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EXPERIMENTAL

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Neurally adjusted ventilatory assist decreases ventilator-induced lung injury and non-pulmonary organ dysfunction in rabbits with acute lung injury

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Abstract *Objective:* To determine if neurally adjusted ventilatory assist (NAVA) that delivers pressure in proportion to diaphragm electrical activity is as protective to acutely injured lungs (ALI) and non-pulmonary organs as volume controlled (VC), low tidal volume (Vt), high positive end-expiratory pressure (PEEP) ventilation. *Design:* Prospective, randomized, laboratory animal study. *Subjects:* Twenty-seven male New Zealand white rabbits. *Interventions:* Anesthetized rabbits with hydrochloric acid-induced ALI were randomized ($n = 9$ per group) to 5.5 h NAVA (non-paralyzed), VC (paralyzed; Vt 6-ml/kg),

or VC (paralyzed; Vt 15-ml/kg). PEEP was adjusted to hemodynamic goals in NAVA and VC6-ml/kg, and was 1 cmH_2O in VC15-ml/kg. *Measurements and main results:* PaO_2/FiO_2; lung wet-to-dry ratio; lung histology; interleukin-8 (IL-8) concentrations in broncho-alveolar-lavage (BAL) fluid, plasma, and non-pulmonary organs; plasminogen activator inhibitor type-1 and tissue factor in BAL fluid and plasma; non-pulmonary organ apoptosis rate; creatinine clearance; echocardiography. PEEP was similar in NAVA and VC6-ml/kg. During NAVA, Vt was lower (3.1 ± 0.9 ml/kg), whereas PaO_2/FiO_2, respiratory rate, and $PaCO_2$ were higher compared to VC6-ml/kg ($p < 0.05$ for all). Variables assessing ventilator-induced lung injury (VILI), IL-8 levels, non-pulmonary organ apoptosis rate, and kidney as well as cardiac performance were similar in NAVA compared to VC6-ml/kg. VILI and non-pulmonary organ dysfunction was attenuated in both groups compared to VC15-ml/kg. *Conclusions:* In anesthetized rabbits with early experimental ALI, NAVA is as effective as VC6-ml/kg in preventing VILI, in attenuating excessive systemic and remote organ inflammation, and in preserving cardiac and kidney function.

Keywords Respiratory therapy · Respiratory distress syndrome · Multiple organ failure · Diaphragm · Electromyography

Introduction

Mechanical ventilation can cause ventilator-induced lung injury (VILI) [1–3] because of alveolar overdistension (volutrauma) and/or cyclic collapse/re-opening of lung units (atelectrauma). The implications of VILI are not simply limited to structural damage to the lungs, but also encompass the activation of systemic inflammatory cascades (biotrauma) [1, 3–5], which may be involved in propagating injury to non-pulmonary organs, potentially resulting in multiple organ failure [6–8]. A number of studies have demonstrated that ventilatory strategies that minimize VILI can decrease mortality in patients with acute lung injury (ALI) [9, 10]. However, these strategies have been criticized on the grounds that they only minimally tailor the mechanical ventilation to the individual patient. A ventilatory strategy that takes into account the inter-patient and temporal differences in pulmonary pathophysiology may further improve outcome.

Neurally adjusted ventilatory assist (NAVA) delivers pressure to the airways (Paw) proportional to inspiratory diaphragmatic electrical activity (EAdi) [11]; the proportionality factor is set on the ventilator by the clinician. The EAdi is influenced by facilitatory and inhibitory, dominantly vagally mediated feedback loops that integrate information from mechano- and chemo-receptors that "sense" the degree of lung stretch, as well as chemical stimuli [12–14]. The EAdi is reflexively up-regulated if the delivered tidal volume (Vt) is below the subject's respiratory demand; down-regulation of EAdi occurs when assist is greater than the subject's demand [12–16]. When the assist level with NAVA satisfies the subject's respiratory demand, Vt remains virtually unchanged despite increases in the proportionality factor [12–16].

Hence, NAVA provides assist on a breath-by-breath basis in synchrony with and in proportion to respiratory demand. Studies in animals and humans demonstrate that NAVA prevents excessive lung distension, efficiently unloads respiratory muscles, and improves patient-ventilator synchrony [12, 13, 15–17].

To test the hypothesis that allowing the animals to "control" their intra-breath assist profile using NAVA would be at least as lung protective as a conventional low Vt strategy, we compared NAVA and two ventilation strategies, one known to be injurious (high Vt with low PEEP) and one known to be protective (low Vt with high PEEP), in an established ALI model.

Methods

The protocol was approved by the local Animal Care and Use Committee of St. Michael's Hospital. For details see the online supplement.

Animal preparation

Thirty adult male New Zealand white rabbits (3.6–4.6 kg) were anesthetized, tracheotomized, and ventilated with a Servo 300 ventilator (Maquet Critical Care, Solna, Sweden) modified for NAVA. Intravenous anesthesia (ketamine hydrochloride 40 mg/kg/h; xylazine 4 mg/kg/h) and fluid (Ringer's lactated solution; 5 ml/kg/h) administration remained constant throughout the experiment. Pulse oxymetry, heart rate, and arterial pressure were continuously monitored.

After induction of neuromuscular paralysis (pancuronium bromide 0.02 mg/kg), hydrochloric acid (pH 1.5) was instilled intratracheally with the rabbit in the lateral position (0.75 ml/kg each side), followed by a ventilation pause at Paw 25 cmH_2O. The procedure was repeated after 5 min. Thirty minutes thereafter, the 27 animals that reached a predefined PaO_2/FiO_2 ratio of 80–200 (on FiO_2 0.5) were randomized ($n = 9$ per group) to 5.5 h ventilation with one of the following strategies:

Experimental protocol

NAVA

No paralysis. NAVA was used as previously described [12, 15, 18]. Briefly, the EAdi derived from an array of electrodes on an esophageal catheter was processed, multiplied by a proportionality factor, and used to control inspiratory assist (Paw = EAdi × NAVA level) [19, 20]. A NAVA level of 0.5 cmH_2O/unit EAdi was used throughout the study based on our previous findings using the same animal model [15].

