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Hepatic effects of lung-protective pressure-controlled ventilation and a combination of high-frequency oscillatory ventilation and extracorporeal lung assist in experimental lung injury

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

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Summary

Background: Ventilation with high positive end-expiratory pressure (PEEP) can lead to hepatic dysfunction. The aim of this study was to investigate the hepatic effects of strategies using high airway pressures either in pressure-controlled ventilation (PCV) or in high-frequency oscillatory ventilation (HFOV) combined with an arteriovenous extracorporeal lung assist (ECLA).

Material/Methods: Pietrain pigs underwent induction of lung injury by saline lavage. Ventilation was continued for 24 hours either as PCV with tidal volumes of 6 ml/kg and PEEP 3 cmH_2O above the lower inflection point of the pressure-volume curve or as HFOV (≥12 Hz) with a mean tracheal airway pressure 3 cmH_2O above the lower inflection point combined with arteriovenous ECLA (HFOV+ECLA). Fluids and norepinephrine stabilized the circulation. The indocyanine green plasma disappearance rate, serum bilirubin, aspartate aminotransferase, alanine aminotransferase, γ-glutamyltransferase, alkaline phosphatase, glutamate dehydrogenase, lactate dehydrogenase and creatine kinase were determined repeatedly. Finally, liver neutrophils were counted and liver cell apoptosis was assessed by terminal deoxynucleotidyl transferase nick end labeling (TUNEL).

Results: Aspartate aminotransferase increased in the PCV group about three-fold and in the HFOV+ECLA group five-fold ($p<0.001$). Correspondingly, creatine kinase increased about two-fold and four-fold, respectively ($p<0.001$). Lactate dehydrogenase was increased in the HFOV+ECLA group ($p<0.028$). The number of neutrophils infiltrating the liver tissue and the apoptotic index were low.

Conclusions: High airway pressure PCV and HFOV with ECLA in the treatment of lavage-induced lung injury in pigs did not cause liver dysfunction or damage. The detected elevation of enzymes might be of extrahepatic origin.

key words: **acute respiratory distress syndrome • positive-pressure respiration • high-frequency oscillation ventilation • arteriovenous extracorporeal lung assist • liver dysfunction • liver function tests**

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Background

Mechanical ventilation is usually necessary to achieve sufficient gas exchange in severe respiratory failure as caused by adult respiratory distress syndrome (ARDS) or acute lung injury. The aggravation of preexisting lung injury by mechanical ventilation was termed ventilator-associated lung injury [1]. Ventilator-associated lung injury is caused by alveolar overdistension due to high inspiratory pressures and tidal volumes, as well as cyclic alveolar closing and reopening by using insufficient positive end-expiratory pressures (PEEP) [2]. Therefore, lung protective ventilation comprises increased PEEP and lower tidal volumes, thus recruiting atelectatic lung areas and reducing driving and plateau pressures [3]. Lung-protective ventilation can reduce inflammatory mediators [4] and mortality [3] in patients with ARDS.

Nevertheless, limiting tidal volumes to 6 ml/kg body weight and plateau pressures to 30 cmH_2O might be insufficient in certain patients [5]. A further reduction of tidal volumes is usually limited by severe respiratory acidosis [6]. High-frequency oscillatory ventilation (HFOV) produces very small tidal volumes at a high mean airway pressure and may improve lung protection, but elimination of carbon dioxide can also be inadequate, especially with the high oscillatory frequencies recommended today [7]. An additional pumpless arteriovenous extracorporeal lung assist (ECLA) can remove carbon dioxide, while having no relevant effect on arterial oxygenation [8]. Hence, strategies enabling a greater reduction of tidal volumes in conventional [6,9] or HFOV [10,11] in combination with an arteriovenous ECLA were developed.

