Flow-controlled expiration: a novel ventilation mode to attenuate experimental porcine lung injury

U. Goebel, J. Haberstroh, K. Foerster, C. Dassow, H.-J. Priebe, J. Guttmann and S. Schumann*

1 Division for Experimental Anaesthesiology, Department of Anaesthesiology; 2 Experimental Surgery, CEMT-FR and 3 Department of Cardiovascular Surgery, University Medical Centre Freiburg, Freiburg, Germany

* Corresponding author. E-mail: stefan.schumann@uniklinik-freiburg.de

Editor’s key points

- The effects of flow-controlled expiration (FLEX) were studied in a porcine model of lung injury.
- Addition of FLEX and volume-controlled ventilation improved lung mechanics and function, and reduced lung injury.
- Further studies are required to determine whether FLEX might improve lung-protective ventilation in humans.

Background. Whereas the effects of various inspiratory ventilatory modifications in lung injury have extensively been studied, those of expiratory ventilatory modifications are less well known. We hypothesized that the newly developed flow-controlled expiration (FLEX) mode provides a means of attenuating experimental lung injury.

Methods. Experimental acute respiratory distress syndrome was induced by i.v. injection of oleic acid in 15 anaesthetized and mechanically ventilated pigs. After established lung injury (P_{aO_2}/F_{IO_2} ratio, 27 kPa), animals were randomized to either a control group receiving volume-controlled ventilation (VCV) or a treatment group receiving VCV with additional FLEX (VCV+FLEX). At predefined times, lung mechanics and oxygenation were assessed. At the end of the experiment, the pigs were killed, and bronchoalveolar fluid and lung biopsies were taken. Expression of inflammatory cytokines was analysed in lung tissue and bronchoalveolar fluid. Lung injury score was determined on the basis of stained tissue samples.

Results. Compared with the control group (VCV; n = 8), the VCV+FLEX group (n = 7) demonstrated greater dynamic lung compliance and required less PEEP at comparable F_{IO_2} (both P < 0.05), had lower regional lung wet-to-dry ratios and lung injury scores (both P < 0.001), and showed less thickening of alveolar walls (an indicator of interstitial oedema) and de novo migration of macrophages into lung tissue (both P < 0.001).

Conclusions. The newly developed FLEX mode is able to attenuate experimental lung injury. FLEX could provide a novel means of lung-protective ventilation.

Keywords: acute respiratory distress syndrome; oleic acid; positive pressure ventilation; pulmonary oedema

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Positive pressure ventilation consists of active insufflation of the lungs followed by passive exhalation as a result of elastic recoil forces of the respiratory system. Where the effects of various modifications of the inspiratory phase (e.g. by varying end-inspiratory volume, peak inspiratory pressure, and flow) have been investigated, with the exception of PEEP, modifications of the expiratory phase have received little attention. Consequently, in routine mechanical ventilation, approximately half of the respiratory cycle (i.e. the expiratory phase) is not utilized for active ventilatory management.

We studied the effects of modification of the expiratory phase of mechanical ventilation by applying a newly developed mode of ventilation, flow-controlled expiration (FLEX). FLEX slows the expiratory peak flow rate and maintains decreased flow throughout expiration, thereby prolonging the non-zero flow phase (and, in turn, total expiratory flow time) and increasing mean airway pressure at otherwise unchanged ventilatory settings. This is expected to reduce airway collapse and oedema formation, especially in injured lungs. We hypothesized that FLEX would attenuate experimental lung injury.

Methods

The study was approved by the Animal Welfare Committees of the University of Freiburg, Germany (Registration No: G-09/17), and was carried out in accordance with the German law for animal protection and the animal care guidelines of the European Community (86/609/EC).

Surgical preparation

Sixteen healthy German Landrace Hybrid pigs of either sex (body weight 62.5 (5.0) kg [mean (sd)]) were starved for 8 h and premedicated with i.m. 0.5 mg kg^{-1} midazolam (Dormicum®, Roche,
Flow-controlled expiration