VC6-ml/kg or 15-ml/kg

Continuous paralysis (pancuronium; 0.25 mg/kg/h). An additional dead space of 25 ml was used with VC15-ml/kg, and the ventilatory rate was adjusted to maintain $PaCO_2$ between 35 and 45 mmHg at similar ventilatory rates in both VC groups.

PEEP was adjusted in NAVA and VC6-ml/kg, aiming to use the highest PEEP possible while maintaining mean arterial pressure (MAP) >60 mmHg. PEEP was lowered in 1 cmH_2O steps if MAP decreased below 60 mmHg. Additional fluid was not administered. PEEP was 1 cmH_2O in VC15-ml/kg. FiO_2 was 0.5 and increased, if needed, to maintain SaO_2 above 90%. Recruitment maneuvers and tracheal suctioning were not performed.

Measurements

Cardio-respiratory parameters were recorded every 30 min; arterial blood gases, lactate, and hemoglobin concentrations were measured hourly. Plasma was collected before ALI induction, 3 h thereafter, and at the end of the protocol. Broncho-alveolar lavage (BAL) fluid, and lung, heart, liver, small intestines, kidney, and spleen tissues were collected after killing the animals. Echocardiography was performed before ALI and hourly thereafter. Oxygen delivery, plasma creatinine clearance, and dynamic respiratory system compliance (CRSdyn) were calculated.

In plasma: interleukin 8 (IL-8), plasminogen activator inhibitor type-1 (PAI-1), tissue factor (TF), and creatinine. *In lung tissue:* wet-to-dry ratio, histological lung injury score, IL-8, PAI-1, and TF concentrations. *In non-pulmonary organs:* IL-8 levels and apoptosis rate.

Analysis of lung histology, of inflammatory and coagulation parameters, and of apoptosis in non-pulmonary organs was only performed in animals in which complete sets of material were available (VC15-ml/kg, $n = 5$; VC6-ml/kg, $n = 7$; NAVA, $n = 7$).

IL-8, PAI-1, TF (plasma, BAL), and lung wet-to-dry ratios were measured in four healthy control animals killed immediately after tracheotomy.

Data and statistical analysis

SigmaStat™ (3.10, Systat Software Inc., San Jose, CA) was used. Data are presented as mean ± SD or median (quartiles) as appropriate. Kolmogorov–Smirnov test was used to assess normal distribution of data. Differences among groups were analyzed using one-way analysis of variance (ANOVA). Repeated measurements were analyzed using either ANOVA on ranks (Friedman test) or two-way ANOVA with the ventilatory mode as the between-group factor and time after randomization as the repeated-measures factor. Holm-Sidak method was used for post-test comparison. Wilcoxon signed rank or *t* test was used to compare groups with paired data. Level of significance was $p < 0.05$.

Results

The protocol was completed in all except for three VC15-ml/kg animals that were killed when MAP decreased <60 mmHg after 4 h. For statistical purposes, the last available values were used in these animals.

The infusion rates of sedative drugs and fluids were equal in all groups. Hemodynamic parameters, arterial lactate and hemoglobin concentrations, and body temperature were not different among groups (Table 1). Arterial lactate concentrations remained unchanged in NAVA, whereas it progressively increased in both VC groups ($p < 0.05$ for NAVA vs. VC15-ml/kg).

Ventilatory pattern

Average Vt during NAVA was 2.7 ± 0.9 ml/kg [coefficient of variation (CV) 33%] during the initial 3 h and increased to 3.4 ± 0.8 ml/kg ($p < 0.001$; CV 23%) during the final 2.5 h (Fig. 1a). Early after ALI induction, the respiratory rate in NAVA was up to three times higher than the ventilatory rate in both VC groups, and thereafter decreased towards values in the VC6-ml/kg group (Fig. 1b). PEEP was decreased from 8.1 ± 2.0 to 4.2 ± 1.6 cmH_2O in NAVA and from 7.0 ± 1.6 to 4.6 ± 1.2 cmH_2O in VC6-ml/kg (p = n.s.; Fig. 1c).

Mean Paw during NAVA was lower compared to both VC6-ml/kg and VC15-ml/kg groups ($p < 0.05$ for both comparisons), while it was not different between VC6-ml/kg and VC15-ml/kg groups (Table 1). Early during NAVA, $PaCO_2$ increased to 57 ± 7 mmHg; arterial pH decreased to 7.29 ± 0.04. Both variables remained unchanged throughout the study. $PaCO_2$ was lower in VC6-ml/kg and VC15-ml/kg ($p < 0.05$ vs. NAVA; Table 1). At the end of the protocol, FiO_2 was unchanged at 0.5 ± 0.0 in NAVA and was 0.6 ± 0.2 in VC6-ml/kg, whereas it was 0.9 ± 0.2 in VC15-ml/kg ($p < 0.001$ vs. NAVA and VC6-ml/kg).

