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

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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 **fl**ow-controlled **ex**piration (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 ($Pa_{O_2}/F_{I_{O_2}}$ 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 $F_{I_{O_2}}$ (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.[1] Whereas the effects of various modifications of the inspiratory phase (e.g. by varying end-inspiratory volume, peak inspiratory pressure, and flow) have been investigated,[2–5] with the exception of PEEP,[6 7] 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, **fl**ow-controlled **ex**piration (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, Grenzach-Wyhlen, Germany) and 20 mg kg^{-1} ketamine hydrochloride (Ketamin® 10%, Intervet, Unterschleißheim, 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}, an *I:E* ratio of 1:1.5, and PEEP of 8 cm H_2O. Inspired oxygen fraction ($F_{I_{O_2}}$) was maintained at 0.21. Respiratory rate and *I:E* ratio were kept constant. Ringer's solution (B. Braun Melsungen AG, Melsungen, Germany) was infused at 10 mg kg^{-1} h^{-1}.

The animals were kept in the supine position. The right carotid artery was cannulated to monitor mean arterial pressure (MAP) and obtain blood samples (arterial blood sampler, Pico 50, Radiometer, Brønshøj, Denmark) for blood gas analysis and haemoximetry (Cobas B 121, Roche Diagnostics, Stuttgart, Germany). The left external jugular vein was cannulated for insertion of a pulmonary artery thermodilution catheter (7 Fr, Edwards, Irvine, CA, USA) via an introducer sheath (8.5 Fr, Arrow, Reading, PA, USA) for measurements of mean pulmonary artery pressure (MPAP), cardiac output (CO), pulmonary capillary wedge pressure (PCWP), and central venous pressure. A second introducer sheath (7 Fr Prelude, Merit Medical, UT, USA) was inserted into the right external jugular vein for administration of oleic acid. A suprapubic catheter was inserted into the bladder for urine collection.

Expiration control

We slowed the expiratory peak flow rate by inserting an expiratory mechanical resistor in the expiratory limb of the ventilator.[8] The resistor contained an aperture that could variably be occluded by a cone that was connected to a computer-controlled linear motor (PS01-23Sx80, LinMot, Spreitenbach, Switzerland) for computer-controlled positioning. Flow data were continuously sampled and analysed by a personal computer that controlled the linear motor system. Once start of inspiration was detected by the flow signal, the cone was moved to a position occluding the aperture. At the start of expiration, the cone was pulled back at constant speed, thereby gradually opening the aperture and decreasing expiratory resistance.

Induction of lung injury

Oleic acid (Oleic acid PhEur, 75096, Sigma Aldrich, Munich, Germany) was emulsified with an equal volume of 5% glucose (Glucose 5%, B. Braun Melsungen AG). Lung injury was induced by repeated i.v. boli of 1 ml of oleic acid emulsion until $Pa_{O_2}/F_{I_{O_2}}$ ratio was <27 kPa at an $F_{I_{O_2}}$ of 1.0. Subsequently, $F_{I_{O_2}}$ and PEEP were adjusted to maintain Pa_{O_2} >8 kPa in accordance with the ARDSnet recommendations.[9] First, $F_{I_{O_2}}$ was reduced to 0.8 to minimize absorption atelectasis. Subsequently, PEEP was reduced stepwise (2 cm H_2O per step) to minimize peak pressure. The response of Pa_{O_2} to these interventions was monitored by blood gas analysis. If Pa_{O_2} was >8 kPa for >30 min, $F_{I_{O_2}}$ and PEEP were further reduced. If Pa_{O_2} was <8 kPa, $F_{I_{O_2}}$ was increased first to 0.8, followed by stepwise increases of PEEP to a maximum of 15 cm H_2O.

Experimental protocol

After established lung injury, lungs were recruited by applying PEEP of 20 cm H_2O 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, München, 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.[10]