Angiotensin-converting enzyme activity in serum and bronchoalveolar lavage fluid after damage to the alveolo-capillary barrier in the human lung

R. F. De Jongh[1], W. A. De Backer[2], R. Mohan[2], P. G. Jorens[2], F. J. van Overveld[2]

[1] Department of Anesthesiology, Sint Jansziekenhuis, Schiepse bos 2, B-3600 Genk, Belgium
[2] Departments of Medicine, University of Antwerp, Antwerp, Belgium

Abstract. *Objective:* Angiotensin-converting enzyme (ACE) is considered as a possible marker for endothelial cell damage in serum or bronchoalveolar lavage fluid. This hypothesis was tested during cardiac surgery and during the adult respiratory distress syndrome.
Design: We used patients with an expected different degree of endothelial cell damage. ACE levels in serum and bronchoalveolar lavage fluid were compared with indirect markers of alveolo-capillary barrier integrity.
Setting: Interdisciplinary team in a university hospital.
Methods: 13 Cardiac surgery patients received no glucocorticoids and 13 others received 2 g methylprednisolone before extracorporeal circulation. Thirteen patients were used as controls and 15 patients had nonseptic adult respiratory distress syndrome. All underwent bronchoalveolar lavage for ACE determination.
Results: At different times during surgery serum angiotensin-converting enzyme levels were not significantly different between the two groups. In post-operative bronchoalveolar lavage fluid, angiotensin-converting enzyme levels were significantly higher in patients who received corticoids (27.8 ± 1.7 U/l, mean ± SEM), compared to patients without corticoids (19.8 ± 1.4 U/l), control patients (18.2 ± 1.3 U/l) or patients with full blown non-septic adult respiratory distress syndrome (18.8 ± 1.1 U/l). There were no correlations between lavage angiotensin-converting enzyme and other parameters for alveolo-capillary membrane integrity in the lavage fluid such as the number of neutrophil cells, albumin or protein concentration, and between lavage angiotensin-converting enzyme and PaO_2/FIO_2 ratio during lavage.
Conclusion: Angiotensin-converting enzyme activity in serum or bronchoalveolar lavage fluid does not reflect damage of endothelial cells or damage of alveolo-capillary integrity in acute pulmonary disease.

Angiotensin-converting enzyme (ACE) is a glycoprotein, which is found in human lungs as a membrane bound ectoenzyme on the luminal surface of endothelial cells of pulmonary vessels [1]. Smaller amounts of ACE are also found in alveolar macrophages [2] and perhaps fibroblasts [3, 4]. Within endothelial cells, ACE is present in enzyme clusters within caveolae on the plasma membrane that is in contact with the vascular lumen [1]. Decreases in serum levels of ACE can result from diminished production or release of the enzyme by pulmonary cells [4]. Reduced pulmonary microvascular competence, possibly by microaggregates of polymorphonuclear cells (PMN) can explain the lower enzyme levels during nonseptic adult respiratory distress syndrome (ARDS) or during cardiac surgery [4, 5]. Since the findings of Hollinger et al. [6], who described elevated ACE levels in lung lavage of rats exposed to thiourea, elevated ACE levels in bronchoalveolar lavage (BAL) fluid have been used as a specific marker for endothelial cell injury in various animal experimental settings [7, 8]. However the source of soluble ACE in BAL fluid and its use as a marker for lung injury still remains uncertain [9, 10].

Extracorpoal circulation (ECC) used during haemodialysis [11] and cardiac surgery [12], induces complement mediated PMN activation. The activated PMNs are considered to be responsible for endothelial cell damage [13]. Patients with ECC can therefore be used as a model for lung injury. Several in vitro studies support the potential protective effects of steroids on endothelial cell damage during cardiac surgery, not by reducing complement activation but by reducing PMN activation [14, 15]. However there is lack of in vivo confirmation of the benefit of steroids.

The present study was designed to evaluate the use of ACE as a marker for endothelial cell injury and to study the eventually protective effects of glucocorticoids on pulmonary endothelial cells during cardiac surgery. The ACE levels in BAL fluid of these patients were compared to those of two reference groups: control patients without endothelial cell damage and patients with nonseptic ARDS. Other variables considered as markers of inflam-

mation and alveolo-capillary integrity damage were correlated with serum-ACE and BAL-ACE.

Patients and materials

In all cases, informed consent was obtained by the patients or their closest relatives in accordance to the revised Helsinki declaration of 1983. The protocol and procedure, initially designed to study cytokine levels and macrophage behaviour in bronchoalveolar lavage fluid after cardiac surgery, were approved by the ethical committee of the University Hospital of Antwerp.