Grenzach-Wyhlen, Germany) and 20 mg kg⁻¹ ketamine hydrochloride (Ketamin®, 10%, Intervet, Unterscheiβheim, Germany). Anaesthesia was induced with i.v. 2–4 mg kg⁻¹ propofol (Propofol®, 1%, Fresenius Kabi, Bad Homburg, Germany) and maintained by infusions of 1–2 mg kg⁻¹ h⁻¹ midazolam, 4–6 mg kg⁻¹ h⁻¹ ketamine hydrochloride, and 10 μg kg⁻¹ h⁻¹ fentanyl citrate (Fentanyl Janssen®, Janssen-Cilag, Neuss, Germany). Muscle relaxation was maintained by i.v. 0.5 mg kg⁻¹ h⁻¹ vecuronium (Vecuronium-Inresa®, Inresa, Freiburg, Germany). After tracheal intubation, the lungs were ventilated (Evita 4, Dräger Medical, Lübeck, Germany) in the volume-controlled mode at a respiratory rate of 15 bpm, a tidal volume of 7–8 ml kg⁻¹ F, and PEEP of 2 cm H₂O. Inspired oxygen fraction (FiO₂) was increased first to 0.8, followed by stepwise increases of PEEP to a maximum of 15 cm H₂O.

Experimental protocol

After established lung injury, lungs were recruited by applying PEEP of 20 cm H₂O for 15 s by end-inspiratory hold. Using a computer-generated randomization sequence, animals were allocated to either the control group receiving volume-controlled ventilation only (VCV, n=8) or to the treatment group receiving VCV plus flow-controlled expiration (VCV+ FLEX; n=8). The observation period lasted 6 h. At the end of the study, sternotomy was performed for right lung lobectomy. The animals were then killed by intracardiac potassium chloride. Bronchoalveolar fluid was collected post-mortem.

Respiratory system mechanics

Airway pressure and flow were read off the ventilator at a sampling rate of 125 Hz. Dynamic compliance was calculated by multiple regression analysis of pressure, volume, and flow curves.

Pulmonary markers of inflammatory response

Pro-inflammatory markers [interleukin (IL)-1β, IL-6, IL-8, tumour necrosis factor (TNF)-α] were measured in serum, bronchoalveolar fluid, and lung tissue using ELISA kits (DuoSet, R&D Systems, Wiesbaden, Germany) according to the manufacturer’s instructions. Protein content was determined by BCA assay (ThermoScientific, Rockford, IL, USA). IL-1β, IL-6, and IL-8 mRNA concentrations were determined in lung tissue. One microgram of RNA was transcribed to cDNA using a reverse transcription kit (iScript, Bio Rad Laboratories, Munich, Germany). Quantitative real-time reverse transcriptase–polymerase chain reaction (qRT–PCR) was performed with a mastermix (ABSolute SYBR Green, ThermoScientific) monitored with an iCycler (Bio Rad Laboratories). Data were normalized to β-actin.

Lung histopathology and lung injury score

Immediately before killing the animals, four lung biopsies were taken from the ventral and dorsal portions of the apical and basal lobes. Samples were either fixed in 4% formalin for histopathology, snap frozen, and stored at −80°C for molecular analysis, or weighed for determination of the wet/dry ratio. Slices of 4 μm thickness were obtained by microtome and stained with haematoxylin/eosin and an antibody [mouse monoclonal (MAC-387) against macrophage-expressed calprotectin; #ab22506, Cambridge, UK] for microscopic examination. This included measurement of alveolar wall thickness and count of de novo migrated macrophages in 10 independent fields of vision in each sample. Two independent outside examiners experienced in lung histopathological assessment and blinded to group assignment analysed the lung tissue sections according to the scoring system of the American Thoracic Society.
Indicators of organ function
Cardiac index (CI), systemic vascular resistance index (SVRI), and pulmonary vascular resistance index (PVRI) were calculated by standard formulae. CO values were measured in triplicate using ice-cold normal saline. Various indicators of organ function (e.g. creatine kinase, NT-pro-BNP, and cystatin C) were analysed in the venous blood. Haemodynamic, haematological, blood chemistry, blood gas, and ventilatory variables were determined immediately before injection of oleic acid (pre-lung injury), after established lung injury (0 h), and then 2 hourly until the end of the 6 h observation period.

Quantitative and statistical analysis
An *a priori* performed power calculation showed that a sample size of *n*=14 would be required to detect a change in respiratory system compliance >15% at *β*=0.2. Respiratory data were analysed by a computerized statistical program (GraphPad Prism 5, GraphPad Software, San Diego, CA, USA). As we did not find statistically relevant changes in the 2 hourly determined *Pao₂/Fio₂* ratios, lung mechanics, and blood gases throughout the 6 h observation period after induction of lung injury, we treated the 6 h observation period as one phase. Serial measurements were averaged by the method of summary measures. These summary data were treated as raw data and analysed by the Mann–Whitney U-test for between-group comparison. Serum cytokines were analysed by repeated-measures analysis of variance (ANOVA). If not indicated otherwise, values are expressed as mean (SD). A *P*-value of <0.05 is considered statistically significant.

