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

R. M. Wösten-van Asperen[1], R. Lutter[2], J. J. Haitsma[3, 4], M. P. Merkus[5], J. B. van Woensel[1], C.M. van der Loos[6], S. Florquin[6], B. Lachmann[3], A. P. Bos[1]

[1]Pediatric Intensive Care Department, Emma Children's Hospital/Academic Medical Center, Amsterdam, The Netherlands; [2]Departments of Pulmonology and Experimental Immunology, Academic Medical Center; [3]Department of Anesthesiology, Erasmus-MC Faculty, Rotterdam; [4]Department of Critical Care Medicine, St. Michael's Hospital, Toronto, Ontario, Canada; [5]Center for Pediatric Clinical Epidemiology, Academic Medical Center; [6]Department of Pathology, Academic Medical Center.

CORRESPONDENCE: R.M. Wösten-van Asperen, Pediatric Intensive Care Department, Emma Children's Hospital/AMC, Meibergdreef 9, 1100 DD Amsterdam, The Netherlands. FAX number: 0031-20-6919338

E-mail: r.m.vanasperen@amc.uva.nl

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ABSTRACT

Ventilator-induced lung injury (VILI) is characterized by inflammation and apoptosis, but underlying mechanisms are poorly understood. A role for angiotensin-converting enzyme (ACE) via angiotensin II (Ang II) and/or bradykinin in acute lung injury was proposed. We 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. Non-ventilated animals served as controls. ACE activity, Ang II and bradykinin, 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 positive cells.

BALF ACE activity, levels of total protein, inflammatory parameters and the number of apoptotic cells were increased in the high pressure amplitudes group as compared to the control group. Blocking ACE activity by captopril attenuated inflammation and apoptosis in this 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.

We conclude that inflammation and apoptosis in VILI is, at least in part, due to ACE-mediated Ang II production.

INTRODUCTION

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 II (Ang II). Ang II is a potent vasoconstrictor. Besides that, Ang II is 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]. Besides 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 is well-established by decreasing caspase-3 activation [14]. Taken

together, ACE may exert its role in the pathogenesis of lung injury via Ang II and/or bradykinin.

Recently, it was shown in a model of acute lung injury induced by acid-aspiration and sepsis, that mice deficient for ACE had reduced pulmonary edema formation and leukocyte infiltration [15]. However, it is unknown whether different ventilation strategies lead to increased pulmonary ACE activity. Therefore we investigated whether during VILI (2 different ventilation strategies) pulmonary ACE activity is increased and if so, whether the ACE inhibitor captopril could attenuate VILI-induced inflammation and apoptosis. Finally, we 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. Care and handling of the animals were in accordance with the European Community guidelines. Animals did either or not receive 500 $mg.l^{-1}$ captopril in their drinking water for 5 days. At day 6, a tracheostomy was performed and the carotid artery was catheterized under inhalation anesthesia (65% nitrous oxide/33% oxygen/ 2% isoflurane (Isoflurane; Pharmachemie BV, Haarlem, The Netherlands)). Inhalation anesthesia was replaced by hourly intraperitoneal injections of pentobarbital sodium (60 $mg.kg^{-1}$, Nembutal®; Algin BV, Maassluis, The

Netherlands). Muscle relaxation was attained with 2 $mg.kg^{-1}$ pancuronium bromide (Pavulon®; Organon, Boxtel, The Netherlands) intramuscular hourly.

Animals were connected to a Servo ventilator 300 (Siemens-Elema, Solna, Sweden) and ventilated in the pressure controlled time-cycled mode, at a fractional inspired oxygen concentration of 1.0, a frequency of 20 to 30 per minute to maintain normocapnia and an inspiratory/expiratory ratio of 1:2. Animals were randomly allocated to two ventilation strategies, for 4 h each, as follows: (1) Group I (without captopril (Cap-) n=9; with captopril (Cap+) n=9) peak inspiratory pressure (PIP)16 cmH_2O and positive end expiratory pressure (PEEP) 5 cmH_2O (tidal volume (TV) approximately 8 ml/kg, low pressure amplitudes), (2) Group II (Cap- n=9; Cap+ n=9) PIP 26 cmH_2O and PEEP 5 cmH_2O (TV app. 18 ml/kg, high pressure amplitudes). Blood gas analysis and blood pressure were recorded just before and hourly after randomization. Non-ventilated animals (Cap- n=9, Cap+ n=9) served as controls. To rule out that exposure to oxygen was underlying the observed effects, 3 non-ventilated 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, we treated rats (n=9 per experimental group) with specific receptor antagonists and ventilated them with the ventilation strategy of group II. For Ang II, rats were exposed to the Ang II type1 receptor antagonist, Losartan (MSD, Haarlem, The Netherlands) during 5 days in their drinking water (200 $mg.l^{-1}$). For bradykinin, rats were pretreated with captopril (see above) that blocks degradation of bradykinin, followed by administration of the bradykinin B_2 receptor antagonist, HOE-140 (1 mg/kg iv, BioMol International, Exeter, England) just before the start of the

mechanical ventilation. One animal of the latter group died during the surgical procedure.