Cardiac surgery patients ($n = 26$) underwent standard anaesthesia (high dose fentanyl, low dose isoflurane, diazepam and pancuronium). Prophylactic antibiotics (cephazoline or cefamandol 2 g), heparin 300 U/kg (Heparin Leo, Leo Pharmaceutical Products, Denmark) and lidoflazine 1 mg/kg were administered before ECC. Priming of ECC (Bentley Oxygenation System CM 50; membrane oxygenator, Baxter, USA) was done with 1800 ml crystalloids (Plasma-Lyte A, Baxter, USA) 400 ml human albumin 20% (Merieux, France) and 5000 U heparin. Intermittent cross clamping and mild hypothermia were used to prevent myocardial damage. After ECC protamine chloride was administered in a 1:1 ratio to heparin. All patients were operated in the morning and divided randomly into two groups: group 1 ($n = 13$) received no corticoids; group 2 ($n = 13$) received 2 g of methylprednisolone sodium succinate (Solu Medrol, Upjohn, Belgium) after induction of anaesthesia. All patients were considered as 'low risk' patients and had uncomplicated operative procedures without post-operative bleeding and normal coagulation tests. None of the cardiac surgery patients developed pulmonary problems or fullblown ARDS afterwards.

Two reference groups were studied. The first group (group ARDS) consisted of 15 patients with non-septic ARDS according to the classical criteria: a history of a predisposing illness, sudden onset of dyspnea, severe hypoxaemia, widespread diffuse lung infiltrates on chest radiograph without evidence of left ventricular failure and a capillary wedge pressure < 15 mmHg. Predisposing factors were: complicated cardiopulmonary bypass ($n = 7$), hypovolaemic shock with massive transfusion ($n = 3$), post-cardiac arrest coma ($n = 2$), disseminated intravascular coagulation ($n = 1$), parathion poisoning ($n = 1$) and paraquat poisoning ($n = 1$). Of the 15 patients 10 died. At the time of lavage all ARDS patients were afebrile and had no other signs of a septic syndrome. None of these patients received cortisoids.

The second reference group (group Cont) consisted of 13 nontreated non-smoking patients, who were referred to the out patient clinic for elective bronchoscopy because of chronic cough. All bronchoscopies were normal. All cell compositions of the BAL fluid could be considered as normal. None of the patients had a history of heavy smoking, pulmonary disease, sarcoidosis, ACE inhibitor or previous glucocorticoid intake.

Methods

Blood samples for determination of haematocrit, haemoglobin, white blood cell count and differentiation, complement factors (C3, C4, C3d) and serum ACE were taken during cardiac surgery: 1) 15 min before ECC, 2) 15 min after starting ECC, 3) at the end of ECC, 4) 10 min after protamine administration, 5) 3 h after operation and 6) 24 h after operation. Samples 2 and 3 were recalculated for haematocrit since significant dilution of the priming fluid occurred. Results of samples obtained after ECC were not recalculated. Samples for complement levels and serum ACE were immediately centrifuged, the plasma was stored at −20°C until determination.

Bronchoalveolar lavage

Three hours after arrival on the intensive care unit the cardiac surgery patients underwent BAL. Surgical patients and ARDS patients were sedated and mechanically ventilated at the moment of lavage. After preoxygenation a flexible bronchoscope (Olympus, type P20D, outer diameter 5 mm, inner diameter 2 mm) was passed through the endotracheal tube of the ventilated patients. In control subjects, bronchoscopy was performed by a standard procedure after premedication with diazepam/ atropine and local anaesthesia with lidocaine. After wedging into the right middle lobe, three successive 50 ml aliquots of 0.9% saline were instilled and immediately aspirated. The aspirate of the first aliquot was discarded to prevent bronchial contamination and the recovered fluid of the second and third aliquots were pooled and kept at 4°C. Blood tinged lavages were not used.

Laboratory

Within half an hour and after gauze filtration, the lavage fluid was centrifuged at 500 g for 10 min and aliquots of the cell-free supernatant were stored at −20°C.

The cells of the pellet were resuspended in 10 ml of Dulbecco's phosphate buffered saline (Gibco Ltd., Paisley, UK) and counted with a Coulter counter; cell differentiation was performed on cytospin preparations, using May Grunwald Giemsa staining. A minimum of 300 cells was examined. For all patients microbiological cultures of both bronchial aspirate and lavage fluid were sterile. Total protein and albumin were determined in the cell free supernatant by means of a nephelometric and a modified colorimetric method respectively.

