementioned *in vitro* studies[1–3] are speculative and multiple and may be a result of the limitations of isolated lung preparations. In our study, high PEEP at high levels of mean airway pressure (OLC group) resulted in a significant release of PGI_2 in the bronchoalveolar lavage–accessible space.

Previous studies were conducted in intact, healthy rats at tidal volumes comparable to the ones used by Tremblay *et al.*[2] in their *ex vivo* isolated and nonperfused rat lungs (tidal volume $\geq$ 40 ml/kg).[9–12] Such studies have shown that the permeability changes as well as the changes in lung mechanics and oxygenation associated with this type of mechanical ventilation can (at least partially) be prevented by application of PEEP or administration of exogenous surfactant before mechanical ventilation.[9–12]

The application of ventilator settings resulting in tidal volumes $\geq$ 40 ml/kg is lethal in intact rats within 1 h, likely because of respiratory failure.[17] However, this period is within the time frame of first assessment of inflammatory mediators in the *in vitro* studies reported previously.[1–3] For this reason, we investigated the effect of different ventilation strategies on inflammatory mediator release in an *in vivo* rat lung lavage model of acute lung injury at more clinically relevant airway pressures.

Bronchoalveolar lavage increases surface tension of the alveolar lining fluid and decreases lung–thorax compliance[18]; bronchoalveolar lavage primarily affects the surfactant system, and the lavage procedure itself does not alter elastic properties of the pulmonary parenchyma.[19] Treatment procedures to improve oxygenation are aimed at the following:

1. Counterbalancing the increased retractive forces by

applying pressure-controlled ventilation that recruits collapsed lung areas by applying an inspiratory pressure that overcomes the opening pressure of collapsed but recruitable lung units. After recruitment, ventilation pressures are reduced, and PEEP is set just above the critical closing pressure of these lung units to prevent end-expiratory collapse. The pressure amplitude is set as small as necessary to maintain normocapnia (OLC).[20]
2. Decreasing alveolar surface tension toward prelavage levels by application of surface active material (exogenous surfactant therapy).[21]
3. PLV, in which ventilation is superimposed on lungs that are filled with perfluorocarbons (which are capable of dissolving high amounts of oxygen and carbon dioxide), thus preventing expiratory alveolar collapse and maintaining gas exchange.[22]

All of these strategies initially improved oxygenation index (arterial oxygen partial pressure/$F_{I_{O_2}}$) $>$ 500 mmHg compared with $\leq$ 85 mmHg after bronchoalveolar lavage; however, oxygenation in the PLV group decreased over time. Results from previous studies have suggested that with PLV, oxygenation decreases over time as a result of evaporation of perfluorocarbon because of its low vapor pressure, with subsequent derecruitment of alveoli over time.[23] In our study, groups in which PEEP was increased by 2 cm H_2O (group 28/8) or in which PIP was increased by 6 cm H_2O (group 32/6), oxygenation did not significantly improve from postlavage values (PEEP, 6 cm H_2O; PIP, 26 cm H_2O). These data indicate that after surfactant impairment, simply increasing PEEP or PIP does not result in mechanical ventilation with higher mean lung volumes and improved oxygenation, but that an active recruitment procedure should be performed or alveolar air–liquid tension should be decreased by surfactant or perfluorocarbon to improve oxygenation. In the present study, oxygenation in healthy animals ventilated with a PIP of 32 cm H_2O without PEEP (group 32/0H) decreased from $>$ 500 mmHg to $<$ 85 mmHg, whereas oxygenation in animals ventilated with 6 cm H_2O (group 32/6H) was preserved. These data confirm previous findings on the beneficial effect of PEEP on oxygenation during mechanical ventilation with moderately high to high PIP.[11,17] Arterial carbon dioxide was maintained within normal limits in all groups except for the animals in group 28/8, which became hypercapnic, and those in group PLV, which became hypocapnic. Differences in arterial carbon dioxide tension are well explained by the interaction between differences in compliance, ventilation pressure, and dead space (group 32/0H), which will result in different tidal volumes and rates of arterial carbon dioxide elimination.