Table 1 Hemodynamic and metabolic parameters; biochemical markers of organ injury

	$n = 9$ per group	Before lung injury	After induction of lung injury				ANOVA, p (t–g)
			30 min	1 h	3 h	5.5 h	
Mean arterial blood pressure (mmHg)	NAVA	76 ± 5	66 ± 5*	64 ± 7*	64 ± 6*	61 ± 3*	0.353
	VC6-ml/kg	78 ± 7	66 ± 6*	67 ± 7*	65 ± 6*	60 ± 3*	
	VC15-ml/kg	71 ± 5	65 ± 9*	66 ± 9*	64 ± 9*	57 ± 7*	
Heart rate (bpm)	NAVA	176 ± 25	192 ± 27*	195 ± 43*	193 ± 28*	185 ± 23	0.568
	VC6-ml/kg	151 ± 24	183 ± 27*	176 ± 26*	175 ± 33*	174 ± 26*	
	VC15-ml/kg	163 ± 32	178 ± 22	185 ± 31*	180 ± 16	191 ± 20*	
Hemoglobin concentration (g/l)	NAVA	111 ± 11	–	113 ± 10	112 ± 10	105 ± 8	0.564
	VC6-ml/kg	107 ± 8	–	109 ± 8	108 ± 7	105 ± 7	
	VC15-ml/kg	106 ± 10	–	110 ± 9	112 ± 11	106 ± 7	
Body temperature (°C)	NAVA	39.4 ± 0.3	39.3 ± 0.6	39.2 ± 0.5	39.5 ± 0.4	39.2 ± 0.3	0.757
	VC6-ml/kg	39.5 ± 0.7	39.3 ± 0.7	39.2 ± 0.6	39.0 ± 0.3	39.0 ± 0.3	
	VC15-ml/kg	39.2 ± 0.4	38.9 ± 0.5	38.9 ± 0.4	39.0 ± 0.4	39.4 ± 0.9	
Mean airway pressure (cmH_2O)	NAVA	4.6 ± 0.5	8.1 ± 2.4*	7.9 ± 2.3	6.4 ± 2.3	4.6 ± 1.6*†‡	<0.001
	VC6-ml/kg	4.8 ± 0.8	7.8 ± 1.8*	7.9 ± 2.1	8.1 ± 2.6	6.9 ± 1.4*	
	VC15-ml/kg	5.0 ± 0.7	6.4 ± 2.2	6.1 ± 1.5	7.7 ± 3.7	8.8 ± 4.0*	
Peak airway pressure above PEEP (cmH_2O)	NAVA	6.4 ± 1.1	1.7 ± 1.3*	1.7 ± 1.7*	3.2 ± 1.1	3.7 ± 2.0†‡	<0.001
	VC6-ml/kg	5.9 ± 1.2	8.3 ± 3.0*	8.8 ± 1.4*	9.9 ± 1.3*	10.3 ± 3.0*§	
	VC15-ml/kg	7.3 ± 1.9	22.7 ± 4.0*	22.3 ± 4.6*	25.2 ± 5.9*	30.4 ± 8.2*	
Arterial oxygen saturation (SaO_2), %	NAVA	99.1 ± 1.1	93.8 ± 3.5*	96.1 ± 2.4*	96.9 ± 2.6*	96.1 ± 1.7*‡	<0.001
	VC6-ml/kg	99.6 ± 0.1	92.4 ± 4.8*	93.8 ± 8.7*	97.3 ± 1.9*	97.6 ± 2.6*§	
	VC15-ml/kg	99.6 ± 0.2	91.9 ± 6.9*	94.0 ± 5.3*	87.7 ± 10.2*	81.8 ± 14.3*	
Arterial pH	NAVA	7.44 ± 0.06	7.37 ± 0.04*	7.29 ± 0.04*	7.29 ± 0.03*	7.28 ± 0.03*†‡	0.026
	VC6-ml/kg	7.48 ± 0.02	7.41 ± 0.04*	7.39 ± 0.09*	7.39 ± 0.03*	7.40 ± 0.03*	
	VC15-ml/kg	7.44 ± 0.05	7.41 ± 0.05	7.39 ± 0.08*	7.37 ± 0.06*	7.33 ± 0.10*	
Arterial PCO_2 (mmHg)	NAVA	42 ± 3	45 ± 3*	57 ± 7*	54 ± 7*	60 ± 4*†‡	<0.001
	VC6-ml/kg	41 ± 5	46 ± 3*	47 ± 8*	42 ± 5	39 ± 5	
	VC15-ml/kg	42 ± 4	43 ± 6	43 ± 8	42 ± 6	39 ± 8	
Arterial lactate concentration (mmol/l)	NAVA	2.1 ± 0.5	–	2.2 ± 0.8	2.2 ± 0.5	2.3 ± 0.7‡	0.003
	VC6-ml/kg	2.2 ± 0.5	–	2.4 ± 0.6	2.7 ± 0.7	3.6 ± 2.1*	
	VC15-ml/kg	2.0 ± 0.8	–	2.0 ± 0.6	2.8 ± 0.8*	4.4 ± 1.1*	
CRSdyn (ml/cmH_2O)	NAVA	3.6 (3.3;4.8)	2.0 (1.7;2.4)*	–	–	2.8 (2.7;3.0)*‡	
	VC6-ml/kg	4.0 (3.7;4.6)	1.9 (1.7;2.3)*	–	–	2.0 (2.0;2.3)*§	
	VC15-ml/kg	3.3 (3.0;3.7)	1.5 (1.4;1.7)*	–	–	1.5 (1.3;1.7)*	
Cardiac output (ml/kg/min) $n = 7$ for all groups	NAVA	61 ± 8	–	58 ± 13	53 ± 9	56 ± 8	0.179
	VC6-ml/kg	58 ± 9	–	52 ± 12	47 ± 12	47 ± 9*	
	VC15-ml/kg	56 ± 7	–	54 ± 10	43 ± 9*	36 ± 7*	
Global DO_2 (ml/kg/min) $n = 7$ for all groups	NAVA	9.4 ± 1.1	–	8.7 ± 2.2	8.2 ± 1.7	7.9 ± 1.4*	0.099
	VC6-ml/kg	9.1 ± 1.7	–	7.7 ± 1.7	7.1 ± 1.7	6.9 ± 1.3*	
	VC15-ml/kg	8.4 ± 1.1	–	8.1 ± 2.0	6.4 ± 1.9*	4.6 ± 1.5*	
Urinary output (ml/kg/h)	NAVA	–	–	–	–	0.61 ± 0.21‡	
	VC6-ml/kg	–	–	–	–	0.65 ± 0.20§	
	VC15-ml/kg	–	–	–	–	0.36 ± 0.12	
Plasma creatinine clearance (ml/min)	NAVA	–	–	–	–	12.9 (10.7;16.0)‡	
	VC6-ml/kg	–	–	–	–	10.0 (6.0;12.6)	
	VC15-ml/kg	–	–	–	–	6.2 (5.7;9.3)	

