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ACE mediates ventilator-induced lung injury in rats *via* angiotensin II but not bradykinin

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ABSTRACT: Ventilator-induced lung injury is characterised by inflammation and apoptosis, but the underlying mechanisms are poorly understood. The present study proposed a role for angiotensin-converting enzyme (ACE) *via* angiotensin II (Ang II) and/or bradykinin in acute lung injury. The authors assessed whether ACE and, if so, Ang II and/or bradykinin are implicated in inflammation and apoptosis by mechanical ventilation.

Rats were ventilated for 4 h with low- or high-pressure amplitudes in the absence or presence of the ACE inhibitor captopril. Nonventilated animals served as controls. ACE activity, Ang II and bradykinin levels, as well as inflammatory parameters (total protein, macrophage inflammatory protein-2 and interleukin-6) were determined. Apoptosis was assessed by the number of activated caspase-3 and TUNEL (terminal deoxynucleotidyltransferase-mediated deoxyuridine triphosphate nick-end labelling)-positive cells.

Bronchoalveolar lavage fluid ACE activity, levels of total protein, inflammatory parameters and the number of apoptotic cells were increased in the high-pressure amplitude group as compared with the control group. Blocking ACE activity by captopril attenuated inflammation and apoptosis in the latter group. Similar results were obtained by blocking Ang II receptors, but blocking bradykinin receptors did not attenuate the anti-inflammatory and anti-apoptotic effects of captopril.

The current authors conclude that inflammation and apoptosis in ventilator-induced lung injury is, at least in part, due to angiotensin-converting enzyme-mediated angiotensin II production.

KEYWORDS: Angiotensin-converting enzyme, apoptosis, captopril, inflammation, ventilator-induced lung injury

Over the last decades, mechanical ventilation has taken an indispensable place in the treatment of critically ill patients. However, mechanical ventilation can cause or aggravate lung damage, often referred to as ventilator-induced lung injury (VILI) [1, 2]. Several pathophysiological mechanisms contribute to VILI, including biotrauma, volutrauma and atelectrauma [2]. Biotrauma involves primarily biological mechanisms of lung injury, including inflammation and apoptosis, whereas the latter two are considered to be due to physical forces [2, 3].

Angiotensin-converting enzyme (ACE) probably plays a pivotal role in the pathogenesis of acute respiratory distress syndrome (ARDS), a form of acute lung injury [4, 5]. ACE is a key enzyme in the renin–angiotensin system (RAS) and the kallikrein–kinin system [6]. In the latter system, ACE degrades the bioactive nonapeptide bradykinin, whereas in the RAS system it generates angiotensin (Ang) II. Ang II is a potent vasoconstrictor and is also involved in key events of inflammation and apoptosis. It increases leukocyte adhesion and migration by inducing endothelial adhesion molecules [7, 8]. Furthermore, Ang II stimulates the production of pro-inflammatory mediators like interleukin (IL)-8 and IL-6, which are known to play a central role in the inflammatory response observed in VILI [9, 10]. In addition to its effect on inflammation, Ang II promotes Fas-induced apoptosis of alveolar epithelial cells *in vitro* [11, 12]. Bradykinin also influences inflammation and apoptosis. Bradykinin has pro-inflammatory actions, including increased vascular leakage and induction of a variety of cytokines [13]. The anti-apoptotic action of bradykinin in decreasing caspase-3 activation is well established [14]. Taken together, ACE may exert its role in the pathogenesis of lung injury *via* Ang II and/or bradykinin.

It was recently shown, in a model of acute lung injury induced by acid aspiration and sepsis, that mice deficient in ACE had reduced pulmonary

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oedema formation and leukocyte infiltration [15]. However, it is unknown whether different ventilation strategies lead to increased pulmonary ACE activity. Therefore, the current authors investigated whether pulmonary ACE activity is increased during VILI (using two different ventilation strategies) and, if so, whether the ACE inhibitor captopril could attenuate VILI-induced inflammation and apoptosis. Finally, the current authors investigated whether the effects of ACE were mediated by Ang II and/or bradykinin. Rats were used as model animals since, in contrast to mice, the physiological contributions of Ang II and bradykinin are more analogous to that documented in humans [16].

METHODS

Experimental protocol

Male Sprague-Dawley rats (292±21 g) were obtained from Harlan CPB (Zeist, the Netherlands). The study was approved by the institutional ethical committee for experiments with animals (Erasmus University Rotterdam, Rotterdam, the Netherlands). Care and handling of the animals was in accordance with the European Community guidelines. Animals either received or did not receive 500 $mg \cdot L^{-1}$ captopril in their drinking water for 5 days. At day 6, a tracheostomy was performed and the carotid artery was catheterised under inhalation anaesthesia (65% nitrous oxide/33% oxygen/2% isoflurane; Pharmachemie BV, Haarlem, the Netherlands). Inhalation anaesthesia was replaced by hourly intraperitoneal injections of pentobarbital sodium (60 $mg \cdot kg^{-1}$, Nembutal; Algin BV, Maassluis, the Netherlands). Muscle relaxation was attained with hourly intramuscular pancuronium bromide (2 $mg \cdot kg^{-1}$ Pavulon; Organon, Boxtel, the Netherlands).

Animals were connected to a servo-ventilator 300 (Siemens-Elema, Solna, Sweden) and ventilated in a pressure-controlled time-cycled mode, at a fractional inspired oxygen concentration of 1.0, a frequency of 20–30 per min to maintain normocapnia and an inspiratory/expiratory ratio of 1/2. Animals were randomly allocated to two ventilation strategies, each for 4 h, as follows. 1) Group I: without captopril (Cap-), n=9; with captopril (Cap+), n=9; peak inspiratory pressure (PIP) 16 cmH_2O (1.6 kPa); and positive end-expiratory pressure (PEEP) 5 cmH_2O (0.5 kPa; tidal volume (V_T) ~8 $mL \cdot kg^{-1}$, low pressure amplitudes). 2) Group II: Cap-, n=9; Cap+, n=9; PIP 26 cmH_2O (2.5 kPa); and PEEP 5 cmH_2O (0.5 kPa; V_T ~18 $mL \cdot kg^{-1}$, high pressure amplitudes). Blood gas analysis and blood pressure were recorded just before and hourly after randomisation. Nonventilated animals (Cap-, n=9; Cap+, n=9) served as controls. To rule out the effects of oxygen exposure underlying the observed effects, three nonventilated animals were exposed to 100% O_2 for 4 h before tracheostomy and carotic artery cannulation.

