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COMPLEMENT ACTIVATION CONTRIBUTES TO VENTILATOR-INDUCED LUNG INJURY IN RATS

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The complement system contributes to ventilator induced lung injury (VILI). We hypothesized that pretreatment with the C1 esterase inhibitor (C1INH) Berinert® constrains complement activation consecutively inducing improvements in arterial oxygenation and histological pulmonary damage. At baseline, male Sprague-Dawley rats underwent mechanical ventilation in a conventional mode (PIP 13 cm H₂O, PEEP 3 cm H₂O). In the Control group, the ventilator setting was maintained (Control, n = 15). The other animals randomly received intravenous pretreatment with either 100 units/kg of the C1-INH Berinert® (VILI-C1INH group, n = 15) or 1 ml saline solution (VILI-C group, n = 15). VILI was induced by invasive ventilation (PIP 35 cm H₂O, PEEP 0 cm H₂O). After two hours of mechanical ventilation, the complement component C3a remained low in the Control group (258 ± 82 ng/ml) but increased in both VILI groups (VILI-C: 1017 ± 283 ng/ml; VILI-C1INH: 817 ± 293 ng/ml; P < 0.05 for both VILI groups versus Control). VILI caused a profound deterioration of arterial oxygen tension (VILI-C: 193 ± 167 mmHg; VILI/C1-INH: 154 ± 115 mmHg), whereas arterial oxygen tension remained unaltered in the Control group (569 ± 26 mmHg; P < 0.05 versus both VILI groups). Histological investigation revealed prominent overdistension and interstitial edema in both VILI groups compared to the Control group. C3a plasma level in the VILI group were inversely correlated with arterial oxygen tension (R = -0.734; P < 0.001). We conclude that in our animal model of VILI the complement system was activated in parallel with the impairment in arterial oxygenation and that pretreatment with 100 units/kg Berinert® did neither prevent systemic complement activation nor lung injury.

Key words: *complement system, ventilator-induced, lung injury, C1 esterase inhibitor, animal model, arterial carbon dioxide tension, arterial oxygen tension*

INTRODUCTION

Mechanical ventilation is life-saving but it includes the risk for ventilator-induced lung injury (VILI). The risk for VILI increases when non-physiologic stress and strain is applied to the lungs (1). Ventilation with high tidal volumes can lead to local overdistension and pulmonary edema (2, 3). Ventilation with too low tidal volume and low levels of PEEP can cause repetitive opening and closing of alveoli thereby increasing shear stress (4). Invasive mechanical ventilation also triggers local and systemic release of inflammatory mediators (5, 6). Among the inflammatory mediators are components of the complement system present (7-9).

The complement system is an integral part of the innate immune system and consists of more than thirty serum and cell proteins (10). Activation of the complement cascade can be triggered *via* the classical, the alternative, and the mannose-

binding lectin pathway (11-13). The three pathways converge to the formation of C3 convertase (14). The C3 convertase cleaves C3 in C3a and C3b, which contributes to the formation of C5a and C5b. C5b initiates the formation of the terminal membrane attack complex with consecutive cell lysis in a cascade-like sequence (15). The split products C3a and C5a are collectively called anaphylatoxins. Anaphylatoxins trigger inflammatory effects like increased vascular permeability, enhanced white blood cell chemotaxis and adherence to the pulmonary endothelium with the subsequent release of lysosomal enzymes and cytokines (16, 17).

Application of C1 esterase inhibitor (C1INH) is best known as treatment for hereditary angioedema. C1INH strictly regulates the classical pathway of the complement system and also reduces the activation of the lectin pathway. Thereby, C1INH offers the opportunity to examine to the contributions of both activation pathways in VILI (18-21).

So far, three investigations explored the role of the complement system in experimental models of VILI (7-9). In a study by Takahashi and colleagues, VILI caused a positive pulmonary C3 staining in mice demonstrating that VILI induces local complement activation (7). The complement component C3 is formed after the conversion of the three activation pathways leaving the initially triggering pathway unclear. Based on enhanced CH50 plasma level Liu and co-workers concluded that the classical pathway contributes to the complement activation in VILI (8). DeBeer and colleagues further investigated the role of the classical pathway of the complement system using a clinically relevant double hit model in rats. After 40 hours of pulmonary *Streptococcus pneumoniae* infection, rats underwent no ventilation, ventilation with low, or ventilation with high tidal volumes, hence VILI (9). In the VILI group they found an increased bronchoalveolar/serum ratio of C4b/c level suggesting an activation of the classical and/or lectin pathway of the complement system (9). However, the role of complement system in a rat model of VILI might be interpreted with caution since *Streptococcus pneumoniae* itself activates the classical and alternative pathway of the complement system (22). Further, in the study of deBeer and colleagues VILI was only mild with no significant associated change in lung weight and arterial oxygenation (9).