Results
All animals survived throughout the observation period. In one animal of the VCV+FLEX group, we were unable to achieve lung injury; data from this animal were excluded from analysis. Before induction of lung injury, the *Pao₂/Fio₂* ratio was comparable between the groups [VCV, 53.9 (5.6) kPa; VCV+FLEX: 54.9 (3.9) kPa]. Induction of lung injury caused a sustained decrease in *Pao₂/Fio₂* ratio by ~80% in both groups [VCV, 13.9 (5.1) kPa; VCV+FLEX, 14.5 (5.2) kPa].

Fig 1 Representative diagrams of flow (A and B), volume (C and D), and airway pressure curves (E and F) in one animal each during ventilation with VCV (A, C, and E) and VCV+flow-controlled expiration (VCV+FLEX) (B, D, and F). Solid vertical lines indicate end-inspiration. Inspiratory curves were comparable during both conditions. During expiration, decreases in peak flow (A), volume (C), and airway pressure (E) were slower during ventilation with VCV+FLEX compared with VCV.
VCV+FLEX: 14.9 (8.5) kPa; each P<0.001. Introduction of FLEX caused characteristic changes in expiratory flow, pressure, and volume curves (Fig. 1). Compared with VCV, VCV+FLEX was associated with slower decreases in flow (Fig. 1a), volume (Fig. 1b), and airway pressure (Fig. 1c) during expiration. Tidal volume [VCV, 6.8 (0.5) ml kg⁻¹ BW; VCV+FLEX, 6.8 (0.7) ml kg⁻¹ BW; P=0.89] and mean airway pressure [VCV, 20 (2) cm H₂O; VCV+FLEX, 19 (3) cm H₂O; P=0.68] were comparable between the groups.

Compared with VCV, expiratory peak flow [VCV, 1109 (88) ml s⁻¹; VCV+FLEX, 331 (33) ml s⁻¹; P=0.0015] and plateau pressures [VCV, 35 (3) cm H₂O; VCV+FLEX, 30 (6) cm H₂O; P=0.024] were lower during VCV+FLEX. FLEX allowed maintaining comparable PaO₂ [VCV, 11.2 (3.3) kPa; VCV+FLEX, 10.4 (1.7) kPa; P=0.121] and FIO₂ (Fig. 2) at ~20% lower PEEP [VCV, 11.5 (1.8) cm H₂O; VCV+FLEX, 9.3 (1.6) cm H₂O; P=0.013]. This was accompanied by a decrease in PCO₂ [VCV, 8.3 (0.9) kPa; VCV+FLEX: 7.2 (0.5) kPa; P=0.0289] and an increase in dynamic lung compliance in the FLEX-treated animals [VCV, 18.4 (1.7) ml cm H₂O⁻¹; VCV+FLEX: 22.4 (3.8) ml cm H₂O⁻¹; P=0.017]. Flow at end-expiration was similar in both groups [VCV, ~6 (16) ml s⁻¹; VCV+FLEX, 0 (1) ml s⁻¹].

Wet-to-dry ratios of lung tissue from both ventral and dorsal segments were lower in the VCV+FLEX group than in the VCV group (P<0.001; Fig. 3). Within lung segments, differences in wet-to-dry ratios between the groups tended to be more pronounced in the ventral (~60%) than in the dorsal segments (~35%). Lung injury scores were lower in the VCV+FLEX group than in the VCV group (P<0.001; Fig. 3). FLEX was associated with lesser alveolar wall thickness (P<0.001; Fig. 4A and B) and lower macrophage count (P<0.001; Fig. 5A and B).