To further delineate whether ACE exerted its effects *via* Ang II, bradykinin or both, rats were treated (nine per experimental group) with specific receptor antagonists and ventilated with the ventilation strategy of group II. For Ang II, rats were exposed to the Ang II type-1 receptor antagonist, losartan (MSD, Haarlem, the Netherlands) for 5 days in their drinking water (200 $mg \cdot L^{-1}$). For bradykinin, rats were pre-treated with captopril, which blocks degradation of bradykinin, followed by administration of the bradykinin B_2 receptor antagonist, HOE-140 (1 $mg \cdot kg^{-1}$ *i.v.*; BioMol International, Exeter, England) just prior to the start of mechanical ventilation. One animal of the latter group died during the surgical procedure.

After the ventilation period, heparinised blood was collected. Animals were sacrificed with an overdose of intra-arterial administered pentobarbital sodium. Bronchoalveolar lavage (BAL; six rats per experimental group) was performed five times with normal saline (30 $mL \cdot kg^{-1}$); the mean recovery for all groups was 90±0.4%. The retrieved BAL fluid (BALF) was centrifuged ($300 \times g$ at 4°C for 10 min). To obtain serum, coagulated blood was centrifuged at $1,350 \times g$ at 4°C for 10 min. BALF and serum were aliquoted and stored at -80°C. Lavage was not performed in animals used for histology and immunohistochemistry. From these animals (three rats per experimental group), lungs were dissected. After recruitment by positive airway pressure (10 cmH_2O (0.98 kPa)), lungs were fixed in 4% buffered formalin and embedded in paraffin.

Assays

ACE activity

ACE activity was measured in BALF and serum by monitoring the degradation of the fluorogenic peptide substrate Mca-R-P-P-G-F-S-A-F-K(Dnp)-OH (R&D Systems, Uithoorn, the Netherlands) over time in a spectrofluorometer (FLUOstar Galaxy; BMG Labtechnologies, Offenburg, Germany) at 320 nm excitation and 405 nm emission. As endothelin-converting enzyme also converts this substrate, ACE activity was distinguished as inhibitable by captopril.

Ang II and bradykinin assays

Ang II and bradykinin were quantified in BALF using a radioimmunoassay (BioSource, Nivelles, Belgium) and a competitive enzyme immunoassay (Bachem, Bubendorf, Switzerland), respectively, according to the manufacturers' instructions.

Lactate dehydrogenase activity

Lactate dehydrogenase (LDH) activity was measured in BALF, frozen in the presence of 10% polyethylene glycol 6000, by following the oxidation of nicotinamide adenine dinucleotide at 340 nm ($\varepsilon_{NADH,\ 1\ cm}$ 6,220) due to the reduction of sodium pyruvate.

Inflammatory mediator assays

Macrophage inflammatory protein (MIP)-2 and IL-6 were measured using rat-specific ELISA (R&D Systems and BioSource, respectively) according to the manufacturer's instructions. Total protein was measured by the Bradford method (BioRad assay, Munich, Germany) using bovine serum albumin as a reference.

Histology and immunohistochemistry

Lung sections were stained with haematoxylin and eosin. The influx of inflammatory cells and oedema were semi-quantitatively scored as none, minimal, light, moderate or severe (scoring 0, 1, 2, 3 or 4, respectively) in 10 fields. The lung injury score was obtained by taking the average score from three animals in each group [17].

Apoptosis in lung sections was quantified by immunostaining using an antibody against activated caspase-3 (Cell Signaling Technology, Beverly, MA, USA) following standard procedures [18].

The number of caspase-3 positive cells was counted at the surface of alveolar walls in 10 fields using the ×40 objective lens. In addition, apoptotic cells in lung tissue sections were identified by the TUNEL (terminal deoxynucleotidyl-transferase-mediated deoxyuridine triphosphate nick-end labelling) method using a commercial kit (Roche Diagnostics Corp., Indianapolis, IN, USA), according to the manufacturer's instructions.

Statistical analysis

Group comparisons were evaluated by two- or one-way ANOVA, followed by Student–Newman–Keuls test for pair-wise multiple comparisons, or the Kruskall–Wallis test, where appropriate. Blood pressure, carbon dioxide tension (P_{a,CO_2}) and arterial oxygen tension (P_{a,O_2}) levels were evaluated by repeated measures ANOVA. Log transformations of the variables were analysed in case of lack of normality and/or homoscedasticity. A p-value <0.05 was considered significant.

RESULTS

Gas exchange and haemodynamic parameters

During the study period P_{a,CO_2} values did not drop below 20 mmHg (2.7 kPa) and there were no significant differences between the different time-points and between the experimental groups (data not shown). Mean P_{a,O_2} levels also remained unchanged in both ventilation groups, irrespective of pre-treatment with captopril (fig. 1a and b).

Blood pressure remained constant in group I during the study period (fig. 1c). In contrast, a significant decline in blood pressure was observed in group II during the ventilation period ($p<0.01$; fig. 1d). Pre-treatment with captopril reduced blood pressure significantly in group II; in group I this reduction was only significant during the first hour (fig. 1c and d).

Oxygen effect in control animals

Exposure of rats to 100% oxygen for 4 h prior to the surgical procedure did not affect BALF protein content, ACE activity or inflammatory parameters compared with the nonexposed control animals (data not shown).