We also aimed at investigating the role of the classical and/or lectin pathway of the complement system in VILI. In our study, the rats received a single intravenous bolus of 100 mg/kg Berinert® (VILI-C1INH) or saline (VILI-C) before VILI was induced. An additional group of rats received conventional mechanical ventilation and served as a further control (Control). Mechanical ventilation was maintained for two hours. Arterial blood gases and serum level of the complement component C3a were measured to estimate the impact of the ventilator settings on pulmonary gas exchange and complement activity, respectively. At the end of the experiments, the lungs were harvested for the measurement of wet lung weight and histological examination.

MATERIALS AND METHODS

All animal experiments were approved by the Animal Committee of the Erasmus University Medical Center Rotterdam. Care and handling of the animals were in accordance with the European Community guidelines. The study was performed in 45 male Sprague-Dawley rats with a body weight of 300 ± 30 g at the Department of Anesthesiology, Erasmus University Medical Center Rotterdam, The Netherlands.

Preparation of the complement inhibitor C1 esterase inhibitor

A pasteurized human C1-INH (Berinert®, CSL Behring, Marburg, Germany) was suspended with normal saline solution to obtain a concentration of 30 U/ml. A dosage of 100 units/kg was administered as a single bolus.

Animal preparation

After inhalative induction of general anesthesia with nitrous oxide, oxygen and isoflurane (66/33/1-2%) the rats received an intraperitoneal injection of pentobarbital sodium (Nembutal®, Algin BV, Maassluis, The Netherlands; 60 mg/kg). A polyethylene catheter (SIMS Portex, UK: 0.8 mm outer diameter) was inserted into the carotid artery for arterial blood sampling. The rats were tracheotomized and muscle relaxation was achieved by intramuscular administration of pancuronium bromide (Pavulon®, Organon, Boxtel, the Netherlands; 2 mg/kg)

before connecting to the ventilator (Servo 300, Siemens-Elema AB, Solna, Sweden). Up to six animals were mechanically ventilated in parallel in a pressure controlled mode. At an inspiratory oxygen fraction of 1.0 the ventilator settings were as follows: peak inspiratory pressure (PIP) of 13 cm H₂O, positive end-expiratory pressure (PEEP) of 3 cm H₂O, frequency of breaths 30/minutes, and an inspiratory/expiratory ratio of 1:2. Anesthesia was continued by hourly intraperitoneal injections of pentobarbital (60 mg/kg). Muscle relaxation was perpetuated with intramuscular injections of pancuronium bromide (2 mg/kg) every hour. The body temperature was kept within normal range with a heating lamp. To reopen atelectatic lung areas PIP was increased to 26 cm H₂O for six breathing circles and reduced to 13 cm H₂O thereafter. Five minutes later baseline parameters were documented, and blood samples taken.

Animals were randomized to one of three groups (n = 15 each). The first group of animals was ventilated with a conventional strategy (Control). The setting of mechanical ventilation (PIP 13 cm H₂O, PEEP 3 cm H₂O) was maintained in the Control group during the whole experiment. In the VILI groups the ventilatory setting was changed as follows: PIP was increased to 35 cm H₂O and PEEP was decreased to 0 cmH₂O. Ventilatory frequency was kept stable at 30 bpm, and the dead space (4 – ml) was increased to maintain a normal arterial carbon dioxide tension. After baseline the Control group received a bolus of 1 ml saline. The second group served as a control group for ventilator induced lung injury (VILI-C). The rats received 1 ml saline before VILI was started. To investigate the impact of the C1INH on VILI the third group received a pretreatment with 100 units/kg Berinert® before the ventilatory setting was changed to VILI (VILI-C1INH).

Blood gas analyses

At baseline, one and two hours later 0.5 ml blood were taken anaerobically from the carotid artery catheter into heparinized tubes and analyzed for blood gases (ABL 505, Radiometer, Copenhagen, Denmark). The arterial blood gas analysis included arterial oxygen tension (PaO₂), arterial carbon dioxide tension (PaCO₂), arterial bicarbonate level (HCO₃⁻), and arterial pH (pH_a).

Plasma levels of the complement split product C3a

At the same three time points (baseline, one and two hours later) 1.0 ml arterial blood samples were taken to measure the plasma levels of the complement split product C3a. The samples were immediately centrifuged at 3000 rpm for 10 minutes at 4°C. The plasma was stored at -20°C for later analyses. C3a levels were determined by the commercially available C3a-enzyme-linked immunosorbent assay for rat (Dianova, Hamburg, Germany).