After the ventilation period, heparinized blood was collected. Animals were sacrificed with an overdose of intra-arterial administered pentobarbital sodium. Bronchoalveolar lavage (6 rats per experimental group) was performed 5 times with normal saline (30 $ml.kg^{-1}$); mean recovery was 90 ± 0.4 % for all groups. The retrieved fluid (BALF) was centrifuged (300 x g at 4°C for 10 min). To obtain serum, coagulated blood was centrifuged at 1350 x 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 (3 rats per experimental group), lungs were dissected. After recruitment by a positive airway pressure (10 cmH_2O), lungs were fixed in 4% buffered formalin and paraffin-embedded.

Assays

ACE-activity

ACE-activity was measured in BALF and serum 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) at 320 nm excitation and 405 nm emission. As endothelin-converting enzyme also converts this substrate, ACE activity was distinguished as captopril inhibitable.

Angiotensin II (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 manufacturer's instructions.

Lactate dehydrogenase (LDH) activity

LDH activity was measured in BALF, frozen in the presence of 10% PEG 6000, by following the oxidation of NADH at 340 nm ($\varepsilon_{NADH,\ 1cm}$ 6220) due to the reduction of sodium-pyruvate.

Inflammatory mediator assays

MIP-2 (macrophage inflammatory protein-2) and interleukin (IL)-6 were measured using rat specific enzyme-linked immunosorbent assays (ELISA) (R&D Systems and BioSource, Nivelles, Belgium, 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 hematoxylin and eosin. The influx of inflammatory cells and edema were semi-quantitatively scored in 10 fields as none, minimal, light, moderate or severe (score 0, 1, 2, 3 or 4, respectively). The lung injury score was obtained by averaging the scores from three animals of each group [17].

Apoptosis in lung sections was quantified by immunostaining using an antibody against activated caspase-3 (Cell Signaling Technology, Beverly, MA) following standard procedures [18]. The number of caspase-3 positive cells was counted at the surface of alveolar walls in 10 fields using an x40 objective. In

addition, apoptotic cells in lung tissue sections were identified by the terminal deoxynucleotidyltransferase-mediated dUTP nick end labeling (TUNEL) method using a commercial kit (Roche Diagnostics Corp. Indianapolis, IN), according to the manufacturer's instruction.

Statistical Analysis

Statistical analysis was performed using SPSS version 11.5.1 (SPSS Chicago, IL). Group comparisons were evaluated by two- or one-way analysis of variance (ANOVA), followed by Student-Newman-Keuls test for pairwise multiple comparisons, or the Kruskall Wallis test, where appropriate. Blood pressure, $PaCO_2$ and PaO_2 levels were evaluated by repeated measures ANOVA. Log transformations of the variables were analyzed in case of lack of normality and/or homoscedasticity. A p-value < 0.05 was considered significant.

RESULTS

Gas exchange and hemodynamic parameters

During the study period $PaCO_2$ values did not drop below 20 torr and there were no significant differences between the different time points and between the experimental groups (data not shown). Mean PaO_2 levels remained also unchanged in both ventilation groups, irrespective of pre-treatment with captopril (Figure 1A & B).

Blood pressure remained constant in group I during the study period (Figure 1C). In contrast, a significant decline of blood pressure was observed in group II during the ventilation period ($p<0.01$; Figure 1D). Pre-treatment with captopril

reduced blood pressure significantly in group II; in group I this reduction was only significant during the first hour (Figure 1C & D).

Oxygen effect in control animals

Exposure of rats to 100% oxygen during 4 hours prior to the surgical procedure did not affect BALF protein content, ACE activity or inflammatory parameters compared to the non-exposed control animals (data not shown).

Protein content

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

ACE activity

In BALF, ACE activity increased 2.5 and 6.5 fold, respectively, in group I and II compared to the control group ($p<0.05$, Figure 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 decreased serum ACE activity albeit not significant (Figure 3B). After pre-treatment with captopril, serum ACE activity increased 4- to 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 in group I and 300 pg/ml in group II ($p<0.05$, Figure 4A). Pre-treatment with captopril

apparently attenuated MIP-2 levels in both ventilation groups, but this reduction was only significant for group II.

IL-6 BALF levels also raised from undetectable and low levels in control animals and group I, respectively, to 200 pg/ml in group II ($p<0.05$, Figure 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 (Figure 5A, C, E and G). Lung injury scores of group I and II were significantly higher compared to that of the control group (Table1). 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 about 400 caspase-3 positive cells mm^{-2} (Figure 5B & D, Figure 5I). This number was about 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 (Figure 6) were in agreement with those of activated caspase-3 staining.

There was no significant difference in LDH activity between the control group, group I or II (Figure 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 (Figure 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 (Figure 7B) but no increase was observed after pre-treatment with captopril.

Contribution of Ang II and/or bradykinin in group II

Captopril attenuates inflammatory and apoptosis markers in group II, but this is not paralleled by attenuation of Ang II levels and/or enhanced bradykinin levels (see above). 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 pretreatment (Figure 8).

If the observed effects after blocking ACE were mediated by increased bradykinin levels, blocking its receptor after 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 with those by captopril pre-treatment alone (Figure 8).