High PEEP ventilation prevented hypoxemia [12] and accelerated weaning from the respirator [13], but did not decrease mortality in acute lung injury and ARDS. PEEP may lower the cardiac output and increase the backpressure to liver venous outflow and liver venous resistance, therefore a reduction of liver blood flow [14] and hepatic congestion [15] can occur. High levels of PEEP used in critically ill patients promoted hepatic dysfunction [16]. Hepatic dysfunction independently increased the length of intensive care unit stay and mortality [17]. After experimental lung injury in pigs, elevations of hepatic enzymes, bilirubin and neutrophil infiltration of liver tissue were detected after 24 hours of ventilation with initial recruitment of the lung by high levels of PEEP [18]. Until now, no study has examined the hepatic effects of airway pressures set consistently above the lower inflection point (LIP) of the pressure-volume curve throughout 24 hours of ventilation.

In a previous publication by our research group, the combination of HFOV and arteriovenous ECLA showed less histopathologic signs of inflammation in the basal lung than pressure-controlled low tidal volume ventilation after experimental lung injury [19]. The PEEP and mean tracheal airway pressure were set above the LIP and ventilation was continued for 24 hours. The aim of the present paper was to demonstrate the effects of those two lung-protective ventilation strategies on liver dysfunction and damage. Those data were gathered during the same experiment as in the companion paper [19]. Liver dysfunction was measured by indocyanine green plasma clearance and bilirubin serum levels. Liver damage was measured by changes of hepatic serum enzymes, liver neutrophil infiltration and liver cell apoptosis.

Material and Methods

Animals and preparation

This study was conducted according to the German Animal Protection Law and was approved by the Laboratory Animal Care and Use Committee of the District of Unterfranken, Germany (ref. no. 21/06) on 11 July, 2006. To yield eight animals per group as in the companion paper [19], a total of 18 animals were studied because one animal per group was unsuitable for the liver analysis. Juvenile female Pietrain pigs negative for malignant hyperthermia were fasted with unlimited water access. Premedication, induction of anesthesia and neuromuscular blockade were done as described before [20]. Intravenous anesthesia was maintained with thiopental (5–10 $mg \cdot kg^{-1} \cdot h^{-1}$), fentanyl (10 $\mu g \cdot kg^{-1} \cdot h^{-1}$) and pancuronium bromide (0.1 $mg \cdot kg^{-1} \cdot h^{-1}$). Pressure-controlled ventilation (PCV) was performed (Evita XL, Dräger, Lübeck, Germany) through a cuffed 7.5 mm inner diameter tracheal tube with an additional tip lumen (Rueschelit®, Rüsch AG, Kernen, Germany). PEEP was 5 cmH_2O and the inspiratory airway pressure was adjusted for tidal volumes of 10 ml/kg body weight at a rate of 8–12 per minute. Inspiratory oxygen fraction (FiO_2) was 1.0 and inspiratory to expiratory time ratio was 1:1.

The left carotid artery was cannulated (Vygon, Ecouen, France). A central venous catheter and a pulmonary artery catheter (131F7, Edwards Lifesciences, Irvine, CA, USA) were inserted through the right internal jugular vein. The urinary bladder was drained. Lactated isotonic electrolyte solution was infused at 4 $ml \cdot kg^{-1} \cdot h^{-1}$ throughout the experiment. Cefazolin (100 mg/kg) was administered every 8 hours. The core temperature was maintained at 38°C.

Experimental protocol

Baseline measurements were done after 30 minutes of stabilization. Lung injury was induced by lavages with prewarmed 0.9% sterile saline solution (30 ml/kg), which were repeated every 10 minutes until the arterial oxygen tension (PaO_2) was less than 60 mmHg. Additional lavages were done until PaO_2 remained stable below 60 mmHg for one hour. This model of surfactant depletion has been shown to produce lung injury stable for at least 24 hours [21]. In order to add ventilator-induced lung injury [22], lavages in the present experiment were applied during continued ventilation with tidal volumes of 10 ml/kg body weight. Then, measurements were done (*Injury*) and the animals were randomized into two groups of nine animals each. An inspiratory and expiratory pressure-volume curve was generated by the PV-tool of the respirator to determine the LIP [20]. A bolus of 500 ml colloid solution (Voluven 6% HES 130/0.4, Fresenius Kabi, Bad Homburg, Germany) was infused before sustained inflation of the lungs was performed by applying 50 cmH_2O for 30 seconds.