Morphological investigations

At the end of the protocols, the animals were sacrificed with an intravenous overdose of pentobarbital. The lungs were removed, prepared and weighted wet. Subsequently, the lungs were fixed in 4% paraformaldehyd, processed through graded alcohol and embedded in Paraplast (Monojestic Scientific, St. Louis, USA). Sections of 4 µm were stained with hematoxylin and eosin for routine histological assessment. The histological parameters included atelectasis, overdistension and necrosis. Bleeding, edema and inflammation were evaluated in the interstitial and alveolar compartment. Cell infiltration was further examined for bacteria and eosinophils. Each variable was evaluated using a 0- to 4-point scale corresponding to an injury

in 0%, 25%, 50%, 75%, or 100% of the investigated tissue, scoring 0, 1, 2, 3, and 4, respectively. The slices were evaluated by a pathologist who was blinded to the animal's group assignment.

Statistical analyses

All data are expressed as mean \pm S.D. All calculations were performed using a standard statistical program (SPSS 20.0, IBM Corporation, Armonk, New York, USA). Statistical significance was accepted at P -values < 0.05 . ANOVA was used to assess differences between the groups. Differences within a group were analyzed by the means of ANOVA for repeated measures. Significant differences of the lung injury score data were detected by the Kruskal-Wallis nonparametric test. In all cases post hoc multiple comparisons were performed using the Bonferroni test. Correlation analysis of C3a plasma level and PaO_2 values of the VILI group was performed with the Spearman rank correlation coefficient.

RESULTS

C1 esterase inhibitor did not improve pulmonary gas exchange in VILI

Induction of VILI was associated with a three-fold increase of the lung tidal volume in the VILI-C and VILI-C1INH group compared to the Control group (both VILI groups: 37 ± 3 ml/kg; Control: 12 ± 1 ml/kg; values at the end of the protocol).

Arterial blood gas tensions were measured at baseline as well as one and two hours later in the Control-, VILI-C, and VILI-C1INH group (Fig. 1, Table 1). The measured values of the arterial oxygen partial pressure (PaO_2) were identical to $\text{PaO}_2/\text{FiO}_2$ quotients since the animals were ventilated with 100% oxygen (FiO_2 1.0) throughout the protocol. At baseline PaO_2 did not differ between the groups. VILI induced a marked decrease in arterial oxygenation. After one hour, the PaO_2 decreased in the VILI-C1INH group compared to baseline ($P <$

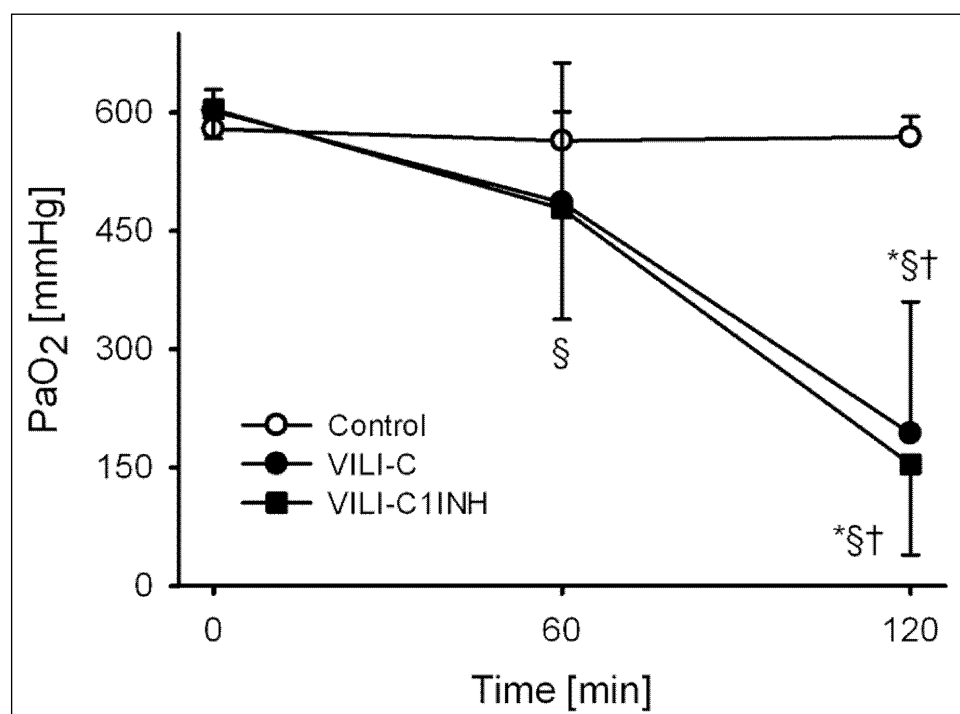


Fig. 1. Arterial oxygenation during the protocol in conventionally ventilated Controls (Control, $n = 15$) and in animals with VILI induced after baseline (0 min) and pretreated either with saline (VILI-C, $n = 15$) or the C1-esterase inhibitor (VILI-C1INH, $n = 15$). VILI resulted in a significant and sustained decrease in arterial oxygenation. * $P < 0.05$ versus Control; §: $P < 0.05$ versus baseline (0 min) of the respective group; † $P < 0.05$ versus 1 hour within the respective group.