DISCUSSION

We here show 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 (rodent homologue of the human neutrophil chemoattractant IL-8) as well as IL-6 were higher in the high pressure amplitude group as compared to the low pressure amplitude group. This indicates that mechanical ventilation with higher pressure amplitudes leads to a local inflammatory response within 4 hours. 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, we 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 decreased blood pressure. However, there is evidence that hemodynamic changes do not play a pivotal role in lung injury [21, 22, 23]. Correction of hypotension induced during high tidal volume ventilation did not change microvascular leak in the lung [23]. In contrast, anti-TNF antibodies prevented lung permeability [21]. This suggests that inflammatory rather than hemodynamic mechanisms are involved in VILI. Indeed, we show that pre-treatment with captopril was associated with an attenuation of inflammation and apoptosis despite causing a significant lower blood pressure in the animals.

Captopril pre-treatment resulted in increased *serum* ACE activity in all experimental groups. This apparent 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 signaling 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. We do not see an increase in BALF levels of ACE suggesting that captopril does not potentiate ACE expression by alveolar epithelial cells. This suggests compartmentalization of ACE expression, as previously suggested for inflammation [2]. An unexpected finding of captopril treatment was a small but significant increase of BALF LDH activity. As yet we have 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, we have found no altered Ang II levels in response to captopril. Local tissue levels of Ang II are difficult to assess because of the short half-life of Ang II due to 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 we showed that the increase of MIP-2 and IL-6 BALF levels and that of the number of apoptotic cells in our *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. We thus 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 edema [15].

Our findings need to be cautiously interpreted. In our model we have 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 we have administered captopril *before* the onset of mechanical ventilation. It remains to be determined whether captopril *after* applying mechanical ventilation is just as effective.

The present study shows that mechanical ventilation activates BALF ACE, leading to an inflammatory response and increased apoptosis within hours. Pre-treatment with an ACE inhibitor reduced BALF ACE activity and pulmonary inflammation and apoptosis. The clinical application of protective ventilatory strategies has resulted in decreased mortality in patients with ARDS [30]. To what extent VILI can be reduced in the clinical setting by ACE inhibitors remains to be elucidated; however,

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 VILI-induced inflammation and apoptosis.

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FIGURE LEGENDS

Figure 1: PaO_2 (A & B) and mean arterial pressure (C & D) during mechanical ventilation of animals ventilated at PIP 16 cmH_2O/PEEP 5 cmH_20 (group I) (A, C) and PIP 26 cmH_2O/PEEP 5 cmH_2O (group II) (B, D), with (closed circles) or without (open circles) pre-treatment with captopril.

Values are means ± SEM; six animals per time point.

*$p < 0.01$ compared to group I; **$p < 0.05$ vs. non pre-treated animals of the same group.