In the PCV group, ventilation was continued with PEEP set 3 cmH_2O above the LIP and reduced tidal volumes of 6 ml/kg body weight. The respiratory rate was adjusted between 30 and 40/min to maintain the arterial carbon dioxide tension

($PaCO_2$) below 50 mmHg and pH >7.2. The rate was decreased if auto PEEP exceeded 1 cmH_2O. In the HFOV+ECLA group, 15- and 17-French cannulas were inserted into the right femoral artery and left femoral vein, respectively. A low-resistance membrane gas exchanger (iLA™, NovaLung®, Talheim, Germany) was filled with sterile isotonic saline solution and was connected to the cannulas after systemic heparinization with 400 units/kg heparin. The infusion of heparin was continued with 20 units·kg^{-1}·h^{-1}. The same doses of heparin were administrated in the PCV group to control for known and unknown effects of heparin. Sweep gas flow with 100% oxygen through the gas exchanger was set between 2 and 14 l/min to keep the $PaCO_2$ within the target range. HFOV was adjusted to achieve a mean tracheal airway pressure (Ptrachea) 3 cmH_2O above the LIP, an amplitude of 60 cmH_2O, an oscillatory frequency between 12 and 15 Hz, and an inspiratory to expiratory time ratio of 1:1 (Sensor Medics 3100 B, Yorba Linda, CA, USA). The bias flow was 30 l/min. Respiratory and hemodynamic variables were determined after sustained inflation and starting the designated respirator treatment (*Recruitment*). In both groups, the FiO_2 was set between 0.3 and 1.0 to maintain PaO_2 within 90–110 mmHg. Respirator settings were adjusted hourly according to arterial blood gases. Additional volume administration with colloid and crystalloid solution in a 1:1 ratio was adjusted to achieve a heart rate below 100/min and a mean arterial pressure (MAP) above 60 mmHg. Norepinephrine was given if volume administration at 8 ml·kg^{-1}·h^{-1} was insufficient to reach the target MAP. The animals were killed after 24 hours by an overdose of thiopental and embutramid combined with mebezoniumiodid (T61, Intervet, Unterschleissheim, Germany).

Measurements

Pressure transducers (xtrans®, pvb® Critical Care GmbH, Kirchseeon, Germany) zeroed at the mid-thorax level measured central venous pressure (CVP) and MAP. A monitor (SMU611, Hellige, Freiburg, Germany) showed pressures and electrocardiographic heart rate. The Ptrachea was measured at the tip of the tube (PM8050®, Draeger, Luebeck, Germany). Blood gases were analyzed in arterial (SaO_2, PaO_2, $PaCO_2$), mixed venous (SvO_2, PvO_2) and in blood after passing the ECLA-membrane (SeO_2, PeO_2) (ABL 505, Radiometer, Copenhagen, Denmark). Cardiac output was determined by cardiopulmonary thermodilution [20]. An ultrasonic flow probe (HT110 Flowmeter and HXL Sensor, Transonic Systems Inc.®, Ithaca, NY, USA) measured the flow within the ECLA (ECLAflow). Oxygen concentrations in arterial (caO_2), mixed venous (cvO_2) and post-ECLA (ceO_2) blood were: cO_2 [ml/l] = (SO_2×13.4× hemoglobin concentration + PO_2×0.031). Body oxygen delivery was: DO_2 [ml/min] = (cardiac output – ECLAflow) × caO_2. Body oxygen uptake was: VO_2 [ml/min] = cardiac output × (caO_2 – cvO_2) – ECLAflow × (ceO_2 – caO_2). The indocyanine green plasma disappearance rate (ICG-PDR) as a measure for the liver excretory function and perfusion was determined noninvasively after injection of 0.25 mg/kg indocyanine green into the right atrium at *Baseline*, *Injury*, 6 and 24 hours after randomization (LiMON and ICG-PULSION®, PULSION Medical Systems, Munich, Germany). Arterial blood samples were simultaneously drawn for immediate determination of serum bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyltransferase (GGT), alkaline phosphatase, lactate dehydrogenase (LDH), creatine kinase, albumin, myoglobin, troponin T, leukocyte count, hemoglobin concentration and lactate using routine laboratory methods. Glutamate dehydrogenase (GLDH) was determined at *Baseline* and 24 hours. The degree of hemolysis was determined by absorption spectrometry.