Table 1. Arterial blood gas analyses during the protocol.

Parameter	Group	Baseline	1 hour	2 hours
PaCO_2 (mmHg)	Control	35 ± 6	37 ± 5	43 ± 11
	VILI-C	31 ± 5	38 ± 12	52 ± 23 §†
	VILI-C1INH	33 ± 7	47 ± 15 §	68 ± 17 *§†
HCO_3^- _a (mmol/l)	Control	27 ± 3	26 ± 3	27 ± 2
	VILI-C	28 ± 2	26 ± 3 §	20 ± 6 *§†
	VILI-C1INH	26 ± 3	26 ± 4	23 ± 4 *§†
pH_a	Control	7.49 ± 0.06	7.45 ± 0.06 §	7.42 ± 0.08 §
	VILI-C	7.55 ± 0.06	7.45 ± 0.10 §	7.23 ± 0.13 *§†
	VILI-C1INH	7.52 ± 0.08	7.38 ± 0.10 §	7.16 ± 0.08 *§†

PaCO_2 , arterial carbon dioxide tension; HCO_3^- _a: arterial bicarbonate level; pH_a , arterial pH. $N = 15$ in each group. *: $P < 0.05$ versus Control; § $P < 0.05$ versus baseline of the respective group; † $P < 0.05$ versus 1 hour of the respective group.

0.05). Two hours of VILI caused a further decrease of the PaO_2 values in VILI-C and VILI-C1INH groups ($P < 0.05$ versus baseline and $P < 0.05$ versus Control for both VILI groups).

There was no difference in the values of arterial partial pressure of carbon dioxide (PaCO_2) between the three groups at baseline. VILI resulted in increased PaCO_2 values. During the protocol PaCO_2 increased in the VILI-C and in the VILI-C1INH group. At 2 hours PaCO_2 values were in both VILI groups significantly different when compared to baseline and the Control group.

The arterial bicarbonate values were comparable at baseline between the three groups. In Controls the bicarbonate levels

remained stable during one and two hours of ventilation whereas the bicarbonate levels decreased in the VILI-C group after one and two hours when compared to baseline ($P < 0.05$ for both). After two of ventilation the bicarbonate values were significantly reduced in both, the VILI-C and the VILI-C1INH group when compared to values at baseline and values in Controls.

The arterial pH values in the Control group were lower after one and two hours of ventilation compared to baseline ($P < 0.05$ for both). There was a significant reduction in the pH values of the VILI-C and VILI-C1INH group at 1 and 2 hours when compared to baseline ($P < 0.05$ for both VILI groups). Comparison between the groups revealed lower pH values in the