Protein content

Protein content in BALF increased significantly with increasing pressure amplitudes (fig. 2). Pre-treatment with captopril had no significant effect on BALF protein content in either control or ventilated animals.

ACE activity

In BALF, ACE activity increased 2.5- and 6.5-fold in groups I and II, respectively, compared with the control group ($p<0.05$; fig. 3a). Pre-treatment with captopril reduced ACE activity in both groups, but this reduction was only significant in group II. In serum, increasing pressure amplitudes apparently

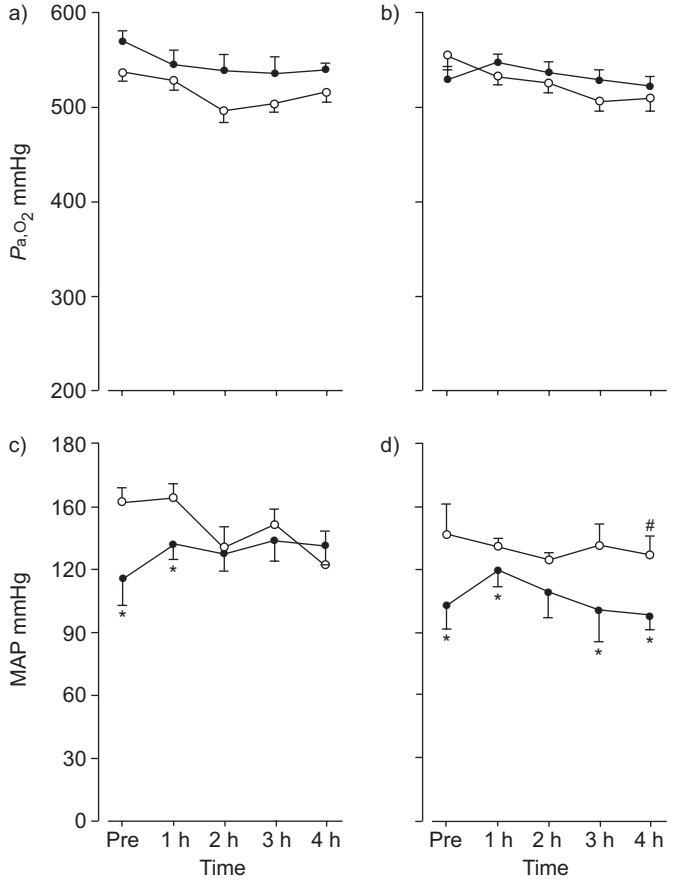

FIGURE 1. Arterial oxygen tension (P_{a,O_2}; a and b) and mean arterial pressure (MAP; c and d) during mechanical ventilation of animals ventilated at peak inspiratory pressure (PIP) 16 cmH_2O/positive end-expiratory pressure (PEEP) 5 cmH_20 (group I; a and c) and PIP 26 cmH_2O/PEEP 5 cmH_2O (group II; b and d), with (●) or without (○) pre-treatment with captopril. Data are expressed as mean±SEM, with six animals per time-point. #: $p<0.01$ compared with group I; *: $p<0.05$ *versus* animals of the same group that were not pre-treated. 1 mmHg=1.36 cmH_2O=0.133 kPa.

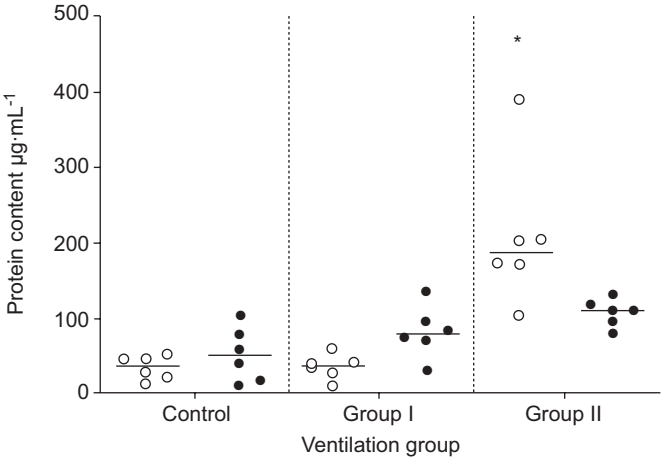

FIGURE 2. Scatter plot of individual protein content in bronchoalveolar lavage fluid of animals ventilated at positive inspiratory pressure (PIP) 16 cmH_2O/positive end-expiratory pressure (PEEP) 5 cmH_20 (group I) and PIP 26 cmH_2O/PEEP 5 cmH_2O (group II), with (●) or without (○) pre-treatment with captopril. Nonventilated animals served as controls. Horizontal bars represent the median. There were six animals per experimental group. The effect of mechanical ventilation was significant ($p<0.001$). *: $p<0.05$ compared with control group. 1 cmH_2O= 0.0978 kPa.

decreased serum ACE activity, albeit not significantly (fig. 3b). After pre-treatment with captopril, serum ACE activity increased 4–7–fold in all groups ($p<0.05$). Differences between groups were not significant.

Inflammatory mediators

MIP-2 BALF levels increased from undetectable in control animals to 80 pg·mL^{-1} in group I and 300 pg·mL^{-1} in group II ($p<0.05$; fig. 4a). Pre-treatment with captopril apparently attenuated MIP-2 levels in both ventilation groups, but this reduction was only significant in group II.

IL-6 BALF levels increased from undetectable and low levels in control animals and group I, respectively, to 200 pg·mL^{-1} in group II ($p<0.05$; fig. 4b). Blocking ACE activity with captopril significantly reduced IL-6 levels in group II.

Lung injury score; apoptosis and necrosis

Histological evaluation of lung tissue from the different ventilation groups showed increased injury with increasing pressure amplitudes (fig. 5). Lung injury scores of group I and II were significantly higher compared with that of the control group (table 1). Pre-treatment with captopril had no significant effect on lung injury scores.