Intra-alveolar protein levels in the PLV, surfactant, and OLC groups were lower than those in groups 28/8 and 32/6. These findings can be explained by differences in lung parenchymal stretch and epithelial stretch, in particular with widening of intracellular junctions.[24] Increased protein infiltration may also be caused by alveolar collapse due to surfactant impairment, which will increase suctioning from the capillary into the direction of the interstitial spaces and alveolus.[12] Therefore, reducing surface tension by exogenous surfactant (surfactant group) or perflubron (PLV group) and preventing alveolar collapse by high PEEP levels (OLC group) are important explanations for the reduced protein concentrations in these animal groups compared with groups 28/8 and 32/6. The lower intra-alveolar protein concentration of group 32/6H compared with those of group 32/0H confirms previous findings on the beneficial effect of PEEP on the permeability of the alveolo-capillary barrier to protein.[9,11] The fact that there was no difference in the protein concentration of groups LPS_{it} and Sal_{it} indicates that TNF-α did not play an important role in promoting intra-alveolar protein infiltration.

Mechanical ventilation with high PEEP levels at high levels of mean airway pressure resulted in an increase in the PGI_2 level of BALF after 240 min of mechanical ventilation (OLC group). It may be speculated that PGI_2 release as a result of mechanical ventilation will protect the lung from reduced capillary perfusion as a result of compression associated with mechanical ventilation at high mean airway pressures. Because the barrier function of the alveolo-capillary membrane in lavaged lungs is lost even to large molecules,[25] PGI_2 may freely diffuse over the alveolo-capillary barrier, which makes its origin unclear. Stretching of both cultured rat lung cells[26] and cultured endothelial cells[27] has been shown to result in PGI_2 production. We do not have an explanation for the consistent finding of a decrease in serum PGI_2 concentration over time. It may be the result of decreased flow speeds in the lung vasculature[28] or depletion of a PGI_2 pool, altered PGI_2 metabolism, or a naturally occurring physiologic vascular adaptation to changes in lung perfusion as a result of mechanical ventilation.

The BALF control levels of TNF-α were on the same order of magnitude as in isolated rat lungs.[2] Our data showed no statistically significant effect of lavage or ventilation on BALF TNF levels *in vivo*. Alveolar macro-

phages are primary candidates for mediator release[29] and TNF-α in particular,[30] which may be induced by mechanical stretch.[6] The failure to observe ventilation-dependent TNF release *in vivo* in this study could, in theory, have been a result of the washout of alveolar macrophages from the alveolar spaces. To exclude this possibility, we included two groups of healthy, nonlavaged rats exposed to comparable PIP levels but different PEEP levels. There was no increase in either serum or lavage TNF-α levels in healthy nonlavaged animals as a result of mechanical ventilation compared with nonventilated controls, and the values were on the same order of magnitude as those in the ventilated and lavaged animals. Therefore, it is unlikely that bronchoalveolar washout of macrophages affected bronchoalveolar TNF-α levels.

The absence of any effect of ventilation on TNF or PGI_2 release raises the question as to the minimum difference in mediator levels that the present study would have detected. Because we used as many as 10 animals per group, the power of the present study was sufficient to note a moderate increase of either PGI or TNF levels in serum ($<$ 170 pg/ml) or BALF (PGI, 175 pg/ml; TNF, 350 pg/ml). Thus the increase that was detectable was much less than that after LPS treatment, which was only used as an internal control. The power of the present study to detect an increase in BALF TNF of 1,000 pg/ml as reported after high-volume zero PEEP ventilation of nonperfused isolated rat lungs[2] was $>$ 99%. These considerations show that the present study had sufficient power to show even moderate changes in mediator levels. However, it should be noted that the absence of increased TNF or PGI levels in mechanically ventilated healthy animals or animals with homogeneous lung injury as induced by the lavage procedure, does not allow prediction of what would happen in inhomogeneously injured lungs, such as those typical for acute respiratory distress syndrome.

In conclusion, different ventilation strategies had a profound effect on lung protein permeability and oxygenation in lungs from lavaged rats *in vivo*. However, except for an increase in the level of PGI_2 in the BALF by mechanical ventilation with high levels of PEEP (OLC), we could not demonstrate any increase in TNF-α or PGI_2 levels in serum or BALF as a result of these different ventilatory strategies in lavaged rats *in vivo*. These results are in contrast to previous findings in isolated perfused lungs.[1–3] We therefore stress that caution is required in the extrapolation of data on ventilation-induced inflammatory mediator expression in isolated lung preparations to *in vivo* preparations and the clinical situation.

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