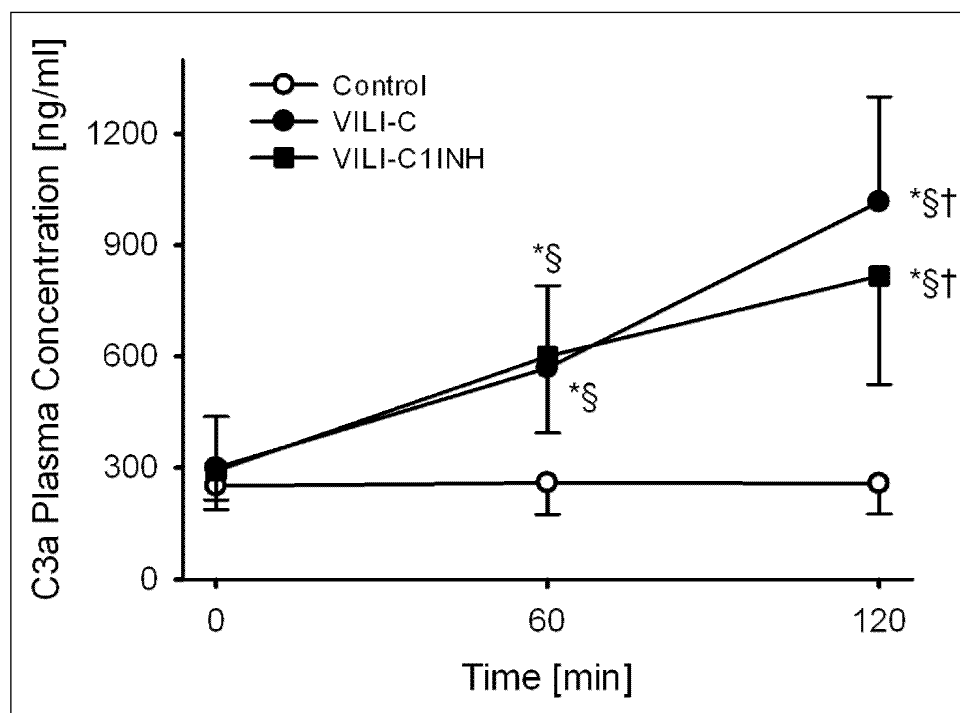


Fig. 2. Plasma levels of the complement split product C3a during the protocol of conventionally ventilated Controls (Control, $n = 15$) and in animals with VILI induced after baseline (0 min) and pretreated either with saline (VILI-C, $n = 15$) or Berinert® (VILI-C1INH). VILI caused a clear and significant increase in C3a plasma levels which was not prevented by Berinert® application. * $P < 0.05$ versus Control; § $P < 0.05$ versus baseline (0 min) within the respective group; † $P < 0.05$ versus 1 hour of the respective group.

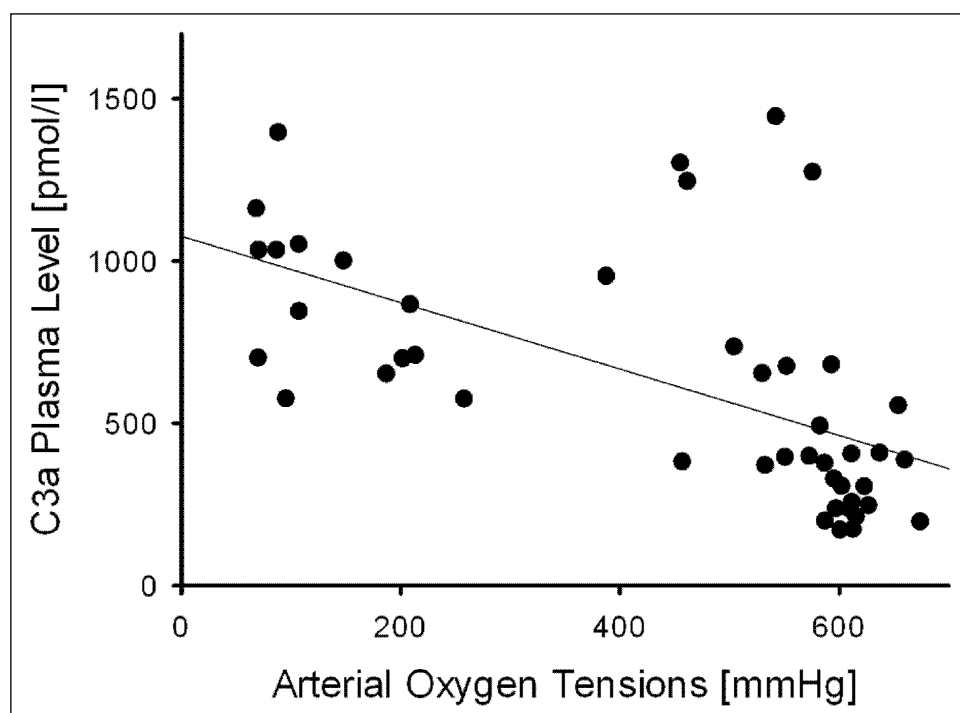


Fig. 3. The figure shows the arterial oxygenation and C3a plasma level at baseline as well as after one and two hours of VILI ($n = 15$ for each time-point). A statistically significant substantial inverse correlation between the arterial oxygen tensions and the level of systemic complement activation is present ($R = -0.734$; $P < 0.001$).

VILI-C and in the VILI-C1INH group at 2 hours when compared to the Controls ($P < 0.05$ for both VILI groups).

Release of high plasma levels of the complement split product C3a in VILI

The plasma levels of the complement split product C3a did not differ between the groups at baseline (Control: 251 ± 64 ng/ml, VILI-C: 300 ± 138 ng/ml, VILI-C1INH: 292 ± 79 ng/ml; Fig. 2). In the Control group the plasma C3a values remained at constant levels after one and two hours (260 ± 85 ng/ml and 258 ± 82 ng/ml, respectively). In contrast, the plasma C3a concentration markedly increased in the VILI-C and VILI-C1INH group after

one hour (VILI-C: 569 ± 222 ng/ml, VILI-C1INH: 600 ± 206 ; $P < 0.05$ compared to Control for both) and at two hours (VILI-C: 1017 ± 283 ng/ml, VILI-C1INH: 817 ± 293 ng/ml; $P < 0.05$ compared to Controls for both groups). Further, intra group comparisons of the C3a values revealed a significant increase from baseline within the VILI-C and the VILI-C1INH group at one and two hours ($P < 0.05$ for both VILI groups).