Values are mean ± SD or median (quartiles). p (t-g) values indicate time–group interaction (two-way ANOVA). Post hoc pairwise comparison procedure between groups: $^{\dagger}p < 0.05$ NAVA versus VC6-ml/kg; $^{\ddagger}p < 0.05$ NAVA versus VC15-ml/kg; $^{\S}p < 0.05$ VC6-ml/kg versus VC15-ml/kg. $^{*}p < 0.05$ values at 6 h versus values at before ALI

There were no differences in cardiopulmonary and metabolic parameters among groups before and 30 min after induction of acute lung injury (ALI). Arterial oxygen saturation (SaO_2) decreased to a similar degree after induction of ALI in all groups. Thereafter, SaO_2 partially recovered in NAVA and VC6-ml/kg, while it progressively decreased in the VC15-ml/kg animals. Arterial carbon dioxide tension ($PaCO_2$) was higher and arterial pH was lower in NAVA compared to both VC groups. Arterial lactate concentration remained at pre-ALI levels in NAVA, whereas it increased when either VC6-ml/kg or VC15-ml/kg was applied after induction of ALI. Respiratory system compliance (CRSdyn; assessed during paralysis) was reduced by about 50% after ALI induction in all groups. CRSdyn was unchanged at the end of the protocol in VC6-ml/kg, increased in NAVA, and decreased in VC15-ml/kg. At the end of the protocol, cardiac output and DO_2 were lower compared to before ALI in all groups, except for NAVA in which cardiac output was preserved throughout the protocol. Plasma creatinine clearance was higher in NAVA as compared to VC15-ml/kg, while it was not different between the two VC groups

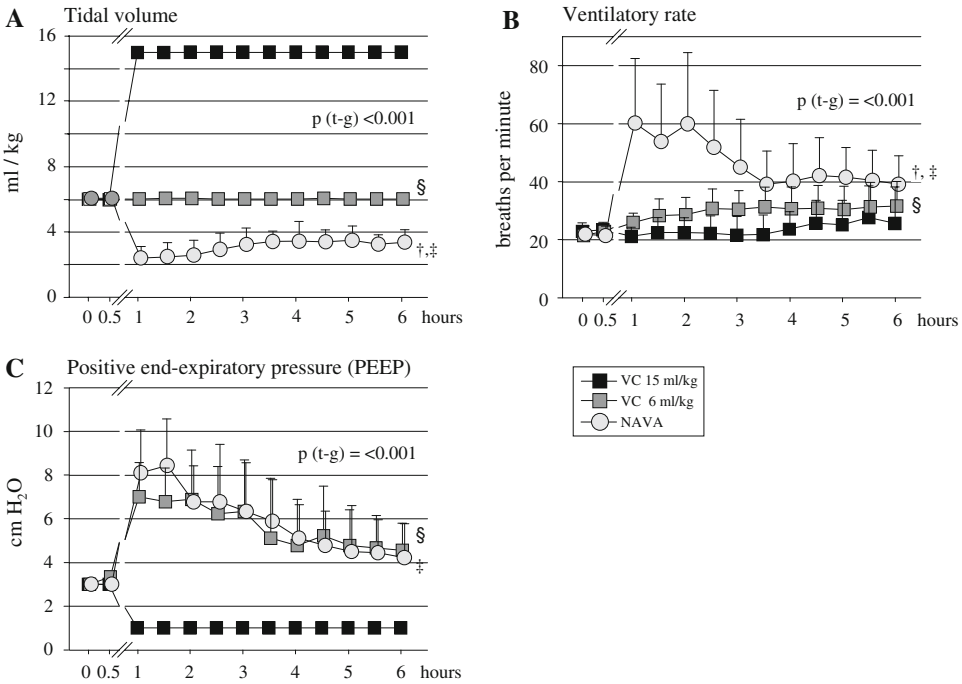

Fig. 1 Ventilatory pattern. PEEP levels, adjusted to maintain mean arterial pressure ≥60 mmHg in NAVA and VC6-ml/kg, were not different between NAVA and VC6-ml/kg. Ventilatory rate was higher in NAVA compared to both VC groups during the first 3 h, but was not different thereafter. *Symbols* represent group mean; *error bars* indicate standard deviation. *t–g* time–group interaction (two-way ANOVA). Post hoc pairwise comparison procedure between groups: $^{\dagger}p < 0.05$ NAVA versus VC6-ml/kg; $^{\ddagger}p < 0.05$ NAVA versus VC15-ml/kg; $^{\S}p < 0.05$ VC6-ml/kg versus VC15-ml/kg

VILI

During the first hours after ALI, the PaO_2/FiO_2 ratio was higher during NAVA than during VC6-ml/kg, whereas no difference was observed later in the protocol (Fig. 2a). SaO_2 remained >93% with NAVA and VC6-ml/kg, while it progressively decreased in VC15-ml/kg (Table 1) despite increasing FiO_2. Lung wet-to-dry ratio was increased for both dependent and non-dependent regions of the right lower lobe in all groups compared to healthy controls. Wet-to-dry ratio during NAVA was lower for both dependent and non-dependent regions compared to VC15-ml/kg, whereas it was only lower for the dependent but not for the non-dependent lung with VC6-ml/kg compared to VC15-ml/kg (Fig. 2b). Thirty minutes after induction of ALI, CRSdyn had decreased by about 50% in all groups ($p < 0.05$ vs. before ALI for all groups). At the end of the protocol CRSdyn had recovered partially during NAVA, whereas it remained unchanged in VC6-ml/kg and VC15-ml/kg (Table 1).