ACE activity in blood and BAL fluid was determined using 3-(2-Furylacryloyl)-L-phenylalanyl-glycyl-glycine (FAPGG) (Bachem Feinchemicalien, Switzerland) as a chromophoric monitor of substrate hydrolysis [16, 17]. Absorbance was measured by a centrifugal analyser (Cobas-Bio®, Roche, Switzerland) at a wavelength of 340 nm.

Peripheral white blood cell count and differentiation were performed by means of counting (Coulter Counter) and staining by May Grunwald Giemsa. Complement factors were measured using N-antiserum from rabbit (Behring, Germany) for nephelometric determination (Nephelometer analyser, Behring, Germany).

Statistical analysis

Data are represented as mean ± standard error of the mean. Statistical significant differences ($p < 0.05$) were determined using the non parametric Mann-Whitney U test. The Spearman rank order coefficient was used to look for correlations between different parameters.

Results

The pump time for both cardiac surgery groups showed no statistical difference (group 1: 131 ± 11 min; group 2: 143 ± 8 min).

Serum ACE levels are shown in Fig. 1., white blood cell count in Fig. 2., and C3d blood levels in Fig. 3. for both cardiac surgery patient groups. There were no statistical significant differences in serum ACE between patients that did or did not receive steroids before ECC, neither in absolute value ($p = 0.20$, $p = 0.44$, $p = 0.83$, $p = 0.58$, $p = 0.56$, $p = 0.84$ for all time intervals respectively), nor in changes of serum ACE between two sampling times. Also no correlations were found between serum ACE levels or its changes and any absolute values or changes of white blood cell count or complement activation (C3, C4, C3d).

The fall in white blood cell count 15 min after starting ECC was significantly higher (decreased to 67%) in group 1 compared to group 2 (decreased to 88%, $p < 0.05$). Any peripheral blood parameters nor their changes correlated with BAL-ACE levels.

BAL fluid measurements for all patient groups are shown in Table 1. BAL fluid volume recovery was comparable between all the groups. BAL-ACE was significantly higher in group 2 (27.8 ± 1.7 U/l) compared to all other

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Table 1. Demographic data and data from the bronchoalveolar lavage

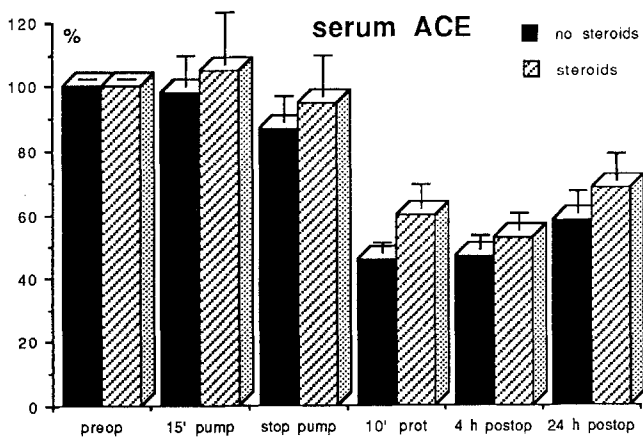|	Group cont n = 13	Group 1 n = 13		Group 2 n = 13		Groups ARDS n = 15	
Recuperated volume (ml)	40.5±4.6	44.5±4.5		45.2±3.8		47.0±3.9	
Age (years)	45±4	59±2	(C)	65±2	(C)	55±4	(C)
Pump time (min)		131±11		143±8			
Recuperated cells (×10⁶)	7.6±1.3	12.7±2.0	(C)	6.3±1.1	(0.1)	19.0±4.3	(C, 2)
Total macrophages (×10⁶)	4.9±1.2	9.4±2.0	(C)	4.2±1.0	(1*)	6.4±1.0	
% Macrophages	60±8	75±5		66±5		42±5	(1*, 2*)
Total PMN (×10⁶)	0.09±0.03	0.5±0.2	(C*)	0.2±0.1	(C*)	10.3±4.0	(C*, 1*, 2*)
% PMN	2±1	5±2		5±1	(C)	45±6	(C*, 1*, 2*)
Albumin (µg/ml)	35±6	50±6	(C)	60±12		231±59	(C*, 1*, 2*)
Protein (µg/ml)	62±10	127±12	(C*)	130±27	(C*)	468±116	(C*, 1*, 2*)
BAL-ACE (U/l)	18.2±1.3	19.8±1.4		27.8±1.7	(C*, 1*)	18.8±1.1	(2*)
BAL-ACE/Alb (U/mg)	0.84±0.19	0.70±0.27	(C)	0.75±0.20		0.20±0.06	(C*, 2*)
BAL-ACE/Prot (U/mg)	0.37±0.04	0.17±0.03	(C*)	0.32±0.07	(1*)	0.09±0.03	(C*, 1*, 2*)
PaO₂/FIO₂ (mmHg)	–	303±26		268±25		138±12	(1*, 2*)