Liver neutrophils and TUNEL

Samples of the left medial liver lobe were fixed in 10% formaldehyde and embedded into paraffin. Chloracetate esterase staining of granulocytes was done as described previously [20]. An investigator blinded to the group assignment counted the neutrophil granulocytes within the liver sinusoids and extravascular in the midlobar and pericentral region in ten high-power fields (magnification ×630). The percentage of terminal deoxynucleotidyl transferase nick end labeling (TUNEL)-positive cells to total cells was determined as described before [20].

Statistical analysis

Results for continuous data are shown as the mean with standard deviation. Serial measurements were tested with two-way repeated measures analysis of variance (ANOVA). For significant results, multiple comparisons versus *Injury* and PCV were tested with the Bonferroni t-test. Results of FiO_2, GLDH, neutrophil counting and TUNEL were not normally distributed and are shown as the median with 25^{th} and 75^{th} percentiles. Intergroup and intragroup comparisons were tested with the Mann-Whitney *U* test and the Wilcoxon signed rank test or Friedman test, respectively. For significant results, multiple comparisons versus *Injury* were tested with Dunn's method. SigmaStat for Windows, version 3.5 (Systat Software Inc., Point Richmond, USA) was used. $P<0.05$ was considered to indicate a statistically significant difference.

Results

One animal of either group was excluded from the analysis: one developed critical hypoxia after lung injury, and another animal showed a liver abscess at autopsy and marked leukocytosis. The remaining 16 animals had a mean weight of 54±4.3 kg. Fourteen of these animals, 7 per group, were also used in the companion paper [19].

Respiratory variables

Lung injury was induced by 5.2±1.6 lavages. Table 1 shows respiratory variables. The lavage number, animal weight and respiratory variables at *Baseline* and *Injury* were not different between both groups. After randomization, a LIP of 20±1 was determined in the PCV group and 19±3 cmH_2O in the HFOV+ECLA group. After *Recruitment*, Ptrachea in the HFOV+ECLA group was lower than in the PCV group, but comparable with the PEEP in the PCV group (22±3 cmH_2O). The PaO_2 was higher at *Recruitment* and the FiO_2 was lower at 6 and 24 hours in the PCV group. Mean $PaCO_2$ increased in the PCV group until 24 hours, while $PaCO_2$ remained in the normal range in the HFOV+ECLA group.

Hemodynamic variables

Table 2 shows the hemodynamic variables. At *Baseline* and *Injury* there were no statistically significant differences

Table 1. Respiratory variables.

		Baseline	Injury	Recruitment	6 h	24 h
Ptrachea	PCV	11±2*	19±5	29±4*	29±3*	30±4*
(cmH_2O)	HFOV+ECLA	11±1*	20±3	22±3#	22±3#	22±3#
FiO_2	PCV	1 (1–1)	1 (1–1)	1 (1–1)	0.3 (0.3–0.3)*	0.3 (0.3–0.3)*
	HFOV+ECLA	1 (1–1)	1 (1–1)	1 (1–1)	0.4 (0.38–0.59)#	0.35 (0.34–0.51)*,#
PaO_2	PCV	565±47*	44±11	327±182*	111±13	107±12
(mmHg)	HFOV+ECLA	574±36*	46±20	115±67#	105±25	104±12
$PaCO_2$	PCV	38.6±2.3	40.2±7.1	43.1±3.1	45.7±4.3	49.1±9.8*
(mmHg)	HFOV+ECLA	38.5±3.2	39.9±6.3	32.4±7.2*,#	36.9±2.9#	38.9±1.5#