Cumulative lung injury scores as well as average scores for each component were lower, albeit not always significantly so, in NAVA and VC6-ml/kg as compared to VC15-ml/kg (Table 2).

IL-8, tissue factor, and PAI-1 concentration

IL-8 concentrations in BAL and in lung tissue of the non-dependent lobe were lower in NAVA and VC6-ml/kg compared to VC15-ml/kg ($p < 0.05$). There was no difference in IL-8 tissue concentration between groups in the dependent regions. Tissue factor in BAL was lower during both NAVA and VC6-ml/kg, whereas PAI-1 concentration in BAL fluid was lower only during NAVA but not during VC6-ml/kg compared to VC15-ml/kg (Fig. 2c/d). Tissue factor and PAI-1 concentrations in lung tissue did not differ from non-ventilated controls.

Plasma IL-8 concentration was increased after 3 h in all groups and continued to increase at the end of the protocol in VC15-ml/kg, whereas it returned to pre-injury levels at the end of the protocol in both NAVA and VC6-ml/kg (Fig. 3a). Compared to VC15-ml/kg, IL-8 concentrations in the spleen and kidney were lower in NAVA but not in VC6-ml/kg. IL-8 concentration in the heart was lower in NAVA and VC6-ml/kg compared to VC15-ml/kg. No difference among groups was found for small intestines and liver (Fig. 3b). Plasma PAI-1 concentration remained elevated until the end of the protocol in both VC groups and tended to decrease in NAVA (Fig. 3c). Plasma tissue factor concentration did not differ from non-ventilated controls before induction of ALI and did not change over time in all groups.

Parameters of non-pulmonary organ function

Cardiac output decreased from before ALI induction to the end of the protocol by 16 ± 23% in VC6-ml/kg and by 36 ± 11% in VC15-ml/kg ($p < 0.05$ vs. before induction of ALI for both), while the decrease in NAVA by 7 ± 16% was not significant (Table 1). Global DO_2 decreased by 21 ± 24% during VC6-ml/kg, by 46 ± 17% during VC15-ml/kg, and by 17 ± 11% during NAVA

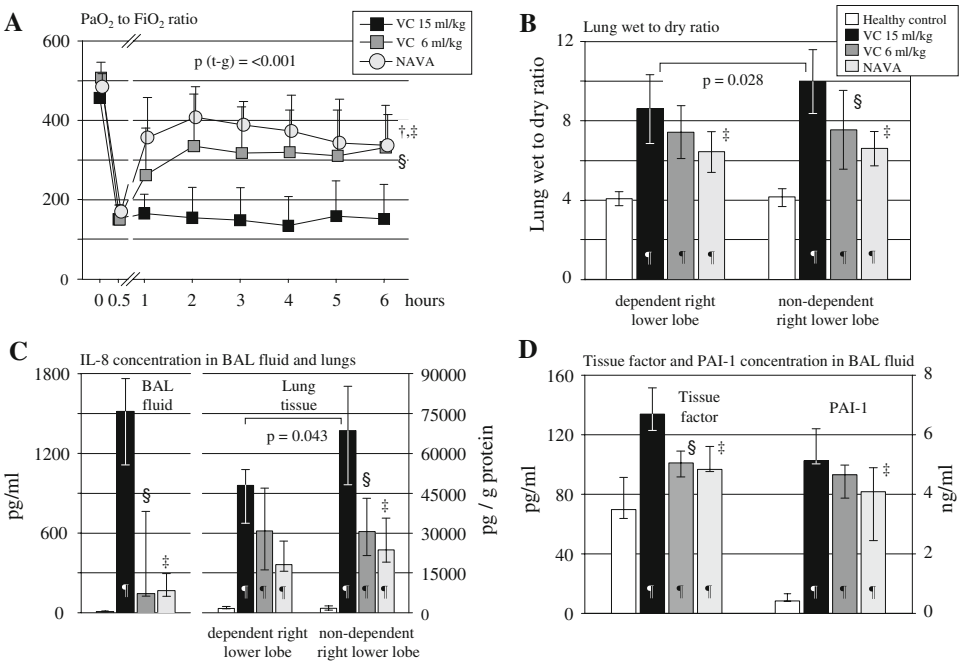

Fig. 2 Parameters indicating ventilation-induced lung injury (VILI). There were no differences in PaO_2/FiO_2 among groups before and 30 min after induction of ALI. The increase in PaO_2/FiO_2 early after switching to the assigned (i.e. before randomization into the treatment groups) ventilation mode was more pronounced in NAVA compared to VC6-ml/kg ($p < 0.05$ in post hoc analysis); however, there were no differences in PaO_2/FiO_2 between NAVA and VC6-ml/kg at the end of the protocol. With VC15-ml/kg, the PaO_2/FiO_2 remained below 200. All PaO_2 values were measured at FiO_2 0.5 and were corrected for body temperature. The lung wet-to-dry ratio in NAVA and in the VC6-ml/kg was lower compared to VC15-ml/kg (albeit not significantly for the dependent lung in VC6-ml/kg animals). Interleukin 8 (IL-8), tissue factor, and plasminogen activator inhibitor type 1 (PAI-1), and concentration in bronco-alveolar (BAL) fluid was higher in all study groups compared to healthy controls and was always higher in VC15-ml/kg compared to the other two groups (except for PAI-1 in VC6-ml/kg). Lung tissue IL-8 concentration was increased in all groups as compared to non-ventilated controls and was highest in the non-dependent lung regions of VC15-ml/kg. In the VC6-ml/kg and NAVA, lung tissue IL-8 concentration was lower compared to VC15-ml/kg (albeit not significant for the dependent lung region). Groups are shown as mean ± SD for **a** and **b**, or as median (quartiles) for **c** and **d**. *Symbols* and abbreviations are the same as in Fig. 1. ¶$p < 0.05$ versus healthy control

($p < 0.05$ vs. before ALI induction ALI for all). Urine output was higher in NAVA and VC6-ml/kg compared to VC15-ml/kg. Plasma creatinine clearance was higher in NAVA compared to VC15-ml/kg, while there was no difference between VC groups. Plasma concentrations for creatinine and urea nitrogen, urine osmolarity, transtubular gradient for potassium, and fractional excretion of sodium were not different among groups.