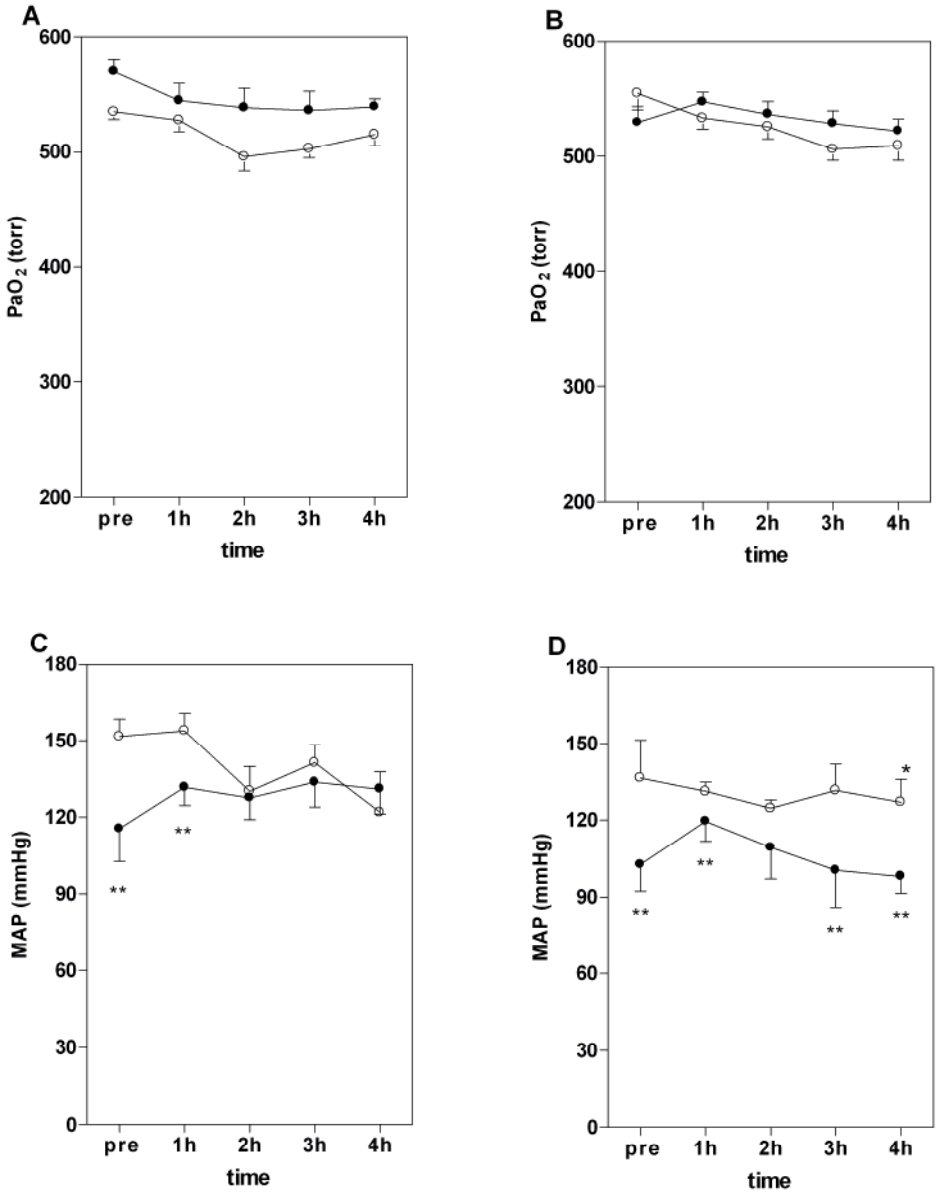

Figure 2: Scatter plot of individual protein content in BALF of animals ventilated at PIP 16 cmH_2O/PEEP 5 cmH_20 (group I) and PIP 26 cmH_2O/PEEP 5 cmH_2O (group II), with (closed circles) or without (open circles) pre-treatment with captopril. Non-ventilated animals served as controls. Horizontal bar represents median; six animals per experimental group. p-values of main effects of mechanical ventilation (MV), captopril (Cap) and interaction are given in the upper left corner.

- p < 0.05 compared to control group.

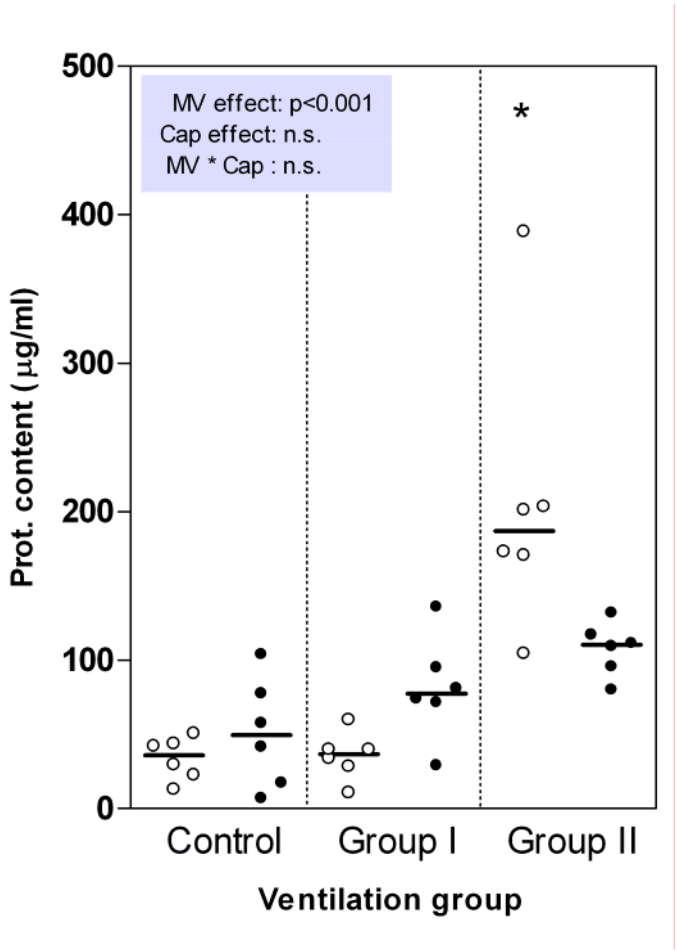

Figure 3: Scatter plot of individual ACE activity in BALF (A) and serum (B) of animals ventilated at PIP 16 cmH_2O/PEEP 5 cmH_20 (group I) and PIP 26 cmH_2O/PEEP 5 cmH_2O (group II), with (closed circles) or without (open circles) pre-treatment with captopril. Non-ventilated animals served as controls. Horizontal bar represents median; six animals per experimental group. p-values of main effects of mechanical ventilation (MV), captopril (Cap) and interaction are given in the upper left corner.

- p < 0.05 compared to control group, † p < 0.05 vs. untreated group II and ‡ p < 0.05 vs. untreated animals.