Pulmonary mRNA and cytokine expression of IL-1β, IL-6, IL-8, and TNF-α did not differ significantly between the groups and lung segments (Table 1). Haematological variables and serum concentrations of various indicators of organ function remained comparable in both groups throughout the observation period (Tables 2 and 3).
the concept of slowing expiration is similar to that of FLEX, the reason for the lack of proven benefit of this technique might be that constant expiratory resistance increases the risk of dynamic hyperinflation (auto-PEEP/intrinsic PEEP) by uniformly increasing the expiratory time constant. This can lead to incomplete expiration if not compensated for by prolonged expiration time. In contrast, the FLEX mode actively modifies the pattern of expiration. Its main characteristic is reduced expiratory peak flow rate during the early phase of expiration. This results in a longer duration of elevated airway pressure during expiration which, in turn, increases expiratory gas flow during late expiration. Both attenuate end-expiratory closure of distal airways and alveolar collapse, thereby facilitating lung emptying.

The shape of the inspiratory flow curve is determined by the ventilator mode. Looking at the ventilator as either a flow source (volume-controlled ventilation) or a pressure source (pressure-controlled ventilation), FLEX can be viewed as volume-controlled expiration with constant flow rate in contrast to the conventional ventilation with pressure-controlled passive expiration. These characteristics of FLEX have the potential of being lung protective by improving airflow dynamics, lung compliance, alveolar stability, and oxygenation.

Oleic acid-induced lung injury caused a significant decrease in compliance. In general, low compliance is the predominant cause for high inspiratory peak pressure. Under such conditions, the driving pressure for passive expiration is high. If
passive expiration is viewed as unloading of a pneumatic capacitor, the process is fast if the capacitance (i.e. compliance) is low. The unloading dynamics are characterized by the expiratory time constant $t_{ex}$, which reflects the time period for exponential unloading from expiratory peak flow to 36.8% ($=1/e$) of this flow. Complete expiration requires about $3t_{ex}$. Exponential decay of expiratory peak flow within three time constants will lead to unphysiological acceleration of lung emptying. This is expected to cause considerable shear forces and airway collapse with resultant adverse effects on oxygenation and lung morphology, especially during underlying lung injury. As the injured lung is characterized by increased collapsibility, lung recruitment can be expected to have only short-lived rather than sustained beneficial effect on lung function. Slowing of expiration and shortening of the zero-flow phase during expiration should stabilize the lung by maintaining airway pressure during expiration and leaving less time for lung collapse. Interventions that favourably affect the dynamics of exponential unloading can thus be expected to be of benefit. In the presence of a pronounced oleic acid-elicited decrease in pulmonary compliance, FLEX reduced the high expiratory peak flow observed during conventional VCV by approximately two-thirds, thereby eliminating $t_{ex}$ as the main determinant of expiratory airflow dynamics. These findings and

![Figure 5](image-url)

Fig 5 (A) Representative lung tissue staining for alveolar macrophage count in ventral and dorsal lung areas. (B) Summary data of alveolar macrophage counts were derived from counts in 10 randomly chosen fields of vision in each of eight lung biopsies in ventral and dorsal areas. Data are presented as box plots, displaying median, 25–75 percentiles, and full range. VCV, volume-controlled ventilation; FLEX, flow-controlled expiration. ***$p<0.001$. 

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VCV VCV+FLEX

Ventricular Dorsal

<table>
<thead>
<tr>
<th>Macrophone count (per field of vision)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VCV</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>50</td>
</tr>
<tr>
<td>100</td>
</tr>
</tbody>
</table>

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Dorsal

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Dorsal

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Dorsal

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Dorsal
data derived in a mathematical model emphasize the importance of time-dependent factors during mechanical ventilation.

Benefits similar to those observed in our study were previously demonstrated for reduced inspiratory peak flow rates in rabbits. The adverse pulmonary effects of mechanical ventilation with high tidal volumes were ameliorated if peak inspiratory flow was reduced. The associated reduced parenchymal shear forces might have been caused by a more homogeneous distribution of air during reduced inspiratory flow. This suggests that limiting peak airway flows during mechanical ventilation might be beneficial for the lungs.

It could be argued that the beneficial effects observed during FLEX were secondary to an increase in mean airway pressure. However, as ventilation with FLEX allowed lowering of PEEP in the VCV+FLEX group, plateau pressure was lower than in the VCV group resulting in comparable mean airway pressures in both groups at comparable tidal volumes. Thus, the beneficial effects of FLEX cannot be attributed to higher mean airway pressures. As cessation of end-expiratory flow rates indicated complete expiration in both groups, neither can intrinsic PEEP have contributed to the findings. Rather, FLEX likely improved lung performance by delaying alveolar closure during expiration, resulting in maintained alveolar pressure and facilitating gas exchange until the late phase of expiration. Improved oxygenation and lower $P_{CO_2}$ in the FLEX group are suggestive evidence of such a mechanism.