Apoptosis rate and histological alterations of non-pulmonary organs

The percentage of apoptotic cells was not different among groups for the heart, liver, and the crypts of the small intestines (Table 3). The kidney cortex had a higher percentage of apoptotic cells in VC6-ml/kg compared to VC15-ml/kg ($p < 0.05$). The villi of small intestines had a higher percentage of apoptotic cells in NAVA compared to VC15-ml/kg ($p < 0.05$).

Histological examination of the heart, kidney, liver, and small intestines of all animals revealed no relevant alterations of the tissue (specifically, no necrosis and intravascular thromboemboli).

Discussion

This is the first study to demonstrate that allowing anesthetized, spontaneously breathing animals with acute lung injury to "control" their ventilatory pattern in synchrony and proportional to their EAdi is at least as protective to the lungs and to non-pulmonary organs as a conventional, low Vt strategy [4, 6, 21, 22]. Similar to previous studies in rabbits with ALI [12, 15], animals in the present study "chose" tidal volumes with NAVA that were approximately half of those frequently described as lung protective in experimental studies exploring the pathophysiology of VILI [4, 6, 21, 22].

Our protocol was designed to compare assisted spontaneous breathing with conventional, lung protective ventilation in the absence of spontaneous breathing. The VC15-ml/kg group acted as a positive control

Table 2 Histological lung injury score

	Cumulative injury score			ANOVA on ranks, *p*
	NAVA ($n = 7$)	VC6-ml/kg ($n = 7$)	VC15-ml/kg ($n = 5$)	
Histological lung injury criteria				
Alveolar edema	4.0 (2.5;6.5)	4.0 (3.0;4.8)	6.0 (4.0;7.0)	0.362
Alveolar collapse	3.0 (1.3;3.0)	0.0 (0.0;1.8)[§]	4.0 (2.0;5.8)	0.024
Intraalveolar hemorrhage	4.0 (3.3;6.5)	4.0 (2.0;4.8)[§]	6.0 (6.0;8.0)	0.033
Bronchial epithelial lesions	0.0 (0.0;0.0)	0.0 (0.0;1.0)	1.0 (0.0;2.3)	0.133
Perivascular/bronchial hemorrhage	0.0 (0.0;0.0)	0.0 (0.0;0.0)	0.0 (0.0;1.5)	0.428
Perivascular edema	2.0 (0.3;4.5)[‡]	2.0 (2.0;2.8)[§]	7.0 (3.8;7.8)	0.016
Vascular congestion	3.0 (2.0;4.0)	3.0 (2.0;4.0)[§]	7.0 (5.0;7.3)	0.033
Alveolar hyaline membranes	0.0 (0.0;0.0)[‡]	0.0 (0.0;0.0)[§]	6.0 (3.0;8.8)	<0.001
Intravascular thrombi	0.0 (0.0;0.8)[‡]	0.0 (0.0;1.0)	3.0 (2.0;6.3)	0.010
Cumulative lung injury score	23 (14;25)[‡]	16 (12;19)[§]	39 (33;48)	<0.001
Intraalveolar cells (average number of cells per alveolus)				
Polymorphonuclear neutrophils	12 (9;12)	11 (8;17)	14 (11;15)	0.662
Macrophages	6.0 (5.3;6.8)	6.0 (5.3;7.8)	5.0 (3.8;6.5)	0.475

Values are median (quartiles). ANOVA on ranks: [†]$p < 0.05$ versus VC6-ml/kg; [‡]$p < 0.05$ versus VC15-ml/kg; [§]$p < 0.05$ VC6-ml/kg versus VC15-ml/kg

Lung injury was assessed by nine different histological criteria that were scored between 0 (=none) to 3 (=maximum). There were no differences among the four lung regions (cranial/caudal dependent and cranial/caudal non-dependent) for any histological lung injury criterion. A cumulative injury score (= sum of injury scores of all four regions) was calculated for each animal with a maximum score per criterion of 12 (i.e., maximal cumulative injury score = 108). The scores for single histological lung injury criteria as well as the cumulative lung injury score (sum of all lung injury criteria) were consistently (albeit not always significantly) higher in the VC15-ml/kg group as compared to the NAVA and VC6-ml/kg groups. There was no difference between NAVA and VC6-ml/kg for any lung injury criterion or for the cumulative injury score. Numbers of intra-alveolar cells (i.e., average number of cells per alveolus) were not different among study groups

demonstrating the increase in VILI with large Vt. Although a number of factors (i.e., level of anesthesia, fluid administration, PEEP, and FiO_2) were controlled using the same protocols in both NAVA and VC6-ml/kg, we did not match the ventilatory patterns of NAVA and VC, as our hypothesis was that VILI would be minimized if animals "chose" their ventilatory pattern (i.e., Vt, intra-breath assist profile, ventilatory rate, including their variability) on a breath-by-breath basis.

Limitations

Based on our previous experience with the same model and to avoid a lengthy titration procedure, we used a single NAVA level [12, 15]. Using higher NAVA levels would have likely resulted in similar Vt and respiratory rates as we previously demonstrated that at higher NAVA levels Vt and respiratory rate reach a plateau due to progressive down-regulation of EAdi once a satisfactory level of respiratory muscle unloading is reached [13–16]. Of note, a uniform NAVA level does not necessarily result in a uniform ventilatory pattern, since the EAdi is continuously adjusted by vagally mediated feedback loops and behavioral inputs [12, 16].