Ptrachea, mean tracheal airway pressure; FiO_2, inspiratory oxygen fraction; PaO_2, arterial oxygen tension; $PaCO_2$, arterial carbon dioxide tension; PCV, group ventilated pressure controlled with positive end-expiratory pressure set 3 cmH_2O above the lower inflection point; HFOV+ECLA, group ventilated with high frequency oscillatory ventilation with Ptrachea set 3 cmH_2O above the lower inflection point and extracorporeal lung assist. Values are shown at baseline, after lung injury, initial measurements after recruitment, 6 hours and 24 hours after randomization. Values are expressed as mean±standard deviation or as median (25–75th percentile). Significant differences ($p<0.05$): * *vs.* Injury; # *vs.* PCV.

Table 2. Hemodynamic variables.

		Baseline	Injury	Recruitment	6 h	24 h
Heart rate	PCV	70±18	67±18	63±8	86±20*	89±10*
(beats/min)	HFOV+ECLA	63±10	66±21	73±18	102±14*	100±11*
MAP	PCV	92±8	87±11	91±12	80±4*	74±7*
(mmHg)	HFOV+ECLA	79±9#	76±10#	81±11#	79±8	72±8
CVP	PCV	7±2	8±3	13±3*	13±2*	16±3*
(mmHg)	HFOV+ECLA	6±2	8±2	9±3#	10±2#	12±2*,#
Cardiac output	PCV	4.9±1.5	4.6±0.9	3.8±0.6	3±0.6*	3.6±0.8
(l/min)	HFOV+ECLA	4.9±1.3	4.3±1.3	5.2±1.1#	4.8±1.1#	4.8±0.8#
ECLAflow	PCV					
(l/min)	HFOV+ECLA			1.5±0.2	1.5±0.2	1.4±0.2
DO_2	PCV	657±221*	440±85	500±48	372±58	436±86
(ml/min)	HFOV+ECLA	640±177*	435±245	462±177	418±169	421±94
VO_2	PCV	166±33	163±17	163±43	151±30	168±29
(ml/min)	HFOV+ECLA	157±34	154±62	146±32	141±21	173±70
Norepinephrine	PCV	0±0	2±2	3±2	3±3	10±12*
(µg/min)	HFOV+ECLA	0±0	4±5	5±5	5±5	13±10*

MAP, mean arterial pressure; CVP, central venous pressure; ECLAflow, blood flow through extracorporeal lung assist; DO_2, body oxygen delivery; VO_2, body oxygen consumption; PCV, group ventilated pressure controlled with positive end-expiratory pressure set 3 cmH_2O above the lower inflection point; HFOV+ECLA, group ventilated with high frequency oscillatory ventilation with mean tracheal airway pressure 3 cmH_2O above the lower inflection point and extracorporeal lung assist. Values are shown at baseline, after lung injury, initial measurements after recruitment, 6 hours and 24 hours after randomization. Values are expressed as mean±standard deviation. Significant differences ($p<0.05$): * *vs.* Injury; # *vs.* PCV.

among groups, except for MAP. The MAP was initially higher in the PCV group, but these differences vanished during the trial. After *Recruitment*, heart rate increased in both groups. CVP was higher in the PCV group. Cardiac output was slightly lower in the PCV group, whereas it was higher in the HFOV+ECLA group according to the ECLAflow. DO_2, VO_2 and norepinephrine dose showed no differences among groups. Total volumes of crystalloid solution

Table 3. Laboratory measurements.