The number of caspase-3- and TUNEL-positive cells at the surface of alveolar walls was taken as a marker of apoptosis, whereas LDH activity in BALF was a marker for necrosis. The control group and group I showed ~400 caspase-3-positive cells·mm^{-2} (fig. 5b, d, i). This number was ~20% higher in group II. Pre-treatment with captopril reduced the number of apoptotic cells significantly in group II. The findings of the TUNEL assay (fig. 6) were in agreement with those of activated caspase-3 staining.

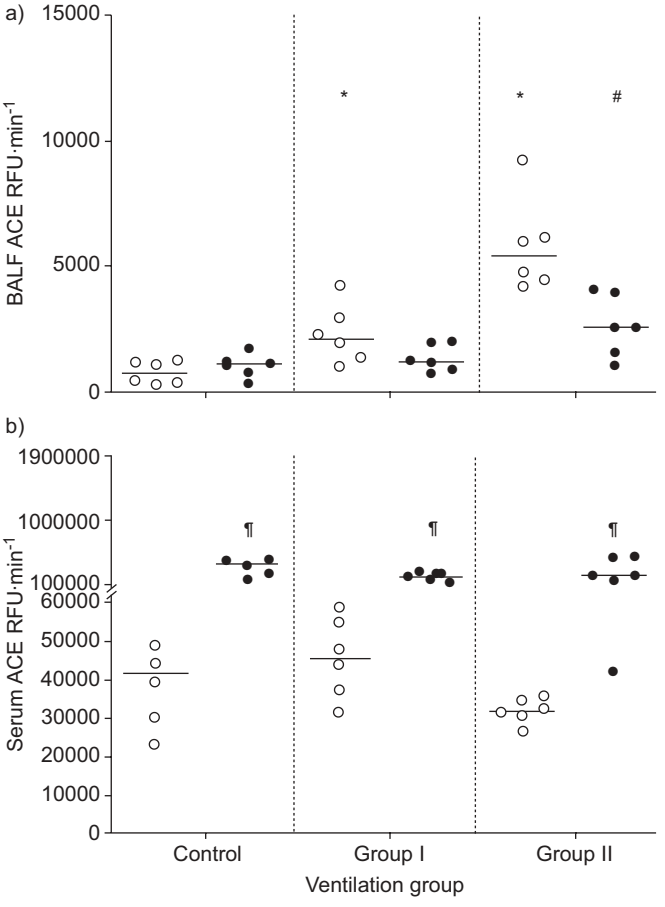

FIGURE 3. Scatter plot of individual angiotensin-converting enzyme (ACE) activity in a) bronchoalveolar lavage fluid (BALF) and b) serum of animals ventilated at positive inspiratory pressure (PIP) 16 cmH_2O/positive end-expiratory pressure (PEEP) 5 cmH_2O (group I) and PIP 26 cmH_2O/PEEP 5 cmH_2O (group II), with (●) or without (○) captoprtil pre-treatment. Nonventilated animals served as controls. Horizontal bars represent the median. There were six animals per experimental group. a) Effect of mechanical ventilation (MV) $p<0.001$, captopril effect $p=0.045$, MV and captopril interaction $p=0.018$. b) No significant effect of MV, captopril effect $p<0.001$ and no significant MV and captopril interaction. *: $p<0.05$ compared with control group; #: $p<0.05$ *versus* untreated group II; ¶: $p<0.05$ *versus* untreated animals. 1 $cmH_2O=0.0978$ kPa.

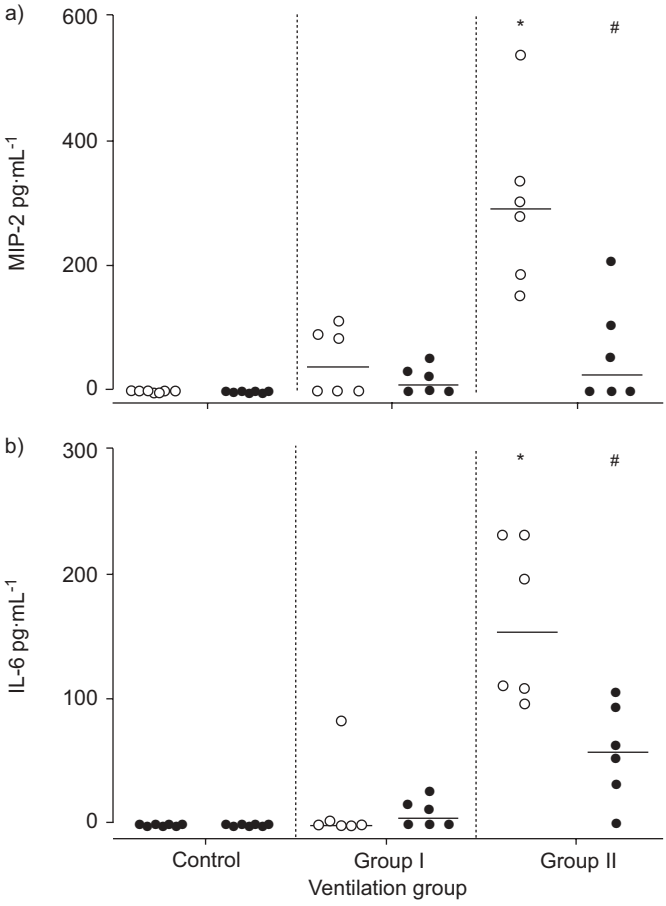

FIGURE 4. Scatter plots of individual a) macrophage inflammatory protein (MIP)-2 and b) interleukin (IL)-6 levels in bronchoalveolar lavage fluid of animals ventilated at positive inspiratory pressure (PIP) 16 cmH_2O/positive end-expiratory pressure (PEEP) 5 cmH_20 (group I) and PIP 26 cmH_2O/PEEP 5 cmH_2O (group II), with (●) or without (○) captopril pre-treatment. Nonventilated animals served as controls. Horizontal bars represent the median. There were six animals per experimental group. a) Effect of mechanical ventilation (MV) $p=0.008$, captopril effect $p=0.030$, MV and captopril interaction $p=0.014$. b) MV effect $p<0.001$, captopril effect $p=0.003$ and no significant MV and captopril interaction. #: $p<0.05$ *versus* untreated group II; *: $p<0.05$ group II *versus* control group and group I. 1 $cmH_2O=0.0978$ kPa.