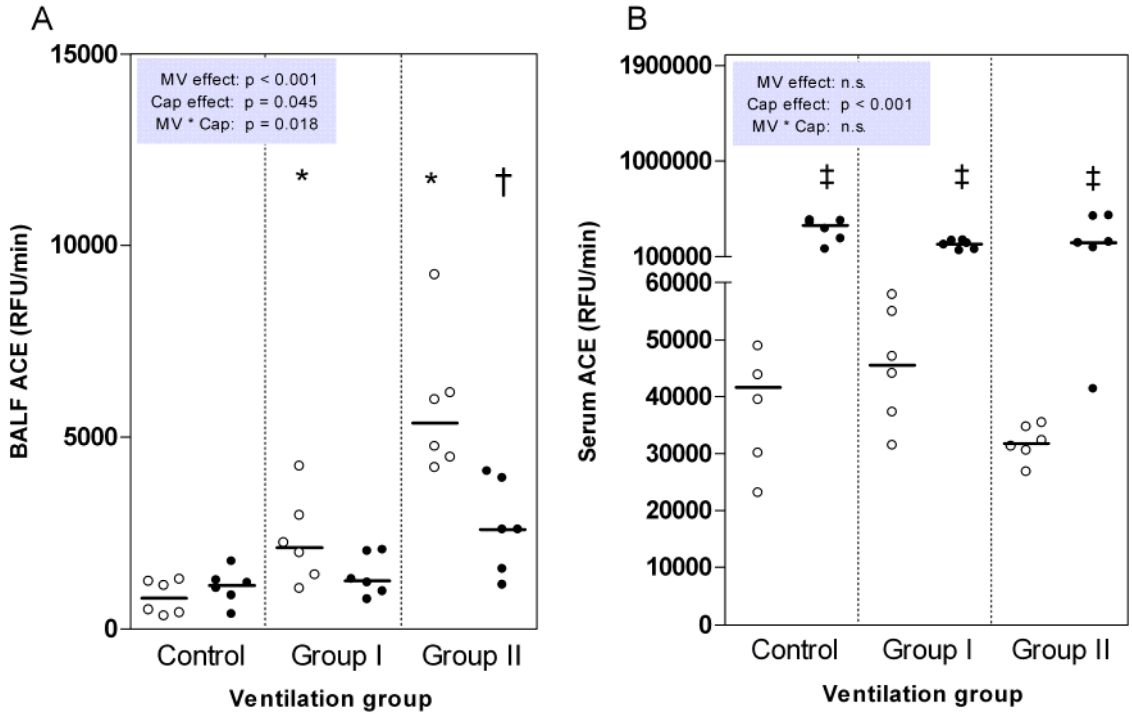

Figure 4: Scatter plot of individual MIP-2 (A) and IL-6 levels (B) in BALF of animals ventilated at PIP 16 cmH_2O/PEEP 5 cmH_20 (group I) and PIP 26 cmH_2O/PEEP 5 cmH_2O (group II), with (closed circles) or without (open circles) pre-treatment with captopril. Non-ventilated animals served as controls. Horizontal bar represents median; six animals per experimental group. p-values of main effects of mechanical ventilation (MV), captopril (Cap) and interaction are given in the upper left corner.

- $p < 0.05$ group II vs. control group and group I, † $p < 0.05$ vs. untreated group II.

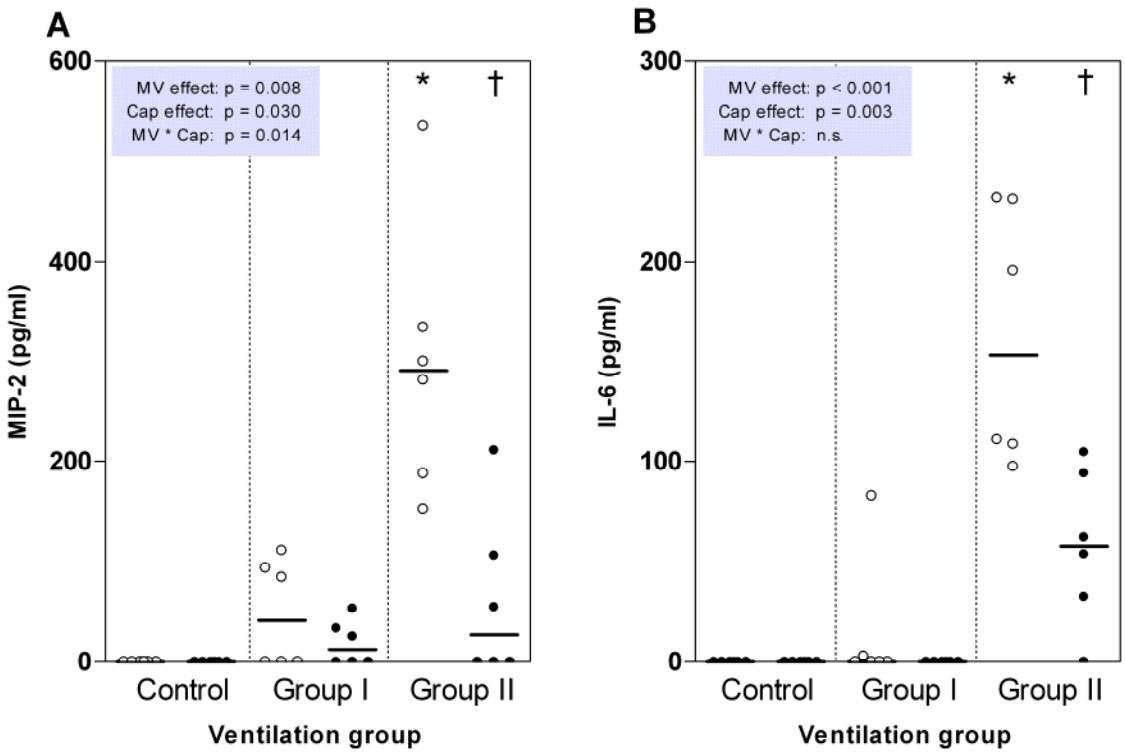

Figure 5: Hematoxylin-eosin (A, C, E & G) and activated caspase-3 staining (B, D, F & H) of lung tissue of the control group (A & B), of group I (C & D) and of group II without (E & F) or with (G & H) captopril pre-treatment (magnification x20 for H-E staining and x40 for caspase staining). Arrows indicate representative positive cells for activated caspase-3.