Plasma level of C3a correlate with arterial oxygen tensions in VILI

VILI induced high level of the complement split product C3a and decreased arterial oxygenation. The statistical analysis

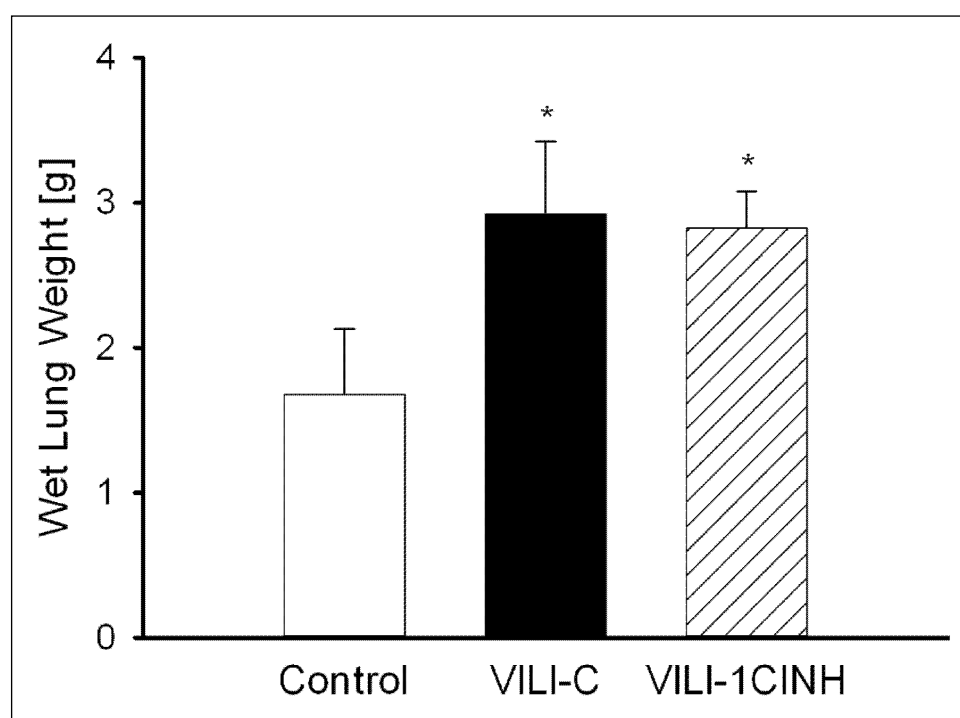


Fig. 4. Wet lung weights were measured at the end of the experiments. VILI caused a significant increase in wet lung weight after pretreatment with either saline (VILI-C, $n = 15$) or C1INH (VILI-C1INH, $n = 15$) compared to conventionally ventilated rats (Control, $n = 15$). * $P < 0.05$ versus Control.