Values are mean ± SEM
Significant differences to the left sided groups are indicated with C (control patients), 1 (to group 1) and 2 (to group 2) if $p<0.05$
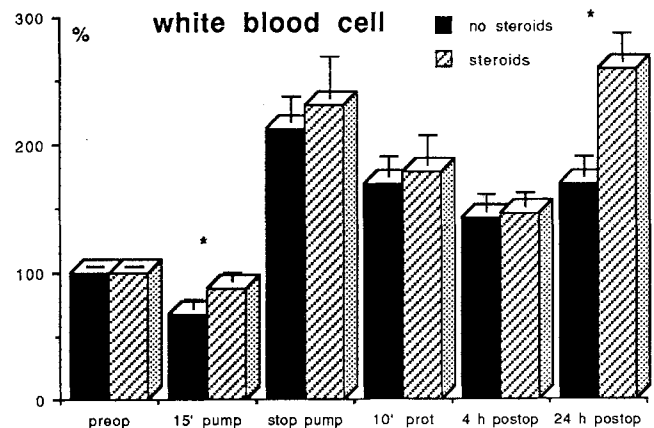C*, 1* or 2* were used if $p<0.01$

groups ($p<0.01$), which showed no intergroup differences (18.9±1.3 U/l).

Both surgical groups showed significant elevation in albumin (group 1: 50±6 µg/ml, $p<0.05$; group 2: 60±12 µg/ml, $p = 0.06$) as well as in protein (group 1: 127±12 µg/ml, $p<0.01$; group 2: 130±27 µg/ml, $p<0.01$) as compared to control patients (albumin: 35±6 µg/ml and protein: 62±10 µg/ml). The levels of albumin (231±59 µg/ml) and of protein (468±116 µg/ml) were significantly higher in the ARDS group ($p<0.01$ for all groups).

ACE/albumin ratio was lowest in the group ARDS (0.20±0.06 U/mg), highest in the control group (0.84±0.19 U/mg) and the surgical groups showed no differences (group 1: 070±0.27 U/mg; group 2: 0.75±0.20 U/mg). ACE/protein patterns were similar,

Fig. 2. Total white blood cell count in the perioperative surgical period, represented as percent of the prepump value. Values of 15'pump and stop pump were recalculated for haematocrit

Fig. 1. Serum angiotensin-converting enzyme activity in the perioperative surgical period, represented as percent of the prepump value. 15' pump: 15 min after starting the pump, 10' prot: 10 min after administration of protamine. Values of 15'pump and stop pump were recalculated for haematocrit
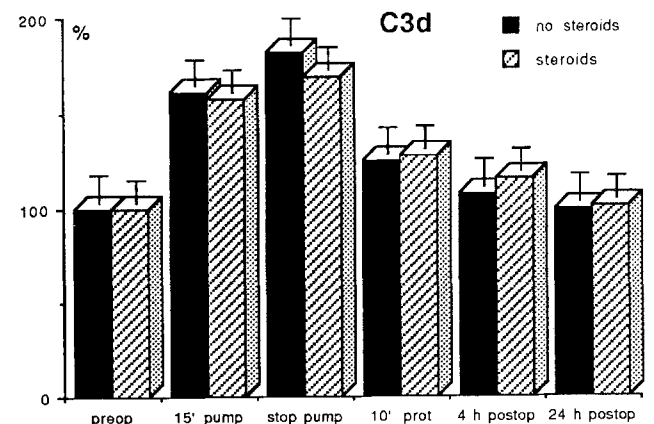
Fig. 3. Serum complement C3d levels in the perioperative surgical period, represented as percent of the prepump value. Values of 15'pump and stop pump were recalculated for haematocrit

but with difference between group 1 (0.17±0.03 U/mg) and group 2 (0.32±0.07 U/mg, $p<0.01$).

The count of recuperated cells was lowest in the control group (7.6±1.3×10⁶ cells) and group 2 (6.3±1.1×10⁶ cells). They were significant higher in group 1 (12.7±2.0×10⁶ cells, $p<0.05$ for both groups) and group ARDS (19.0±4.3×10⁶ cells, $p<0.05$ for both groups). In group 1 this was due to the higher count of macrophages (9.4±2.0×10⁶ cells), as compared to group 2 (4.2±1.0×10⁶ cells, $p<0.01$) and the control group (4.9±1.2×10⁶ cells, $p<0.05$). Neutrophil cell influx into the alveoli of the ARDS patients (10.3±4.0×10⁶ cells) is responsible for the higher cell count compared to other groups ($<1.0×10^6$ cells, $p<0.01$).