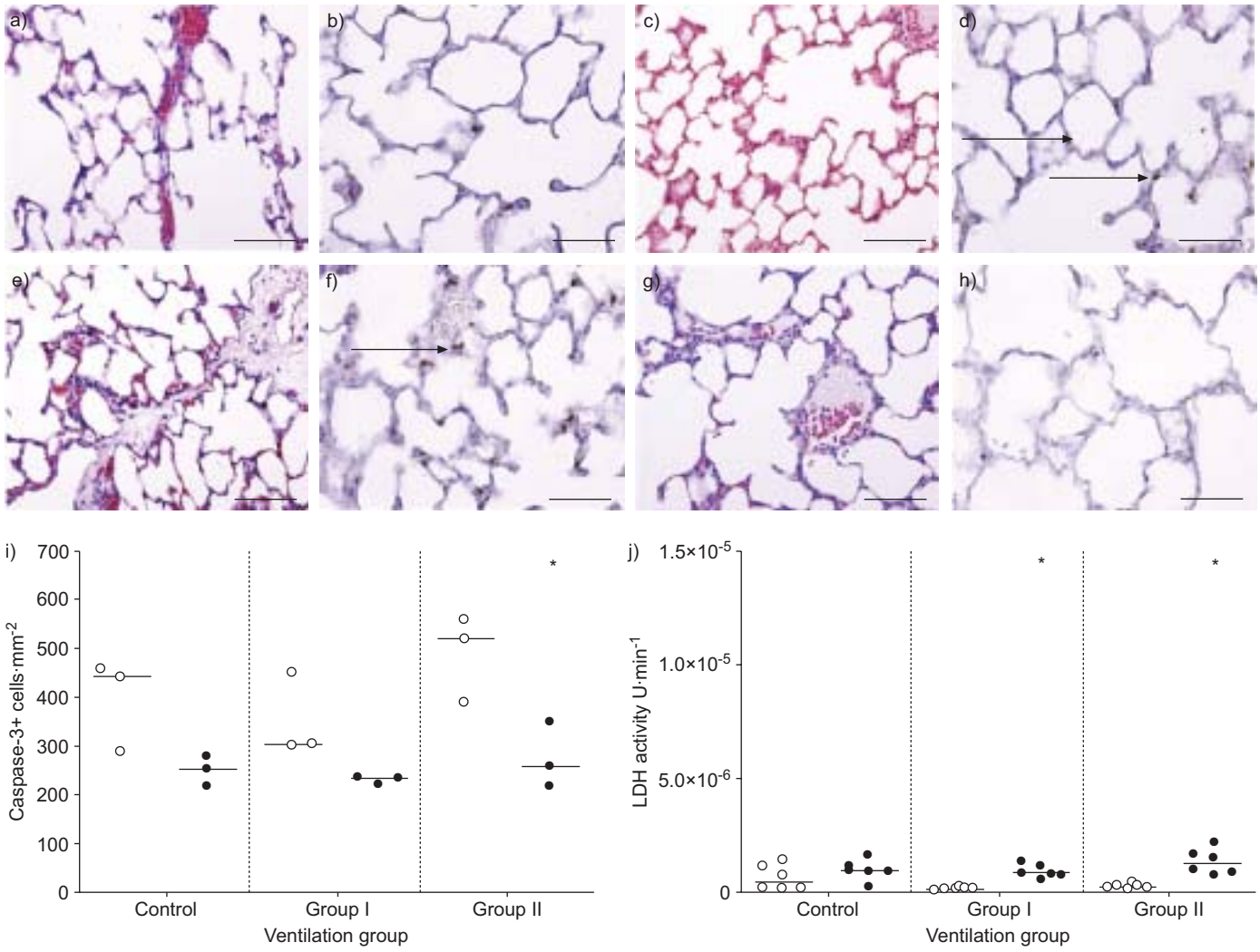

FIGURE 5. Haematoxylin and eosin (a, c, e, g) and activated caspase-3 staining (b, d, f, h) of lung tissue of the control group (a, b), of group I (c, d) and of group II without (e, f) or with (g, h) captopril pre-treatment. Arrows indicate representative positive cells for activated caspase-3. i) Scatter plot of individual caspase-3-positive (+) cells·mm^{-2} in lung tissue (n=3 per group) and j) lactate dehydrogenase (LDH) activity in bronchoalveolar lavage fluid (BALF) of animals ventilated at positive inspiratory pressure (PIP) 16 cmH_2O/positive end-expiratory pressure (PEEP) 5 cmH_2O (group I) and PIP 26 cmH_2O/PEEP 5 cmH_2O (group II), with (●) or without (○) captoril pre-treatment (n=6 per group). Nonventilated animals served as controls; horizontal bars represent the median. Captopril had a significant effect on the number of caspase-3-positive cells and LDH activity ($p<0.001$) *: $p<0.05$ *versus* animals without pre-treatment in the same group. Scale bars=2 μm (a, c, e, g) and 4 μm (b, d, f, h). 1 cmH_2O=0.0978 kPa.

There was no significant difference in LDH activity between the control group, group I or II (fig. 5j). However, in group I and II a slight, but significant, increase in LDH activity was observed after pre-treatment with captopril ($p<0.05$).