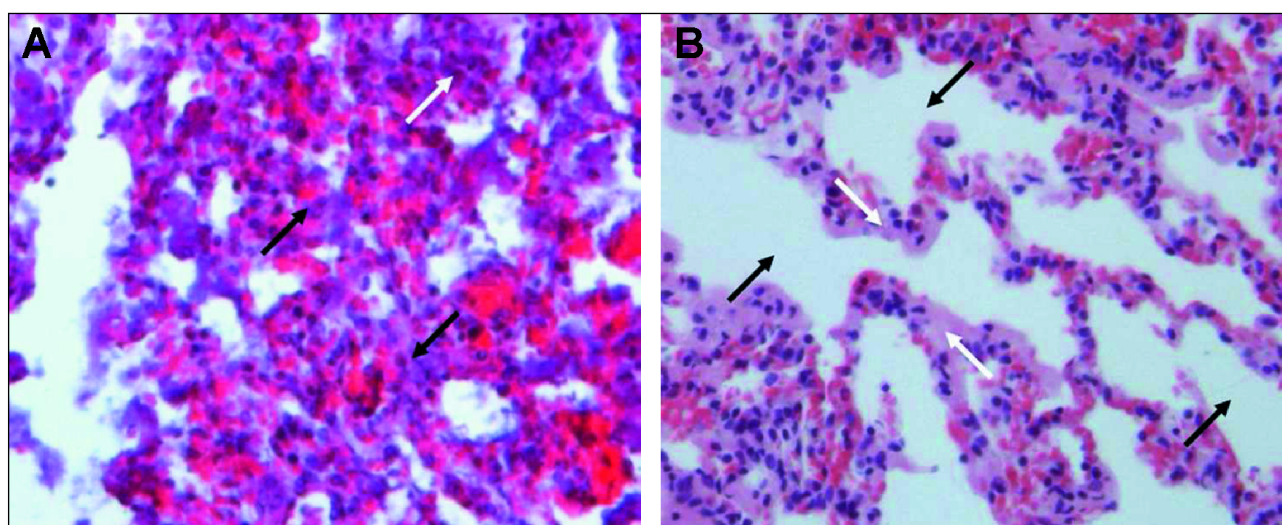


Fig. 5. Lung tissue was stained with hematoxylin & eosin and examined under light microscopy. Panel A: representative slide of the Control group with atelectasis (black arrow) and some interstitial inflammatory cells (white arrow); magnification $\times 200$. Panel B: representative slide of the VILI group with profound overdistension (black arrow) and interstitial edema (white arrow); magnification $\times 200$.

Table 2. Histological lung tissue score at the end of the protocol.

	Control	VILI-C	VILI-C1INH
Interstitial bleeding	1.4 ± 0.5	1.4 ± 0.6	1.2 ± 0.9
Alveolar bleeding	0.6 ± 0.6	0.9 ± 0.5	0.7 ± 0.5
Overdistension	0.1 ± 0.4	1.4 ± 1.1*	1.9 ± 0.6*
Atelectasis	2.5 ± 1.1	0.2 ± 0.6*	0.2 ± 0.4*
Necrosis	0	0	0
Interstitial edema	0	1.6 ± 1.0*	2.3 ± 0.5*
Alveolar edema	0	0.6 ± 0.8	0
Interstitial inflammation	2.3 ± 1.0	1.6 ± 0.7	1.9 ± 0.3
Alveolar inflammation	0.1 ± 0.4	0	0
Eosinophils	0	0	0
Bacteria	0	0	0

Histological examination of lung sections after two hours of mechanical ventilation. The rats were subjected to conventionally ventilation (Control) or ventilator-induced lung injury treated with saline (VILI-C) or treated with C1 esterase inhibitor (VILI-C1INH); n = 15 in each group. *P < 0.05 versus Control.

revealed a substantial inverse correlation between C3a plasma level and PaO₂ values (R = -0.765, P < 0.001) suggesting that enhanced complement activation is associated with a deterioration of arterial oxygenation (Fig. 3).

A single dose of the C1 esterase inhibitor did not prevent lung tissue damage in VILI

VILI caused a profound increase in wet lung weight in the VILI-C (2.92 ± 0.50g) and VILI-C1INH (2.83 ± 0.25g) group compared to Controls (1.68 ± 0.45g; P < 0.05 for both comparisons; Fig. 4).

Atelectases occurred only in Controls and were absent in VILI-C and VILI-C1INH group (P < 0.05 versus Control for both VILI groups; Fig. 5a). The most prominent features of the histological examination were interstitial edema and alveolar overdistension in the VILI-C and VILI-C1INH group compared to Controls (P < 0.05 for both parameters in VILI-C and VILI-C1INH, Fig. 5b). In all three groups interstitial bleeding and to a minor degree alveolar bleeding were present. Evaluation of inflammation showed no difference between the groups. Scattered inflammatory cells were present in the interstitium but nearly absent in the alveoli. No bacteria and eosinophils were detectable. Data of the lung tissue score are presented in Table 2.

DISCUSSION

In our study we intended to investigate the classical and / or lectin pathway of the complement system by the pre-treatment with 100 mg/kg of the inhibitor C1 esterase inhibitor Berinert® in VILI. We found that VILI caused significant systemic complement activation that was inversely correlated with arterial oxygenation. Pretreatment with a single dose of C1INH had no beneficial effects on systemic complement activation, pulmonary gas exchange or lung tissue damage.

Our study included the impact of VILI on complement activation and arterial blood gas tensions. Mechanical ventilation was scheduled for two hours to facilitate repetitive blood samples. VILI was induced by an inspiratory peak pressure 35 cm H₂O and end-expiratory pressure of 0 cm H₂O. The ventilator setting was chosen based on previous investigations showing that ventilation with a tidal volume of 40

ml/kg without positive end-expiratory pressure induces inflammatory mediator expression and impairs arterial oxygenation within two hours of ventilation (23-25).