Scatter plot of individual number of caspase-3 positive cells mm^{-2} in lung tissue (I) and LDH activity in BALF (J) of animals ventilated at PIP 16 cmH_2O/PEEP 5 cmH_20 (group I) and PIP 26 cmH_2O/PEEP 5 cmH_2O (group II), with (closed circles) or without (open circles) pre-treatment with captopril. Non-ventilated animals served as controls. Horizontal bar represents median; caspase-3 staining (I) three animals per experimental group, BALF LDH activity (J) six animals per experimental group.

p-values of main effects of mechanical ventilation (MV), captopril (Cap) and interaction are given in the upper left corner.

- p < 0.05 vs. non pre-treated animals of the same group.

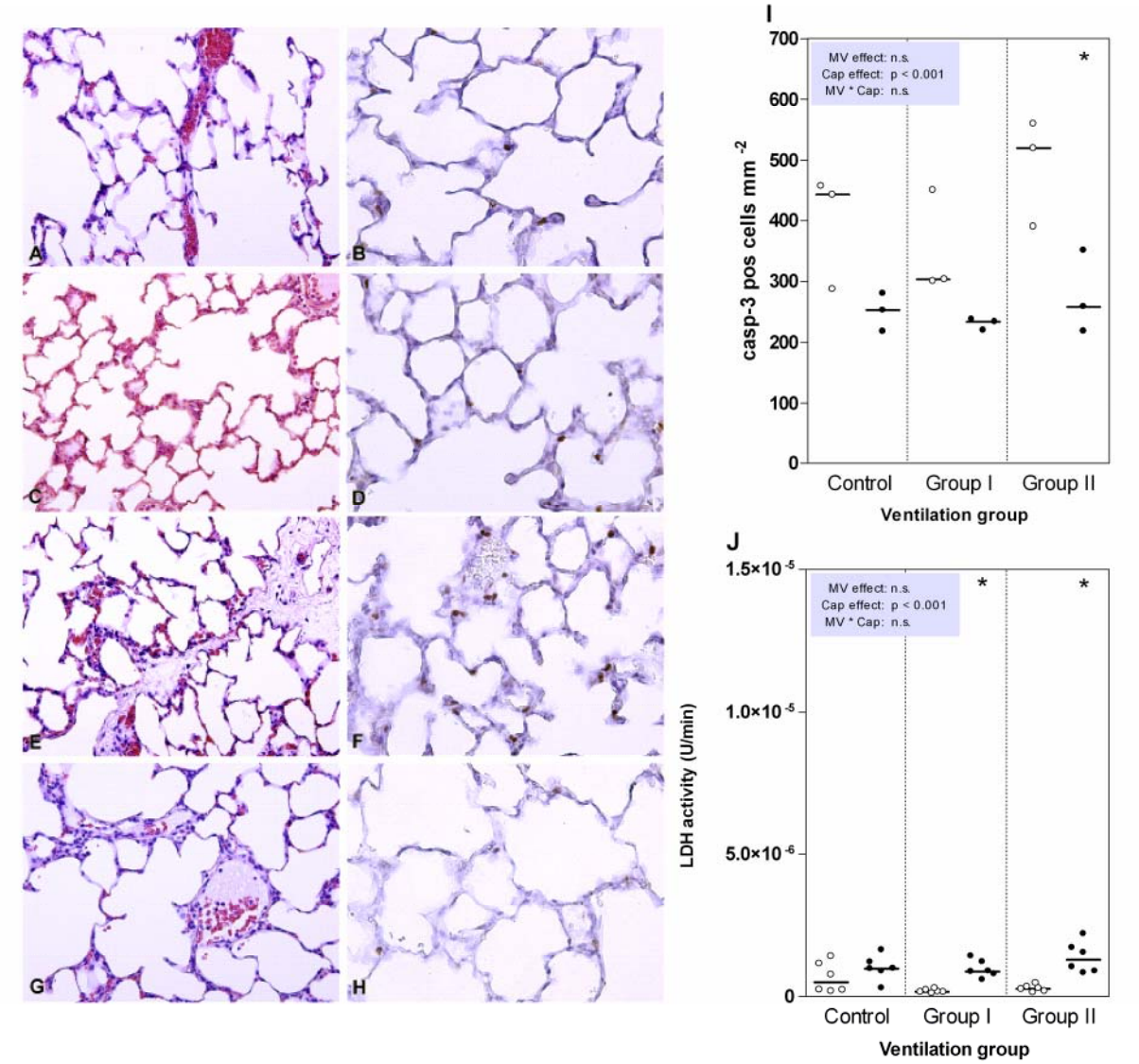

Figure 6: TUNEL staining of lung tissue of the control group (A), group II without (B) or with (C) captopril pre-treatment (magnification x40). Arrows indicate representative TUNEL positive cells.