		Baseline	Injury	6 h	24 h
ICG-PDR	PCV	11.2±2.4	12.3±2.7	13.2±3.4	15.1±2.1*
(%/min)	HFOV+ECLA	10.8±2.4	12.7±3.4	12.8±3.1	16.1±3.3*
Bilirubin	PCV	3.8±1.5	2.4±0.9	2.8±0.9	3.2±0.6
(µmol/l)	HFOV+ECLA	3.4±2	2.8±1.6	4.1±1.3	3.8±1.2
AST	PCV	27.1±5.1	35.1±5.9	52.6±19.1	103.6±47*
(U/l)	HFOV+ECLA	27.6±9.5	32.8±7.5	50.7±12	178.7±66.5*,#
ALT	PCV	40.9±14.1	40.3±13.3	34.5±14.3*	24.8±10*
(U/l)	HFOV+ECLA	42.4±15.2	38.8±15.5	28.4±11.7*	24.6±8.5*
GGT	PCV	37.9±8.2	37.9±5.8	36.6±13.3	25.3±7.9*
(U/l)	HFOV+ECLA	32.7±5.5	38.6±4.7	30.5±3.7	24.5±4.8*
Alkaline	PCV	77±23	79±18	77±22	68±18
phosphatase (U/l)	HFOV+ECLA	65±9	67±14	61±9	63±16
LDH	PCV	541±128	487±197	516±135	496±138
(U/l)	HFOV+ECLA	490±69	484±67	450±79	648±181*,#
Creatine kinase	PCV	570±185	965±350	984±313	2067±474*
(U/l)	HFOV+ECLA	509±107	725±196	995±461	2988±1090*,#
Albumin	PCV	25±3	25±3	21±3*	15±3*
(g/l)	HFOV+ECLA	25±2	23±3	17±3*,#	14±4*
Lactate	PCV	3±1.7*	1.6±0.4	1.8±0.7	2±1.1
(mmol/l)	HFOV+ECLA	2.8±2.1	2.1±0.7	2.5±0.6	2.1±0.7

ICG-PDR, indocyanine green plasma disappearance rate; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, γ-glutamyltransferase; LDH, lactate dehydrogenase; PCV, group ventilated pressure controlled with positive end-expiratory pressure set 3 cmH_2O above the lower inflection point; HFOV+ECLA, group ventilated with high frequency oscillatory ventilation with mean tracheal airway pressure 3 cmH_2O above the lower inflection point and extracorporeal lung assist. Values are shown at baseline, after lung injury, 6 hours and 24 hours after randomization. Values are expressed as mean±standard deviation. Significant differences ($p<0.05$): * *vs.* Injury; # *vs.* PCV.

were 10750±886 ml and 10938±563 ml in the PCV and HFOV+ECLA groups, respectively. Total volumes of colloid solution were 5188±2478 ml and 4813±651 ml, respectively.

Laboratory measurements

Table 3 shows laboratory measurements. At *Baseline* and *Injury* there were no statistically significant differences among groups. After randomization the ICG-PDR slightly increased in both groups. AST increased in the PCV group about three-fold and in the HFOV+ECLA group about five-fold. Creatine kinase increased in the PCV group about two-fold and in the HFOV+ECLA group about four-fold. LDH was increased in the HFOV+ECLA group after 24 hours. There was a difference among groups for AST, creatine kinase ($p<0.001$) and LDH ($p=0.028$). Bilirubin, ALT, GGT, alkaline phosphatase and lactate did not increase or dropped to lower values, as did albumin.

GLDH did not increase, and there was no statistically significant difference between the PCV and the HFOV+ECLA group at 24 hours: 0.4 (0.4–0.5) and 0.7 (0.5–1.1) U/l. Leukocyte number (data not shown) and hemoglobin concentration did not change and were not different between both groups. Hemoglobin concentration at *Baseline* and 24 hours was 8.6±0.6 g/dl and 9.5±1.4 in the PCV group, while it was 8.4±0.5 g/dl and 8.8±2.3 in the HFOV+ECLA group. Myoglobin (detection limit 21 µg/l) and Troponin T (detection limit 0.01 µg/l) reaxhed maximum values of 52 µg/l and 0.03 µg/l, respectively. The degree of hemolysis at 24 hours tended to higher values in the HFOV+ECLA group (4.9±3.1 *vs.* 3.4±2.6 arbitrary units; $p=0.312$).