C1 esterase inhibition blockades mainly the classical pathway and in part the lectin pathway of the complement system thereby offering the opportunity to clarify the role of these pathways in VILI-induced complement activation (26). In our rodent model of VILI, pretreatment with 100 units/kg Berinert® failed to inhibit systemic complement activation which could be related to various reasons. First, Berinert® is extracted out of human plasma. The efficiency of human C1INH might be less than the natural rat analogue. We feel, however, that the above arguments are put into perspective since human C1 esterase inhibitor has been proven to affect complement activation in serum of male Sprague-Dawley rats (27). Secondly, the single dosage might have been too small to be effective. It is short-coming of our study that we did not perform a dose-response investigation in our VILI model. Nevertheless, Berinert® in doses of 50 and 100 U/kg has been demonstrated to sufficiently inhibit activation of the classical pathway of the complement system in rat model of ischemia reperfusion injury (27). Based on this results our dose of 100 U/kg was chosen. Thirdly, the protective effects of C1INH might depend on inflammatory cell infiltration. On the one hand, in thermal injury and ischemia reperfusion injury, the successful inhibition of complement activation was associated with reduced neutrophil infiltration (27, 28). On the other hand, in the double hit model of streptococcus pneumonia infection and mandatory ventilation, VILI was associated with increased pulmonary neutrophil influx, but treatment with the C1INH failed to diminish inflammatory cell infiltration (9). Fourthly, the complement system can be activated *via* the classical, lectin and alternative pathway. C1INH inhibits the classical and constrains the lectin pathway so that activation of the alternative pathway or its amplification loops might have overwhelmed the inhibitory capacity of C1INH.

Because we cannot exclude the above listed arguments for the failure of 100 mg/kg Berinert® to inhibit systemic complement, the discussion focuses on the results of the VILI-C group. The complement system can be activated *via* the classical, lectin and alternative pathway. The studies by Liu, deBeer, and colleagues showed that VILI induces the classical pathway of the complement system (8, 9). However, the alternative pathway can be activated *via* distracted host cells (29). In our study the histological sections show overdistension as a prominent feature

in both VILI groups supporting a possible activation of alternative pathway. Furthermore, the alternative pathway functions primarily as an amplifying process resulting in increased C3b and consecutively C3a levels within minutes (30). Taken together, it appears reasonable that VILI activates both, the classical and the alternative pathway of the complement system. The inefficiency of C1INH pretreatment on complement activation and lung injury in the study by deBeer and in our study may also support the relevance of the alternative pathway since C1INH mainly inhibits the classical and in part the lectin pathway (9).

The three activation pathways converge at the level of C3. VILI causes positive pulmonary C3 demonstrating local complement activation (7). In our study VILI induced increased plasma C3a level supporting VILI-induced systemic complement activation. To explore the functional relevance of complement activation on arterial oxygenation, C3a plasma level and arterial oxygen tensions were measured after one and two hours of VILI. Statistical analysis revealed that the level of complement activation was substantially correlated with the deterioration of arterial oxygenation in our rat model of VILI.

The anaphylatoxins C3a and C5a cause increased chemotaxis of neutrophils (16, 17). In double hit model of pneumonia and mechanical ventilation, VILI was associated with enhanced complement activation and increased pulmonary neutrophil influx leaving the contribution of pneumonia- or anaphylatoxins-induced neutrophil recruitment unclear (9). In our study the histological sections did not show differences in cell infiltration between the groups suggesting that neutrophils are not mandatory to activate the complement system in VILI.

The proteolytic cascade of the complement system finally leads to the formation of the membrane attack complex C5b6789 (15). In rat pulmonary microvascular endothelial cell cultures, Liu and co-workers showed that C5b6789 contributes to increased vascular permeability and thus pulmonary edema (8). In addition, the anaphylatoxins C3a and C5a are known to increase vascular permeability (16, 17). In accordance, we found increased C3a plasma level, increased wet lung weight and profound interstitial edema in the VILI group. However, the main determinant of pulmonary edema is the pulmonary strain caused by mechanical ventilation (1, 31). In this line, overdistension was a prominent feature in the histological sections of the VILI group. The results collectively indicate that the activated complement system might contribute to the strain-induced pulmonary edema in VILI.

In summary, our study confirms that VILI causes systemic complement activation since increased C3a plasma level were paralleled by deterioration of arterial oxygenation. The histological sections of the Control and VILI groups did not reveal differences in inflammation suggesting that the complement system contributes to pulmonary edema independently of cell infiltration. The failure of the pretreatment with 100 mg/kg Berinert® to prevent systemic complement activation, deterioration of arterial oxygenation or lung tissue damage confirms the results of deBeer and colleagues (9) and might point towards the alternative pathway of the complement system. Further studies should clarify the primary activation pathway of the complement system in VILI.

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Conflict of interests: None declared.

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