We cannot exclude that the assist with the NAVA level we used was insufficient. However, in previous experiments using the same rabbit ALI model, we showed that the NAVA level used reduced the transdiaphragmatic pressure time product by more than 50% [15]. Although $PaCO_2$ was elevated and pH was lower during NAVA compared to the VC groups, both parameters remained stable during NAVA, suggesting that there was no progression to respiratory failure due to inadequate low assist.

VC animals were paralyzed to ensure absence of spontaneous breathing and lung recruitment by tonic EAdi [12], a strategy frequently employed in animal research on VILI [6, 23], but used less often in clinical practice. Although unlikely, we cannot exclude the possibility that pancuronium affected the degree of VILI.

One has to be cautious when extrapolating from our short-term experimental ALI model performed under controlled laboratory conditions to much more complex clinical scenarios. Our model may only reflect the very first hours of an acute pulmonary insult such as aspiration of gastric content, but not the fundamentally different processes involved when ALI/ARDS results from an other cause.

Implications

Despite lower mean Paw and Vt with NAVA, we found that lung injury scores, lung wet-to-dry ratio, and lung and systemic biomarkers indicating VILI were similar to VC6-ml/kg, whereas other parameters (i.e., PaO_2/FiO_2 ratio, cardiac output, arterial lactate concentration, and

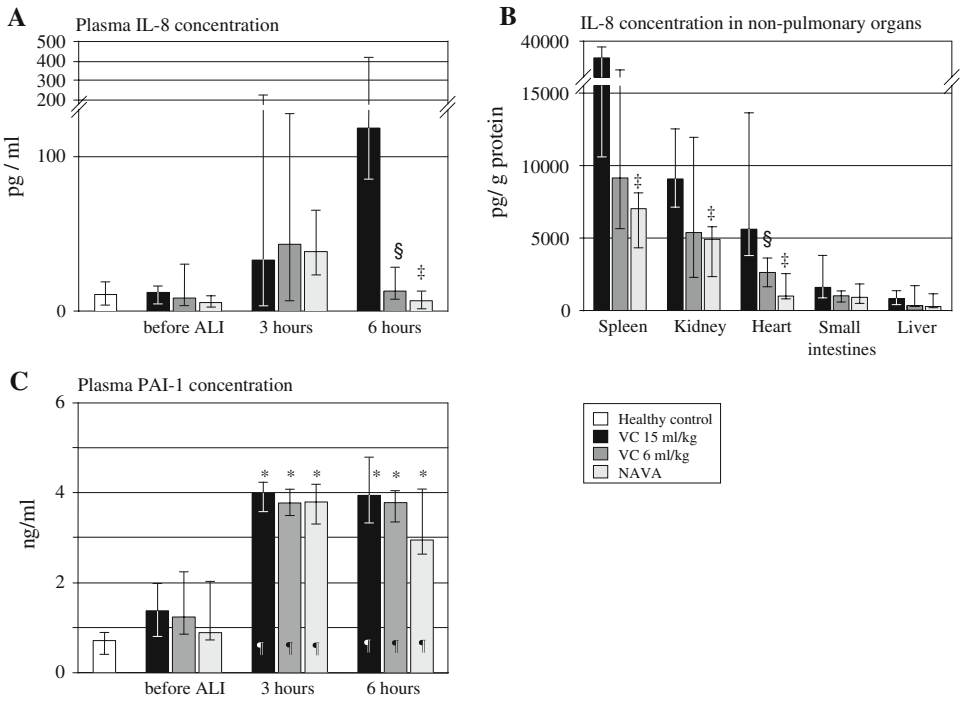

Fig. 3 Plasma and non-pulmonary organ IL-8 levels. Plasma PAI-1 levels. Plasma IL-8 concentration was similarly increased in all groups after 3 h and further increased in VC15-ml/kg, while returning to baseline values in VC6-ml/kg and NAVA at the end of the protocol. Tissue IL-8 concentration was highest in spleen, followed by kidney, heart, small intestines, and liver. While tissue IL-8 concentration was higher in VC15-ml/kg as compared to NAVA and VC6-ml/kg for all organs, the difference was not always statistically significant. Before induction of ALI, plasma levels of PAI-1 were not different from those of non-ventilated controls in all groups, and there was no difference among the study groups. PAI-1 concentrations in plasma were increased 3 h after induction of ALI in all groups and remained elevated until the end of the protocol in both VC groups, whereas it tended to decrease in NAVA. Groups are shown as median (quartiles). Symbols and abbreviations are the same as in Fig. 1. $^{\P}p < 0.05$ versus healthy control. $^{*}p < 0.05$ versus before induction of ALI

Table 3 Apoptosis in non-pulmonary organs

	Average apoptosis rate, %			ANOVA on ranks, p
	NAVA ($n = 7$)	VC6-ml/kg ($n = 7$)	VC15-ml/kg ($n = 5$)	
Left cardiac ventricle	0.35 (0.11;0.75)	0.19 (0.02;1.36)	0.14 (0.07;0.42)	0.708
Right cardiac ventricle	4.27 (0.00;10.6)	1.09 (0.26;8.54)	0.28 (0.09;0.47)	0.408
Kidney cortex	9.3 (4.0;23.8)	15.4 (10.2;18.2)§	0.25 (0.04;4.18)	0.013
Liver	0.35 (0.28;0.37)	0.09 (0.06;0.17)	0.30 (0.08;3.95)	0.154
Small intestines				
Vili	6.81 (3.76;8.00)‡	3.85 (1.67;9.05)	1.01 (0.66;1.30)	0.013
Crypts	0.35 (0.28;0.37)	0.09 (0.06;0.17)	0.30 (0.08;3.95)	0.154

Values are median (quartiles). ANOVA on ranks: $^{\dagger}p < 0.05$ versus VC6-ml/kg; $^{\ddagger}p < 0.05$ versus VC15-ml/kg; $^{\S}p < 0.05$ VC6-ml/kg versus VC15-ml/kg