Ang II and bradykinin levels in BALF

No differences were observed in BALF Ang II levels between the different groups (fig. 7a), irrespective of pre-treatment with captopril. Spiking BALF samples with a known amount of recombinant Ang II showed full recovery after 15 min incubation at room temperature, suggestive of no relevant proteolytic activity in BALF (data not shown). Bradykinin levels in BALF increased significantly with increasing pressure amplitudes (fig. 7b) but no increase was observed after pre-treatment with captopril.

Contribution of Ang II and/or bradykinin in group II

Captopril attenuated inflammatory and apoptosis markers in group II, but this was not paralleled by attenuation of Ang II levels and/or enhanced bradykinin levels. To assess the contribution of either product to inflammation and apoptosis in VILI, specific receptor antagonists were used.

If the effects of ACE on inflammation and apoptosis were mediated by Ang II, blocking its receptor would generate the same effect as blocking ACE with captopril. Indeed, blocking Ang II receptors with losartan resulted in a similar decrease of BALF protein content, MIP-2 and IL-6 levels and caspase-3 positive cells as observed after captopril pre-treatment (fig. 8).

If the observed effects after blocking ACE were mediated by increased bradykinin levels, blocking its receptor after

TABLE 1 Lung injury scores

Group	Oedema		Leukocytes			Total injury score
	Peri-vascular	Alveolar	Peri-bronchial	Intra-vascular	Intra-alveolar	
Control						
Without captopril pre-treatment	0.0±0.0	0.7±0.6	0.0±0.0	0.3±0.6	0.7±0.6	1.7±1.5
With captopril pre-treatment	0.7±0.6	0.0±0.0	0.0±0.0	0.3±0.6	1.0±0.0	2.0±1.0
I						
Without captopril pre-treatment	1.3±0.6	1.2±0.3	0.3±0.6	1.7±0.6	2.0±0.0	6.5±1.5*
With captopril pre-treatment	1.0±0.0	0.3±0.6	0.3±0.6	0.7±0.6	1.3±0.6	3.7±1.5
II						
Without captopril pre-treatment	2.0±0.0	1.0±0.0	0.0±0.0	0.7±0.6	1.7±0.6	5.3±1.2*
With captopril pre-treatment	1.5±0.7	1.0±0.0	0.0±0.0	1.0±0.0	0.5±0.7	4.0±0.0

Data are presented as mean±SD. Lung injury score of animals (n=3) after 4 h of ventilation at positive inspiratory pressure (PIP) 16 cmH_2O/positive end-expiratory pressure (PEEP) 5 cmH_20 (group I) and PIP 26 cmH_2O/PEEP 5 cmH_2O (group II). Nonventilated animals served as a control. *: $p<0.05$ compared with control group without captopril (two-way ANOVA and *post hoc* Student–Newman–Keuls test). 1 $cmH_2O=0.0978$ kPa.

captopril pre-treatment would restore inflammation and apoptosis. Upon addition of HOE-140 the blood pressure increased during the ventilation period (data not shown). Blocking the bradykinin receptor by HOE-140 after captopril pre-treatment, however, showed no difference compared with those pre-treated with captopril alone (fig. 8).

DISCUSSION

The present study has demonstrated that VILI-induced inflammation and apoptosis is mediated, in part, by ACE activity, which involves Ang II production rather than bradykinin degradation.

Rats were subjected to two ventilatory strategies. BALF levels of ACE activity, protein and MIP-2 (the rodent homologue of the human neutrophil chemoattractant IL-8), as well as IL-6, were higher in the high-pressure amplitude group compared with the low-pressure amplitude group. This indicates that mechanical ventilation with higher pressure amplitudes leads to a local inflammatory response within 4 h. This is in accordance with *ex vivo* and *in vivo* experiments, where a similar rise in cytokines and chemokines during injurious ventilation was observed [19, 20]. The increase in ACE, IL-6 and MIP-2 may be due to production and/or release from local alveolar cells but could also be due to leakage of plasma across the alveolar–capillary membrane. This is an important issue to settle, but needs to be addressed in another study using *in situ* analyses of relevant mRNAs and the encoded proteins. However, the current authors consider it unlikely that leakage of plasma is implicated in reduced numbers of caspase-3-positive cells in captopril-treated group II animals. Another potential confounding factor is the captopril-induced decrease in blood pressure. However, there is evidence that haemodynamic changes do not play a pivotal role in lung injury [21–23]. Correction of hypotension induced during high V_T ventilation did not change microvascular leak in the lung [23]. In contrast, anti-tumour necrosis factor antibodies prevented lung permeability [21]. This suggests that inflammatory rather than haemodynamic mechanisms are involved in VILI. Indeed, the present study has shown that pre-treatment with captopril was associated with an attenuation of inflammation and apoptosis despite causing a significantly lower blood pressure in the animals.

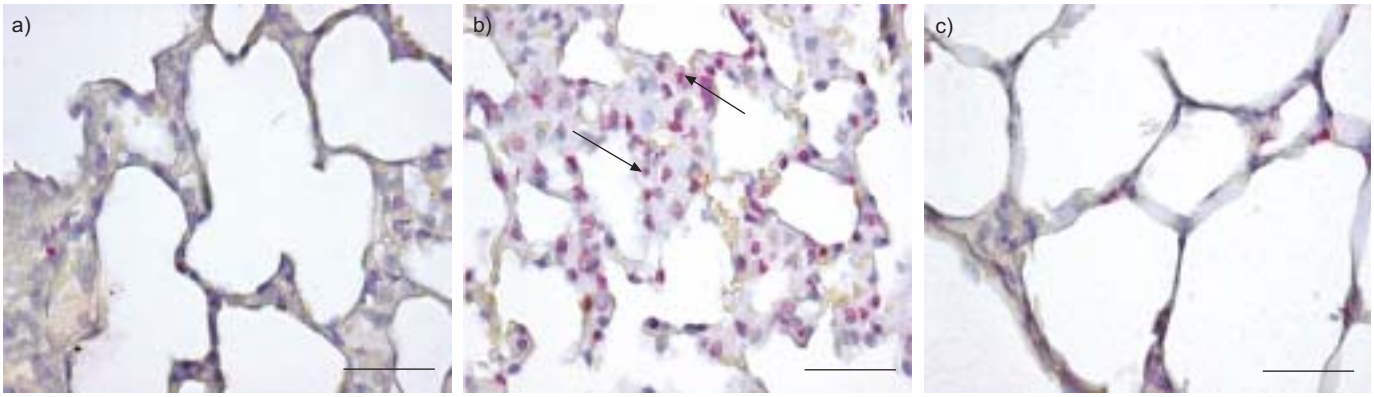

FIGURE 6. TUNEL (terminal deoxynucleotidyltransferase-mediated deoxyuridine triphosphate nick-end labelling) staining of lung tissue in a) the control group and group II b) without or c) with captopril pre-treatment. Arrows indicate representative TUNEL-positive cells. Scale bars=4 μm.