As a clinical parameter, PaO_2/FIO_2 ratio was significantly lower in the group ARDS (138±12 mmHg) compared to both cardiac surgery groups (group 1: 303±26 mmHg, $p<0.01$; group 2: 268±25 mmHg, $p<0.01$), that showed no difference.

Discussion

In this study the only significant difference found in peripheral blood of the cardiac surgery patients was the smaller fall in neutrophil cells after starting the ECC and a higher neutrophil cell count at the first postoperative day in patients receiving corticosteroids. These differences can be explained by decreased degree of leukosequestration and decreased formation of microaggregates of PMN, by altered neutrophil chemotactic responses, altered neutrophil degranulation and inactivation or possibly by increased release of PMN out of the bone marrow [15, 18]. Confirming other studies, methylprednisolone did not alter complement activation measured by consumption of C3 and C4 or production of C3d [19, 20]. The values of serial serum ACE level determinations during cardiac surgery were not influenced by methylprednisolone. Since it was shown that methylprednisolone did not affect the ACE assay [21], our results show that methylprednisolone administered before ECC did not influence ACE production or ACE release into the peripheral blood. Moreover, concerning the absence of any correlation between absolute values or changes of serum ACE with absolute values or changes of any peripheral blood parameters as are white blood cells and complement factors, or with BAL parameters or with postoperative PaO_2/FIO_2, serum ACE levels do not predict pulmonary injury during cardiac surgery.

Since albumin and protein concentrations in BAL fluid, the presence of PMNs and the postoperative PaO_2/FIO_2 ratio did not differ significantly between the two cardiac surgery patient groups, glucocorticoids do not seem to influence damage of the alveolo-capillary barrier. BAL-ACE levels in our non-septic ARDS patients with endothelial cell damage were similar to control patients without endothelial cell damage, according to the results of Idell et al. [22]. On the other hand, BAL-ACE levels in the surgical group treated with corticoids were unexpectedly higher compared to the other surgery patients, the control patients and the ARDS patients.

Several explanations for higher BAL-ACE levels in cardiac surgery patients pretreated with glucocorticoids can be sought, however most of them remain speculative. Use of glucocorticoids can diminish formation of PMN microaggregates, allowing microperfusion in the lung and thus increasing production and release of ACE by the pulmonary endothelial cell [4, 18].

In bovine endothelial cells production of ACE was augmented by corticoids [23]. Moreover, in rabbit alveolar macrophages de novo transcription and ACE synthesis was induced by corticosteroids [2, 24]. However no information is available describing the presence of methylprednisolone in BAL fluid and its influence on the activity of alveolar macrophages after its intravenous administration. It is also known that inhaled budesonide in humans does increase the presence of ACE in BAL fluid [25].

Several factors can influence the ACE assay, e.g. substance P and fractions of albumin [26]. Neutral endopeptidase, an enzyme located on the surface of several human cells can be upregulated by corticoids. This enzyme cleaves neuropeptides such as substance P, a potent competitive inhibitor of ACE in vitro [27]. So the use of corticoids may diminish the concentration of substance P, possibly leading to increased levels of measured ACE activity.

The difference in BAL-ACE between the two surgery patient groups can not be explained by differences in serum ACE or differences in leakage of the alveolo-capillary membrane, since protein or albumin concentrations did not differ between both groups. Little is known about deactivation or removal of proteins found in the alveolar space, so influences of steroids on ACE metabolism or lymphatic drainage are not known.

In conclusion, since BAL-ACE, BAL-ACE/albumin or BAL-ACE/protein ratio did not correlate with any parameter of inflammation (white blood cell behaviour or complement activation), or correlated with parameters of alveolo-capillary membrane integrity (albumin or protein concentration, PaO_2/FIO_2 ratio). BAL-ACE does not reflect damage of pulmonary endothelial cells or leakage of intravascular components into the alveoli. Glucocorticoids seem only to inhibit PMN fall in peripheral blood after starting extracorporeal circulation, but no complement activation, serum ACE, PaO_2/FIO_2 ratio, BAL albumin or protein concentration in the perioperative cardiac surgical period.

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Prof. Dr. W. De Backer
Department of Respiratory Medicine
University of Antwerp (UIA)
Universiteitsplein 1
B-2610 Antwerp-Wilrijk
Belgium