Improved ventilatory characteristics during FLEX were accompanied by morphological improvements. Lesser alveolar wall thickening and lower alveolar macrophage count in the VCV+FLEX group than in the VCV group are consistent with attenuation of alveolar and interstitial oedema formation. This might have been related to increased intrapulmonary pressure caused by the FLEX-induced retarded expiration, resulting in a lower transalveolar filtration pressure through lower MPAP and PCWP on the vascular (up-stream pressure) side, and a longer duration of elevated airway pressure above PEEP level during expiration on the airway (down-stream pressure) side.

Airway inflammation is a concomitant phenomenon of both experimental and human lung injury, and is possibly involved in the pathogenesis of airway remodelling in lung injury. FLEX-associated improved airflow dynamics and respiratory system compliance, and the likely resultant reduced mechanical stress on the small airways, might further reduce the stimuli for parenchymal remodelling. Interestingly, functional and morphological improvements were not accompanied by changes in pulmonary mRNA and cytokine expression. This is in accordance with previous studies, suggesting that lack of agreement between morphological and molecular effects might be typical of oleic acid-induced lung injury.

### Critique of methods

I.V. injection of oleic acid is an established method of inducing stable experimental lung injury. It is characterized by elevated airway pressure, reduced lung compliance, alveolar oedema, impaired gas exchange and pulmonary hypertension. I.V.
Flow-controlled expiration

Table 3  Serum concentrations of variables reflecting various organ functions. VCV, volume-controlled ventilation; VCV + FLEX, VCV plus flow-controlled expiration; AST, aspartate aminotransferase; ALT, alanine aminotransferase; AP, alkaline phosphatase; CK, creatine kinase; CK-MB, creatine kinase muscle and brain subunit; LDH, lactate dehydrogenase; NT-proBNP, N-terminal pro-brain natriuretic peptide. Values are given as mean (SD). No significant differences between the groups were identified for any variable by repeated-measures ANOVA. 