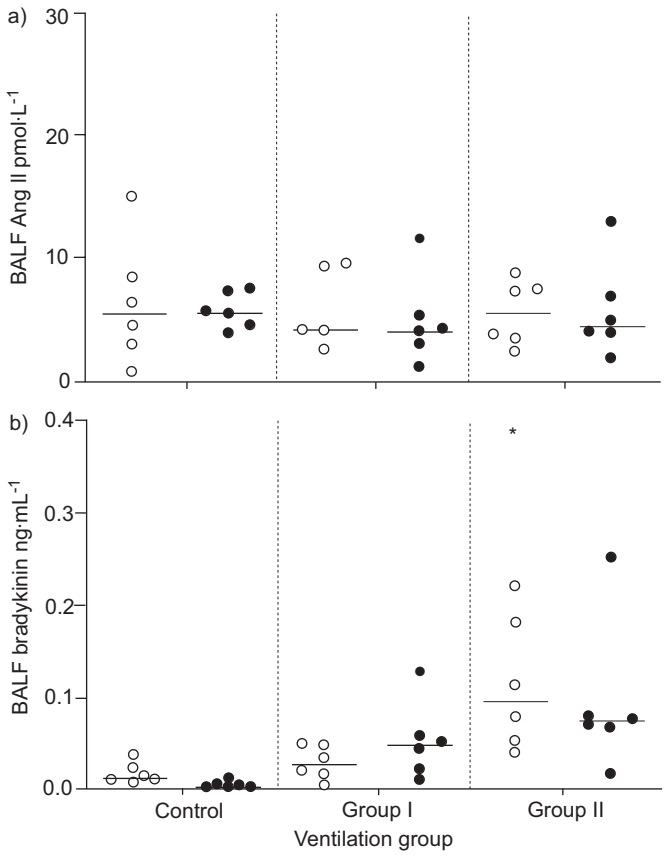

FIGURE 7. Scatter plot of individual a) angiotensin (Ang) II levels and b) bradykinin levels in bronchoalveolar lavage fluid (BALF) of animals ventilated at positive inspiratory pressure (PIP) 16 cmH_2O/positive end expiratory pressure (PEEP) 5 cmH_2O (group I) and PIP 26 cmH_2O/PEEP 5 cmH_2O (group II), with (●) or without (○) captopril pre-treatment. Nonventilated animals served as controls; horizontal bars represent the median with six animals per experimental group. In the untreated group I, one sample for Ang II measurement was lost. Mechanical ventilation had a significant effect on BALF bradykinin levels ($p=0.005$). *: $p<0.05$ compared with control group. 1 $cmH_2O=0.0978$ kPa.

Captopril pre-treatment resulted in increased serum ACE activity in all experimental groups. This apparently inconsistent finding has been reported in a number of studies [24, 25] and is probably explained by phosphorylation of the short C-terminal cytoplasmic domain of ACE by captopril [26, 27]. This leads to outside-in signalling and an increased endothelial ACE expression. Shedding of the enzyme from the endothelial cells may thus account for increased serum ACE activity after captopril pre-treatment. There was no increase in the BALF levels of ACE, suggesting that captopril does not potentiate ACE expression by alveolar epithelial cells. This also suggests a compartmentalisation of ACE expression, as previously suggested for inflammation [2]. An unexpected finding of captopril treatment was a small but significant increase in BALF LDH activity. As yet, there is no explanation for this increase but possibly captopril promotes necrosis, although this was not apparent on histological examination.

ACE generates Ang II and degrades bradykinin. However, no altered Ang II levels were demonstrated in response to captopril. Local tissue levels of Ang II are difficult to assess due to the short half-life of Ang II as a result of proteolysis, degradation by ACE2 and binding to the Ang II receptor [28]. Incubation of recombinant Ang II with BALF, however, showed no degradation and suggests that there is no proteolytic activity in BALF present that degrades Ang II. In the high-pressure amplitude group, a small increase in BALF bradykinin levels was found. However, blocking ACE, thereby blocking bradykinin degradation, did not result in increased levels. Pre-treatment with the Ang II receptor blocker losartan, showed the same attenuation of inflammation and apoptosis as was found after pre-treatment with captopril. So, indirectly the present study showed that the increase of MIP-2 and IL-6 BALF levels and that of the number of apoptotic cells in this *in vivo* model are likely to be mediated by Ang II. Blocking bradykinin receptors with HOE-140 did not counteract the observed effect of captopril, suggesting that degradation of bradykinin plays no significant role in the observed effects of ACE on inflammation and apoptosis. The present authors, therefore, conclude that Ang II is the main effector peptide in VILI-induced inflammation and apoptosis.

This finding is in line with recent studies with similar and other models of acute lung injury. Inhibition of the Ang II receptor significantly prevented an increase in the number of apoptotic lung epithelial cells during mechanical ventilation of surfactant-depleted lungs in rats [29]. Increased lung tissue angiotensinogen mRNA suggested activation of pulmonary RAS. The role of Ang II has been highlighted further by an *in vivo* acute lung injury model with ACE2, a homologue of ACE which degrades Ang II. Administration of recombinant human ACE2 protein to acid-treated mice attenuated lung injury, as assessed by lung function and by formation of pulmonary oedema [15].