Liver neutrophils and TUNEL

Table 4 shows the results of neutrophil counting. Neutrophil numbers in the sinusoids of the midlobar liver regions were not different between both groups. The number of extravascular neutrophils was low in both groups. In the sinusoids of the pericentral regions there was a slight trend to higher neutrophil numbers in the HFOV+ECLA group. This trend was more prominent for extravascular neutrophils, but their numbers were low. The median index of apoptotic cells as determined by TUNEL labeling was low and not

Table 4. Liver neutrophils.

	PCV	HFOV+ECLA	*P*-value
Midlobar region			
Sinusoidal	9.4 (6.6–12.2)	8.1 (5.1–11.7)	0.80
Extravascular	0.6 (0.2–1.0)	0.6 (0.3–0.8)	0.65
Pericentral region			
Sinusoidal	10.9 (4.9–17.5)	17.1 (6.7–40.8)	0.28
Extravascular	1.1 (0.4–3.9)	4.6 (2.9–8.0)	0.08

PCV, group ventilated pressure controlled with positive end-expiratory pressure set 3 cmH_2O above the lower inflection point; HFOV+ECLA, group ventilated with high frequency oscillatory ventilation with mean tracheal airway pressure set 3 cmH_2O above the lower inflection point and extracorporeal lung assist. Average numbers of neutrophil granulocytes in ten high power fields (magnification ×630) found within the liver sinusoids and extravascular in the midlobar and pericentral region were used. Values are expressed as median (25–75th percentile). *P*-values: Results of intergroup comparisons.

different between the PCV and the HFOV+ECLA group: 0.08% (0–0.21) and 0.14% (0.13–0.24).

Discussion

We examined the hepatic consequences of high airway pressure ventilation with PCV using protective low tidal volumes and HFOV combined with ECLA. No obvious liver dysfunction or damage was found; however, AST and creatine kinase increased. In the HFOV+ECLA group these changes were more prominent and LDH was also elevated. Neutrophil accumulation in the liver sinusoids was shown in both groups, as well as a small number of neutrophils infiltrating the liver parenchyma.

There are several possible explanations for the enzyme elevations. Since no significant levels of Troponin T and myoglobin were found, creatine kinase probably did not originate from muscle tissue. Creatine kinase serum levels were elevated after ischemic liver injury in rats [23]. This creatine kinase was the BB isoform, which was found in liver endothelial and Kupffer cells. Imai et al found that injurious ventilation with high tidal volumes elicited the elevation of AST and LDH and more apoptosis in the kidney and small intestine, but not in the liver [24]. One-lung ventilation caused elevations of serum AST, ALT and liver myeloperoxidase activity, liver cell apoptosis and microscopic signs of hepatic damage [25]. Injurious mechanical ventilation in mice increased the expression of adhesion molecules associated with recruitment of granulocytes into the liver and kidney [26]. Therefore, ventilation or the lung injury might have elicited hepatic changes in the present study. But since more specific enzymes, as ALT and GLDH, and also bilirubin were not elevated, the pattern of laboratory results did not confirm liver damage. Serum ALT and GGT were reduced, most probably as a consequence of hemodilution due to the fluid volumes infused, since albumin decreased similarly. Nevertheless, the hemoglobin concentration did not change. Neutrophil accumulation in the liver does not implicate liver damage. Neutrophils can adhere to the sinusoids for rheological reasons, but only cells that emigrate into the parenchyma can damage the tissue [27]. In fact, the number of extravascular neutrophils was very low compared to the number of neutrophils found within the sinusoids. Moreover, hepatocyte apoptosis as detected by TUNEL was sparse. In summary, clinically relevant liver damage cannot be concluded from the results.