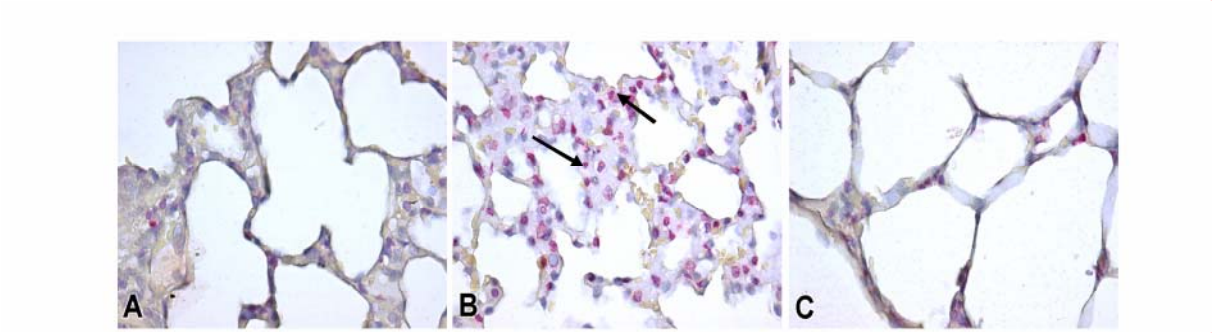

Figure 7: Scatter plot of individual Ang II levels (A) and bradykinin levels (B) in BALF of animals ventilated at PIP 16 cmH_2O/PEEP 5 cmH_20 (group I) and PIP 26 cmH_2O/PEEP 5 cmH_2O (group II), with (closed circles) or without (open circles) pre-treatment with captopril. Non-ventilated animals served as controls. Horizontal bar represents median; six animals per experimental group. In the untreated group I one sample for Ang II measurement was lost. p-values of main effects of mechanical ventilation (MV), captopril (Cap) and interaction are given in the upper left corner.

- $p < 0.05$ compared to control group

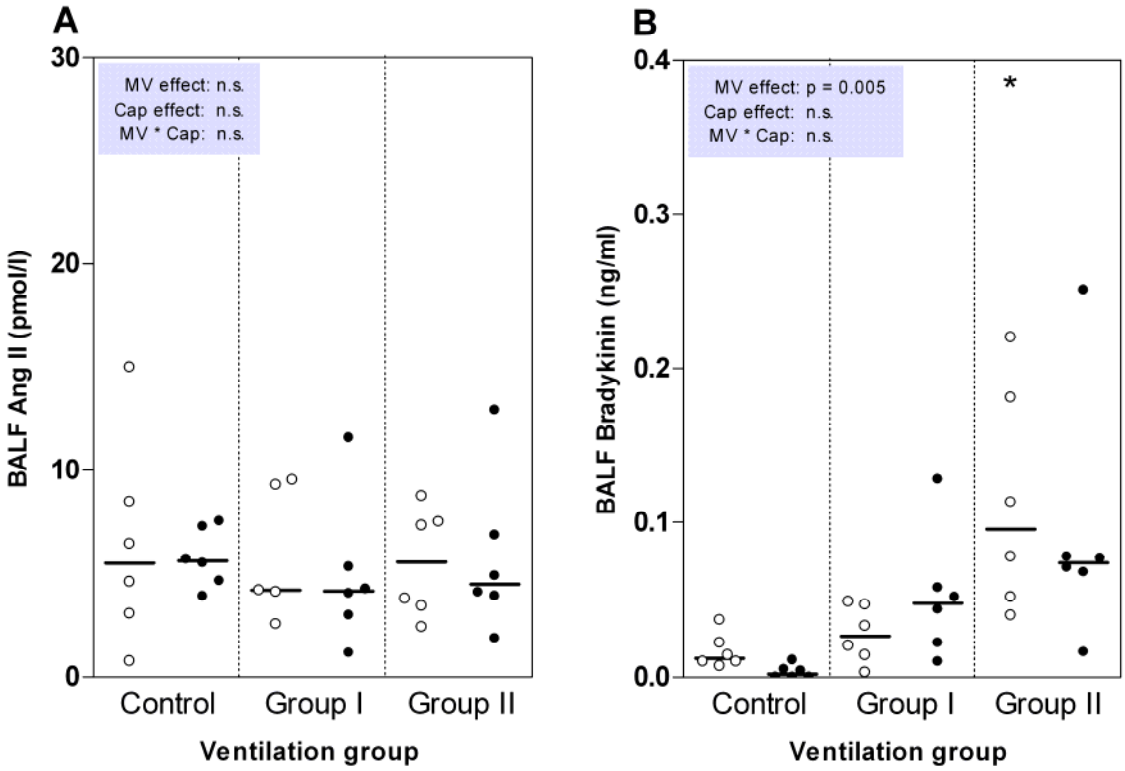

Figure 8. Scatter plot of individual levels of total protein content (A), MIP-2 (B) and IL-6 (C) levels in BALF and number of caspase-3 positive cells mm^{-2} in lung tissue (D) of animals ventilated at PIP 26 cmH_2O/PEEP 5 cmH_20 (group II). Open circles represent animals without pretreatment, closed circles pretreatment with captopril, closed triangles pretreatment with losartan and closed diamonds pretreatment with captopril and HOE-140. Horizontal bar represents median; n = 6 animals for each experimental group (except for group Cap/HOE n=5) and n = 3 animals for the immunohistochemistry.

- $p < 0.05$ compared with untreated group.