The current findings need to be cautiously interpreted. The present model used a relatively short period of mechanical ventilation. Although ACE activity appears an initial trigger of inflammation and apoptosis in VILI, the effect of captopril over a prolonged period remains to be elucidated. In this model, captopril was administered before the onset of mechanical ventilation; however, it remains to be determined whether the application of captopril after mechanical ventilation is just as effective.

The present study shows that mechanical ventilation activates bronchoalveolar lavage fluid angiotensin-converting enzyme, leading to an inflammatory response and increased apoptosis within hours. Pre-treatment with an angiotensin-converting enzyme inhibitor reduced bronchoalveolar lavage fluid angiotensin-converting enzyme activity, pulmonary inflammation and apoptosis. The clinical application of protective ventilatory strategies has resulted in decreased mortality in patients with acute respiratory distress syndrome [30]. The extent to which ventilator-induced lung injury can be reduced in the clinical setting by angiotensin-converting enzyme inhibitors remains to be elucidated; however, the therapeutic value of pre-treatment must be balanced with possible negative effects of afterload reduction. These findings imply that therapeutic intervention may lead to attenuation of ventilator-induced inflammation and apoptosis.

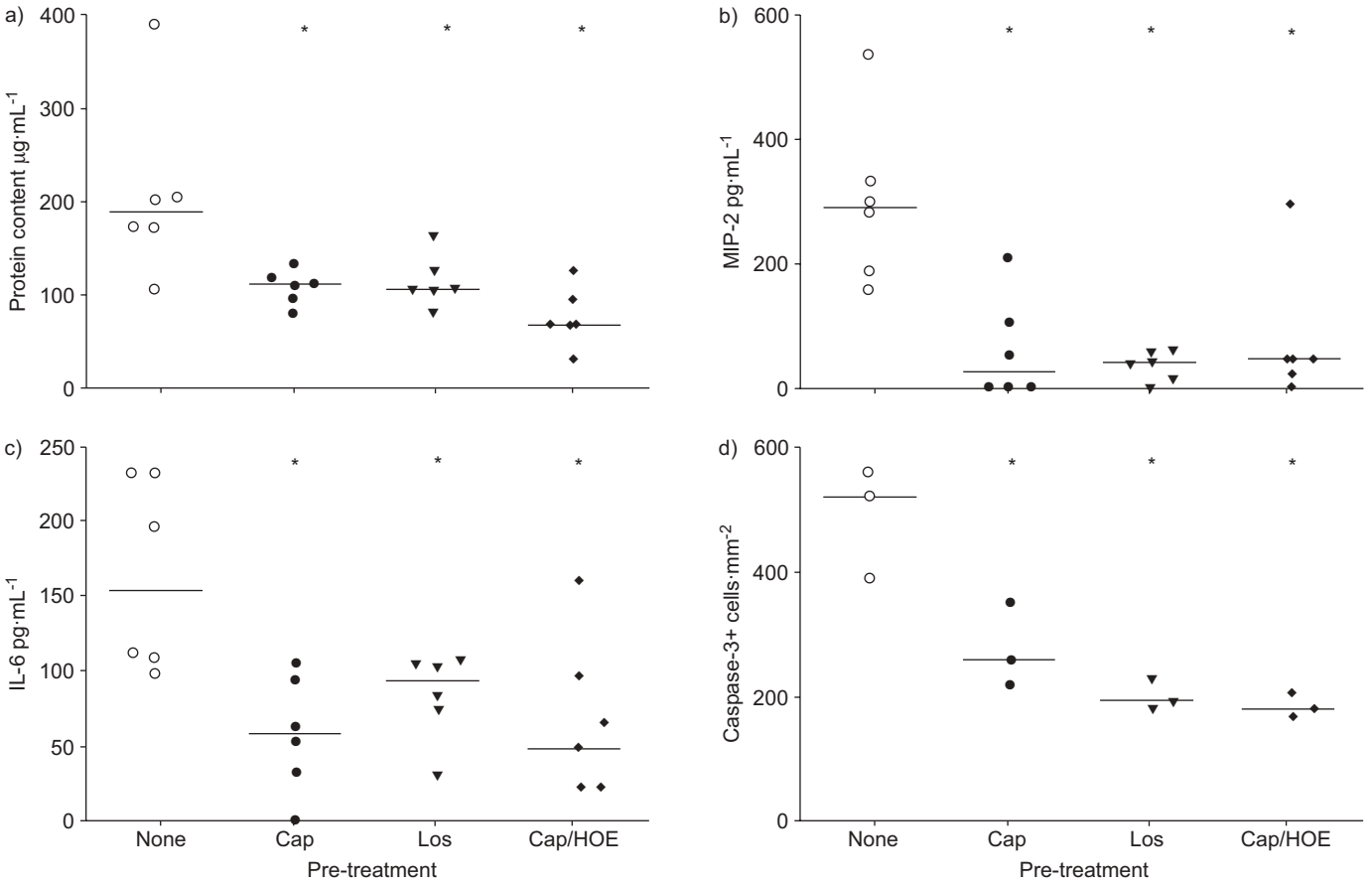

FIGURE 8. Scatter plot of individual levels of a) total protein content, b) macrophage inflammatory protein (MIP)-2, c) interleukin (IL)-6 levels in bronchoalveolar lavage fluid and d) number of caspase-3-positive cells·mm^{-2} in lung tissue of animals ventilated at positive inspiratory pressure 26 cmH_2O/positive end-expiratory pressure 5 cmH_2O (group II). Horizontal bars represent the median; n=6 animals for each experimental group (except for group Cap/HOE n=5). *: $p<0.05$ compared with untreated group. Cap: captopril; Los: losartan; HOE: bradykinin B2 receptor antagonist HOE-140.

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