The membrane gas exchanger might have caused some hemolysis. Absorption spectrometry showed a trend toward more hemolysis in the HFOV+ECLA group. LDH, AST and less prominently, creatine kinase, are elevated in hemolytic blood samples [28]. Therefore, hemolysis might explain the group differences in these enzymes, but not the elevation of AST and creatine kinase in both groups.

Other studies have also tested liver changes in pigs during ventilation after lavage-induced lung injury. Dembinski and colleagues published a similar 24-hour trial that, however, used relatively low airway pressures [9]. They compared ventilation with tidal volumes of 6 ml/kg and ventilation with 3 ml/kg in combination with ECLA. They reported no differences in bilirubin, AST, ALT, alkaline phosphatase, creatine kinase and histologic liver injury between the groups. However, it was not reported whether these measurements changed during the trial in both groups. In our previous investigation, prolonged recruitment with an incremental and decremental PEEP trial was associated with elevations of AST, LDH and bilirubin as soon as after 6 hours [18]. These findings were more prominent after 24 hours, but minor elevations of AST and LDH were also present in a group with 5 cmH_2O of PEEP without recruitment. The peak PEEP levels were higher than in the present study and additional fluids were only given in critical hypotension. This might have yielded the more striking changes in laboratory results. In a recent study comparing an open lung strategy with high PEEP and a strategy with low PEEP, we found elevations of AST until the end of the trial after 6 hours, but no increase of LDH or bilirubin [20]. Both studies [18,20] showed a more prominent granulocyte infiltration of the liver with higher PEEP. The present study extends the findings of our recent publication [20], because the duration of ventilation was longer, the strategy of circulatory stabilization was different, and HFOV combined with an arteriovenous ECLA was investigated.

In our present study, the Ptrachea in the HFOV+ECLA group was comparable to the PEEP in the PCV group. Thus, the mean airway pressure and CVP were lower in the HFOV+ECLA group. The liver blood flow during conventional positive pressure ventilation and high-frequency ventilation was shown to depend on the mean airway pressure, not on the mode of ventilation [29]. Cardiac output, DO_2 and VO_2 were comparable between both groups if ECLAflow was considered. Consequently, we assume that the hepatic blood flow was similar or less impaired in the HFOV+ECLA group. During expiration, the effective airway pressures were presumably comparable in the PCV and the HFOV+ECLA group. Negligible pressure swings were probably transmitted outside the respiratory system due to the high oscillation frequencies in the HFOV+ECLA group. Therefore, less or at least similar liver venous congestion was expected in this group. Thus, hemodynamic differences could not explain the differences in enzyme measurements.

The hemodynamic support with volume administration in combination with norepinephrine administration provided a better stabilization of cardiac output than in a group with volume restriction in our recent study [20]. In that study, we used a similar high PEEP ventilation strategy as in our PCV group. However, splanchnic oxygen uptake was not reduced in this study because of the high oxygen extraction [20]. Therefore, we presume that liver perfusion and oxygen supply was sufficient in the present experiment. The enzyme elevations could be caused by hepatic congestion or had a different source.

This study had certain limitations. It was performed in young pigs and not in human patients, and the lung injury was not necessarily comparable to human ARDS. Because we included no animals without lung injury, we cannot exclude that the induction of the lung injury or even the lung injury itself was in part responsible for the changes found in our study. Sampling hepatovenous blood would have allowed determining whether the elevated enzymes originated from the liver. The effects of the HFOV cannot be separated from the effects of the ECLA. Therefore, including an ECLA to the PCV group would enhance comparability between both strategies.

Conclusions

Lung protective ventilation above the lower inflection point of the pressure-volume curve with PCV or HFOV+ECLA for 24 hours in pigs with lavage-induced lung injury did not cause obvious liver dysfunction or damage. A prerequisite might be the applied strategy of fluid administration and catecholamine therapy resulting in a nearly sustained cardiac output. The detected elevation of AST, LDH and creatine kinase might be of extrahepatic origin. This study cannot exclude the possibility that hepatic effects may be present in animals or humans receiving insufficient circulatory stabilization or suffering from preexisting impairment of liver function or perfusion.

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