For each animal and organ, an average apoptosis percentage [= TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling) positive nuclei per total nuclei per high power field; expressed in %] was calculated for 12 high power fields of the heart (left and right ventricle separately), kidney cortex, and liver (original magnification ×200) and for 15 high power fields of small intestines vili and crypts (original magnification ×400)

There were no differences in apoptosis percentages among groups for left and right cardiac ventricle, for liver, and for small intestines crypts. The apoptosis percentage in the kidney cortex and the villi of the small intestine were not different between NAVA and the VC6-ml/kg, but was higher in NAVA and 6-ml/kg compared to VC15-ml/kg (not significant for kidney cortex in the NAVA and for villi in the VC6-ml/kg)

CRSdyn) reflecting functional aspects of the cardiopulmonary system were less affected with NAVA compared to VC6-ml/kg. We cannot exclude that the seemingly lung protective effects of lower Paw and Vt on VILI might have been negated by the high respiratory rate with NAVA, while the positive effects of preserved

diaphragmatic contraction on cardiopulmonary function were still detectable [24–27]. Our results are comparable to previous reports suggesting an association between preserved spontaneous breathing and better cardiac performance [27, 28].

The NAVA animals' breathing pattern response can be explained by vagally controlled reflexes [12] and seems physiologically plausible [29–33]. The high respiratory rate in combination with a high variability in Vt (partially due to frequent sighs) early after ALI induction may have led to some intrinsic PEEP with lung recruitment. The initially higher PaO_2/FiO_2 ratio with NAVA compared to VC6-ml/kg despite similar levels of extrinsic PEEP would support this speculation. However, measurement of intrinsic PEEP under these conditions is very difficult, and the current study did not address this possibility.

The method of applying PEEP to a level that did not reduce mean arterial pressure below 60 mmHg in the present study differs from the approach normally used for ALI/ARDS. Our previous work has shown that ALI increases tonic EAdi and reduces phasic EAdi, while recruitment of the lungs with PEEP reduces tonic EAdi and restores phasic EAdi [12]. The approach used in the present study represents a compromise among suppressing tonic EAdi, promoting phasic EAdi, and preventing excessive hemodynamic side effects.

The NAVA group had higher $PaCO_2$ levels than the VC groups. Previous studies have suggested that acute, hypercapnic respiratory acidosis may attenuate VILI and increase cardiac output [34–36]. Of note, our protocol did not specifically control $PaCO_2$ in NAVA animals; $PaCO_2$ levels resulted from the effect of the animals' respiratory drive on the delivered Vt and minute ventilation. It was not the goal of the present study to ascertain which component (i.e., Vt, respiratory rate including their variability, and $PaCO_2$) led to a decrease in VILI. Whether a lung protective VC strategy with even lower Vt and/or permissive hypercapnia, or whether different sedation levels in NAVA animals would have affected our measures of VILI remains speculative and requires further investigation.

Although there were few statistically significant differences between NAVA and VC6-ml/kg with regards to variables indicating VILI, the group's average or median values as well as the variability (as evidenced by the group's SD or the inter-quartile range) of most of these variables was consistently lower with NAVA compared to VC6-ml/kg. This is in accord with our hypothesis that NAVA tailors the ventilatory pattern to the specific physiology/biology of the subject.

The higher concentrations of biomarkers involved in coagulation (e.g., TF and PAI-1) in BAL fluid of VC15-ml/kg compared to NAVA and VC6-ml/kg, as well as increased plasma PAI-1 levels early after induction of ALI are compatible with previous studies [37, 38]. Nevertheless, the significance of these findings is unclear. We were unable to detect histological evidence of increased intravascular coagulation in any non-pulmonary organ.

Our data support previous studies with respect to the development of biotrauma [5, 6, 10]. There was a profound increase in plasma IL-8 levels as well as in tissue IL-8 concentration in spleen, kidney, and heart, but not in the liver and small intestines. Whether spill-over from the lungs and/or local production accounts for the IL-8 concentrations in tissue of non-pulmonary organs remains unclear. Although IL-8 concentrations may indicate the extent of a pro-inflammatory response in the various compartments and organs studied, a causal relationship between VILI, plasma, or local IL-8 concentrations, and non-pulmonary organ injury cannot be determined from our data.

In our model, kidney function was impaired with VC15-ml/kg and, consistent with the lowest tissue IL-8 levels, was best preserved with NAVA. How an injurious ventilation strategy ultimately translates into dysfunction of non-pulmonary organs is not fully understood. Kidneys are very susceptible to blood flow restriction. Even small decreases in renal blood flow as well as blood flow redistribution within the kidneys can affect kidney function [39, 40]. In the present study, we suggest that combined effects of impaired perfusion and oxygen delivery to the kidneys along with upregulation of a systemic and local inflammatory response accounted for the more pronounced reduction in kidney function in the injuriously ventilated animals.

Our results are different from those we observed in a previous study in which an injurious ventilatory strategy led to epithelial cell apoptosis in non-pulmonary organs such as the kidney [6]. We are unsure of the exact reason(s) for the difference in results, but it may be related to differences in length of the experiments and/or severity of injury in the two studies. In the study by Imai et al. the animals were ventilated for 8 h prior to sacrifice, compared to 5.5 h or less in the present study. In addition, the PaO_2/FiO_2 ratio in the high Vt group was less than 100 in the Imai study and 100–200 in the present study. Finally, BAL levels of IL-8 in the study by Imai et al. were about 40 times greater than in the present study. Of note, the assay used does not definitively allow discrimination between apoptotic and necrotic cells.

Conclusions

We present evidence suggesting that allowing animals with lung injury to choose their respiratory pattern is at least as effective in preventing various manifestations of VILI as conventional, volume-controlled ventilation using a Vt of 6-ml/kg in an experimental model of early ARDS. Both strategies similarly prevent VILI, attenuate excessive systemic as well as extra-pulmonary organ

inflammation and injury, and preserve cardiac and kidney function.

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