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment group</th>
<th>Time – 1 h</th>
<th>Time 0 h</th>
<th>Time 2 h</th>
<th>Time 4 h</th>
<th>Time 6 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leucocytes (× 1000 µl$^{-1}$)</td>
<td>VCV</td>
<td>3.5 (0.7)</td>
<td>10.2 (3.8)</td>
<td>7.2 (4.5)</td>
<td>7.9 (3.9)</td>
<td>9.6 (4.3)</td>
</tr>
<tr>
<td></td>
<td>VCV + FLEX</td>
<td>3.8 (1.0)</td>
<td>11.4 (4.3)</td>
<td>7.9 (3.7)</td>
<td>8.3 (3.5)</td>
<td>8.3 (4.1)</td>
</tr>
<tr>
<td>Platelets (10$^6$ µl$^{-1}$)</td>
<td>VCV</td>
<td>124 (25)</td>
<td>168 (57)</td>
<td>213 (63)</td>
<td>221 (78)</td>
<td>199 (93)</td>
</tr>
<tr>
<td></td>
<td>VCV + FLEX</td>
<td>117 (32)</td>
<td>132 (69)</td>
<td>197 (72)</td>
<td>241 (51)</td>
<td>218 (76)</td>
</tr>
<tr>
<td>Creatinine (mg dl$^{-1}$)</td>
<td>VCV</td>
<td>0.87 (0.11)</td>
<td>0.77 (0.13)</td>
<td>0.77 (0.16)</td>
<td>0.76 (0.14)</td>
<td>0.78 (0.13)</td>
</tr>
<tr>
<td></td>
<td>VCV + FLEX</td>
<td>0.83 (0.15)</td>
<td>0.72 (0.15)</td>
<td>0.81 (0.21)</td>
<td>0.78 (0.12)</td>
<td>0.81 (0.19)</td>
</tr>
<tr>
<td>Uric acid (mg dl$^{-1}$)</td>
<td>VCV</td>
<td>12 (5)</td>
<td>14 (5)</td>
<td>15 (3)</td>
<td>12 (5)</td>
<td>16 (4)</td>
</tr>
<tr>
<td></td>
<td>VCV + FLEX</td>
<td>14 (3)</td>
<td>13 (7)</td>
<td>13 (6)</td>
<td>13 (5)</td>
<td>13 (5)</td>
</tr>
<tr>
<td>Cystatin C (mg litre$^{-1}$)</td>
<td>VCV</td>
<td>0.7 (0.1)</td>
<td>0.73 (0.1)</td>
<td>0.81 (0.14)</td>
<td>0.75 (0.1)</td>
<td>0.77 (0.1)</td>
</tr>
<tr>
<td></td>
<td>VCV + FLEX</td>
<td>0.7 (0.1)</td>
<td>0.79 (0.2)</td>
<td>0.76 (0.2)</td>
<td>0.77 (0.14)</td>
<td>0.76 (0.12)</td>
</tr>
<tr>
<td>AST (U litre$^{-1}$)</td>
<td>VCV</td>
<td>27 (4)</td>
<td>27 (4)</td>
<td>38 (8)</td>
<td>41 (13)</td>
<td>35 (12)</td>
</tr>
<tr>
<td></td>
<td>VCV + FLEX</td>
<td>27 (6)</td>
<td>26 (5)</td>
<td>35 (12)</td>
<td>38 (12)</td>
<td>34 (8)</td>
</tr>
<tr>
<td>ALT (U litre$^{-1}$)</td>
<td>VCV</td>
<td>40 (2)</td>
<td>41 (3)</td>
<td>44 (6)</td>
<td>54 (12)</td>
<td>47 (13)</td>
</tr>
<tr>
<td></td>
<td>VCV + FLEX</td>
<td>38 (2)</td>
<td>40 (2)</td>
<td>41 (5)</td>
<td>48 (13)</td>
<td>45 (7)</td>
</tr>
<tr>
<td>AP (U litre$^{-1}$)</td>
<td>VCV</td>
<td>86 (13)</td>
<td>88 (16)</td>
<td>90 (13)</td>
<td>82 (10)</td>
<td>83 (14)</td>
</tr>
<tr>
<td></td>
<td>VCV + FLEX</td>
<td>73 (15)</td>
<td>81 (10)</td>
<td>83 (12)</td>
<td>81 (7)</td>
<td>79 (7)</td>
</tr>
<tr>
<td>γ-GT (unit litre$^{-1}$)</td>
<td>VCV</td>
<td>28 (13)</td>
<td>30 (12)</td>
<td>29 (10)</td>
<td>28 (9)</td>
<td>29 (12)</td>
</tr>
<tr>
<td></td>
<td>VCV + FLEX</td>
<td>32 (16)</td>
<td>33 (8)</td>
<td>31 (13)</td>
<td>30 (8)</td>
<td>28 (8)</td>
</tr>
<tr>
<td>CK (unit litre$^{-1}$)</td>
<td>VCV</td>
<td>1437 (122)</td>
<td>3341 (452)</td>
<td>3145 (298)</td>
<td>2978 (301)</td>
<td>3012 (396)</td>
</tr>
<tr>
<td></td>
<td>VCV + FLEX</td>
<td>1618 (141)</td>
<td>3093 (510)</td>
<td>3412 (534)</td>
<td>3125 (464)</td>
<td>3225 (485)</td>
</tr>
<tr>
<td>CK-MB (unit litre$^{-1}$)</td>
<td>VCV</td>
<td>250 (87)</td>
<td>306 (65)</td>
<td>341 (54)</td>
<td>278 (58)</td>
<td>257 (50)</td>
</tr>
<tr>
<td></td>
<td>VCV + FLEX</td>
<td>298 (59)</td>
<td>343 (86)</td>
<td>365 (67)</td>
<td>301 (73)</td>
<td>299 (88)</td>
</tr>
<tr>
<td>LDH (unit litre$^{-1}$)</td>
<td>VCV</td>
<td>421 (76)</td>
<td>1134 (211)</td>
<td>1285 (542)</td>
<td>1054 (356)</td>
<td>867 (564)</td>
</tr>
<tr>
<td></td>
<td>VCV + FLEX</td>
<td>402 (63)</td>
<td>1345 (368)</td>
<td>1096 (593)</td>
<td>1104 (502)</td>
<td>945 (437)</td>
</tr>
<tr>
<td>NT-proBNP (pg ml$^{-1}$)</td>
<td>VCV</td>
<td>&lt; 5</td>
<td>7 (1)</td>
<td>7 (2)</td>
<td>7 (2)</td>
<td>7 (2)</td>
</tr>
<tr>
<td></td>
<td>VCV + FLEX</td>
<td>&lt; 5</td>
<td>8 (2)</td>
<td>6 (2)</td>
<td>7 (2)</td>
<td>6 (1)</td>
</tr>
</tbody>
</table>