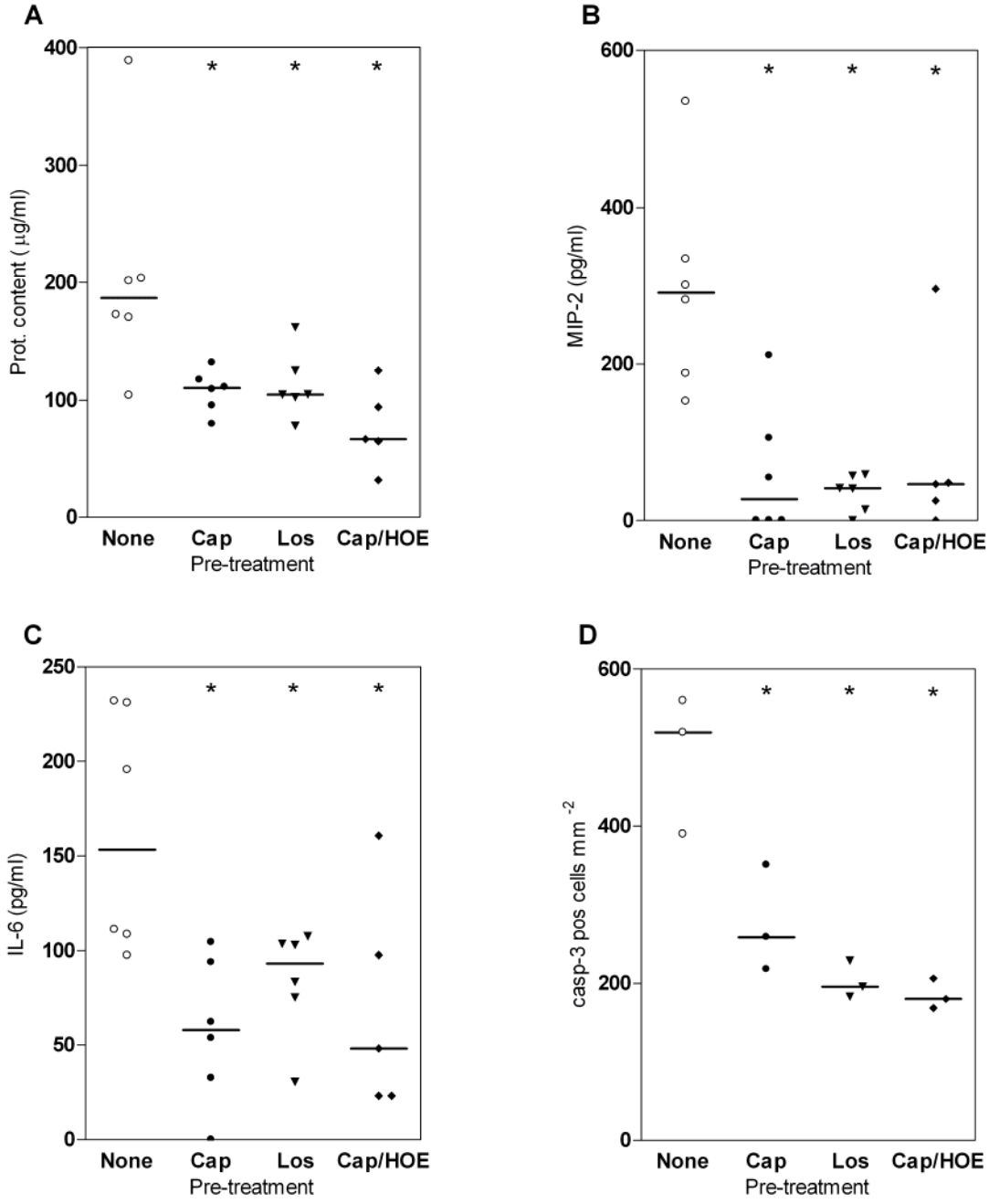
A
Prot. content (µg/ml)
400
300
200
100
0
None
Cap
Los
Cap/HOE
Pre-treatment
B
MIP-2 (pg/ml)
600
400
200
0
None
Cap
Los
Cap/HOE
Pre-treatment
C
IL-6 (pg/ml)
250
200
150
100
50
0
None
Cap
Los
Cap/HOE
Pre-treatment
D
casp-3 pos cells mm -2
600
400
200
0
None
Cap
Los
Cap/HOE
Pre-treatment

TABLE 1. LUNG INJURY SCORE[1]

Group	Captopril	Edema		Leukocytes			Total injury score
		Peri-vascular	Alveolar	Peri-bronchial	Intra-vascular	Intra-alveolar	
Control	-	0.0 (0.0)	0.7 (0.6)	0.0 (0.0)	0.3 (0.6)	0.7 (0.6)	1.7 (1.5)
	+	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	-	1.3 (0.6)	1.2 (0.3)	0.3 (0.6)	1.7 (0.6)	2.0 (0.0)	6.5 (1.5)*
	+	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	-	2.0 (0.0)	1.0 (0.0)	0.0 (0.0)	0.7 (0.6)	1.7 (0.6)	5.3 (1.2)*
	+	1.5 (0.7)	1.0 (0.0)	0.0 (0.0)	1.0 (0.0)	0.5 (0.7)	4.0 (0.0)

[1] Lung injury score of animals (n=3), with (+) or without (-) pre-treatment with captopril, after 4 hours of ventilation at PIP 16 cmH_2O/PEEP 5 cmH_20 (group I) and PIP 26 cmH_2O/PEEP 5 cmH_2O (group II). Non-ventilated animals served as a control. Values represent means ± SD.

* $p < 0.05$ compared to control group without captopril (two-way analysis of variance and post hoc Student-Newman-Keuls test).