Table 4  Haemodynamic variables in the VCV and VCV + FLEX groups before (t = – 1 h) and during (t = 0–6 h) lung injury. VCV, volume-controlled ventilation; VCV + FLEX, VCV plus flow-controlled expiration; HR, heart rate; MAP, mean arterial pressure; CVP, central venous pressure; CI, cardiac index; MPAP, mean pulmonary artery pressure; PCWP, pulmonary capillary wedge pressure; SVRI, systemic vascular resistance index; PVRI, pulmonary vascular resistance index. Values are means (SD). During lung injury, data are averaged from 2 hourly measurements using the method of summary measures. *P < 0.05 compared with preceding VCV value.

<table>
<thead>
<tr>
<th>Variable</th>
<th>VCV Before (−1 h) lung injury</th>
<th>VCV + FLEX During (0–6 h) lung injury</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (beats min$^{-1}$)</td>
<td>90 (22)</td>
<td>93 (26)</td>
</tr>
<tr>
<td>MAP (kPa)</td>
<td>10.3 (2.4)</td>
<td>9.6 (0.9)</td>
</tr>
<tr>
<td>CVP (kPa)</td>
<td>0.8 (0.4)</td>
<td>0.7 (0.4)</td>
</tr>
<tr>
<td>CI (ml min$^{-1}$ kg$^{-1}$)</td>
<td>97 (23)</td>
<td>96 (25)</td>
</tr>
<tr>
<td>MPAP (kPa)</td>
<td>2.5 (0.4)</td>
<td>2.5 (0.5)</td>
</tr>
<tr>
<td>PCWP (kPa)</td>
<td>1.2 (0.3)</td>
<td>0.9 (0.1)</td>
</tr>
<tr>
<td>SVRI (dyn s cm$^{-1}$ kg$^{-1}$)</td>
<td>63 (25)</td>
<td>60 (19)</td>
</tr>
<tr>
<td>PVRI (dyn s cm$^{-5}$ kg$^{-1}$)</td>
<td>9 (2)</td>
<td>11 (4)</td>
</tr>
</tbody>
</table>

administration of oleic acid promotes alveolar, intestinal, or both flooding by increasing endothelial permeability and blocking active sodium transport. It might cause structural disruptions in the hydrophobic lipid bilayer core, and disturb membrane fluidity and lipid–protein interactions. Comparable PaO2/FIO2 ratios in both groups throughout the observation period are indicative of comparable and sustained lung injury.

In the presence of oleic acid-induced lung injury, addition of FLEX to VCV did not adversely affect gene expression of inflammatory mediators or their release into lung tissue, serum, and bronchoalveolar fluid, or various biochemical indicators of organ function when compared with VCV alone. Despite increased intrathoracic pressure caused by FLEX-associated retardation of exhalation, there were no significant differences between the groups in most haemodynamic variables. MPAP and PCWP were even lower in the VCV + FLEX group compared with the VCV group.

We assessed the effects of FLEX during the early phase of acute, oleic acid-induced experimental lung injury. Thus, our findings cannot automatically be translated to treatment of human lung injury. We used FLEX in combination with VCV. Although it can be applied in any controlled ventilatory mode (it merely requires adjustment of expiratory flow to avoid intrinsic PEEP), the effects of FLEX-associated increased expiratory
resistance during ventilator modes supporting spontaneous breathing remain to be investigated.

Conclusions
Compared with conventional mechanical ventilation, addition of our newly developed FLEX attenuated lung injury in a porcine model of oleic acid-induced lung injury. This novel treatment mode has the potential to improve the therapeutic effectiveness of respiratory support in human lung injury.

Authors’ contributions
All authors drafted the article and added substantial intellectual content; U.G.: experimental procedure and analysis of histological data; J.H.: experimental procedure and data analysis; K.F.: experimental procedure; C.D.: experimental assistance and molecular biological data analysis; H.-J.P.: study design and data analysis; J.G.: study design and experimental assistance; S.S.: study design, technical developments, experimental assistance, and data analysis.

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Declaration of interest
None declared.

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