Pulmonary Capillary Endothelium-Bound Angiotensin-Converting Enzyme Activity in Acute Lung Injury

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Background—Pulmonary capillary endothelium-bound (PCEB) angiotensin-converting enzyme (ACE) activity alteration is an early, sensitive, and quantifiable lung injury index in animal models. We hypothesized that (1) PCEB-ACE alterations can be found in patients with acute lung injury (ALI) and (2) PCEB-ACE activity correlates with the severity of lung injury and may be used as a quantifiable marker of the underlying pulmonary capillary endothelial dysfunction.

Methods and Results—Applying indicator-dilution techniques, we measured single-pass transpulmonary hydrolysis of the synthetic ACE substrate 3H-benzoyl-Phe-Ala-Pro (BPAP) in 33 mechanically ventilated, critically ill patients with a lung injury score (LIS) ranging from 0 (no lung injury) to 3.7 (severe lung injury) and calculated the kinetic parameter \( \frac{A_{\text{max}}}{K_m} \). Both parameters decreased early during the ALI continuum and were inversely related to APACHE II score and LIS. Hydrolysis decreased with increasing cardiac output (CO), whereas 2 different patterns were observed between CO and \( \frac{A_{\text{max}}}{K_m} \).

Conclusions—PCEB-ACE activity decreases early during ALI, correlates with the clinical severity of both the lung injury and the underlying disease, and may be used as a quantifiable marker of underlying pulmonary capillary endothelial dysfunction. (Circulation. 2000;102:2011-2018.)

Key Words: lung endothelium angiotensin enzymes acute lung injury

Acute lung injury (ALI) is an acute, diffuse, and severe alteration of lung structure and function that occurs after exposure to noxious external or endogenous agents. ALI represents a pathological continuum, with various degrees of arterial blood gas and chest radiograph abnormalities.1 The most severe extreme of this continuum is the acute respiratory distress syndrome (ARDS), an overt noncardiogenic pulmonary edema.2 ALI/ARDS pathogenesis is only partly understood; however, pulmonary endothelium plays a major role by (1) altering its metabolic activity, thus affecting pulmonary and systemic homeostasis; (2) mediating cell-cell adhesion, especially with neutrophils; and (3) changing its barrier permeability, thus promoting pulmonary edema formation.3

Pulmonary endothelium participates in numerous important physiological and pharmacokinetic processes.4,5 Ectoenzymes located on the endothelial luminal surface are directly accessible to blood-borne substrates, and their activities may be measured in vivo by means of indicator-dilution techniques.5-7 Among them, angiotensin-converting enzyme (ACE; kininase II; EC 3.4.15.1), a major regulator of vascular tone, hydrolyzes angiotensin I and deactivates bradykinin.8 ACE molecules are uniformly distributed along the luminal pulmonary endothelial surface, including the membrane caveolae.9

Pulmonary capillary endothelium-bound (PCEB)-ACE activity has been extensively studied by means of indicator-dilution techniques in animals: Under physiological conditions, PCEB-ACE activity allows estimations of dynamically perfused capillary surface area (DPCSA)7,10,11; under toxic conditions, PCEB-ACE dysfunction is an early and sensitive index of lung vascular injury.6,12-15 The PCEB-ACE indicator-dilution technique has recently been validated in humans, providing evidence that it can be performed safely at the bedside and establishing the range of PCEB-ACE activity values for humans without lung disease.16

We therefore tested the hypothesis that PCEB-ACE activity (1) is altered in critically ill patients with ALI, (2) correlates with the severity of lung injury and the...
underlying disease, and (3) may be used as a quantifiable marker of the underlying pulmonary capillary endothelial dysfunction.

Methods

Subjects

Thirty-three critically ill patients, 22 men and 11 women, 14 to 87 years old, suffering from illnesses that can cause ARDS participated in a protocol approved by our Hospital Ethics Committee. Informed written consents were obtained. All patients were hospitalized in a general Intensive Care Unit (ICU), were mechanically ventilated, had no history of chronic lung or heart disease, and were not on ACE inhibitors. Patients were recruited at the first ICU visit of the assigned investigator. An initial PCEB-ACE activity estimation was performed for each patient. Eleven patients had 2 additional estimations (total 3) 48 hours apart.

Patients were grouped according to their lung injury score (LIS), introduced by Murray et al., as having no lung injury (nLI; LIS = 0), mild-to-moderate lung injury (mLI; LIS = 0.1 to 2.5), and severe lung injury (sLI; LIS > 2.5). To allow comparisons with other studies, patients were also grouped according to the American-European Consensus criteria as not having acute lung injury (NoALI) and having ALI/ARDS.

To validate the technique reproducibility, 7 other mechanically ventilated, head-trauma patients with LIS = 0 were studied. Two PCEB-ACE activity estimations were performed per patient, 30 minutes apart.

All patients had catheters placed in either the subclavian or the internal jugular vein and in the radial artery as part of their routine treatment. On the day of PCEB-ACE activity measurements, the following were performed or recorded: full hematological-biochemical profile, chest radiographs, vital signs, nutrient/drug administration, and central nervous system status assessment (Glasgow Coma Scale). Immediately before measurement, heart rate, mean systemic arterial pressure (MAP), ventilator mode/settings,
positive end-expiratory pressure (PEEP), and the inspiratory oxygen fraction (FIO₂) were recorded; arterial blood was withdrawn for arterial blood gas and hematocrit determinations. Disease and lung injury severity were estimated by assessing the acute physiology, age, and chronic health evaluation (APACHE II),¹⁸ and LIS (chest-roentgenogram score, PaO₂/FIO₂, and PEEP), respectively.¹⁷ Survival was subsequently recorded at ICU discharge.

Using indicator-dilution techniques, we estimated under first-order reaction conditions the single-pass transpulmonary hydrolysis of the synthetic ACE substrate³H-benzoyl-Phe-Ala-Pro (³H-BPAP) by PCEB-ACE. The methodology used is described in detail elsewhere.¹⁶ Briefly, for each determination, a 1.5-mL normal saline solution was prepared containing 20 µCi of ³H-BPAP (22.2 Ci/mmol). From it, 1.3 mL (~17 Ci) was injected as a bolus into the pulmonary circulation through either the distal port of a central vein catheter or the proximal port of a Swan-Ganz catheter. Simultaneously, arterial blood was withdrawn with a peristaltic pump through the radial artery catheter into a fraction collector (1.2 mL blood per tube, 16 tubes). Blood was collected into 1.75 mL of normal saline containing 5 mmol/L EDTA and 6.8 mmol/L 8-hydroxyquinoline-5-sulfonic acid to prevent further activity of blood ACE, and heparin 1000 IU/L (“stop” solution). Four additional tubes containing 1.75 mL stop solution, 1.2 mL blood withdrawn before isotope administration, and 0.02 mL of the isotope mixture were used to calculate the exact amount of radioactivity administered.

Blood samples were collected and centrifuged (3000 rpm, 10 minutes). From the supernatant, a 0.5-mL aliquot of a given sample was transferred into a scintillation vial, and total ³H radioactivity was measured in the presence of 5 mL Ecoscint (National Diagnostics). For the determination of metabolite-associated radioactivity, another 0.5 mL of the supernatant was transferred into a separate vial containing 2.5 mL HCl (0.12N); 3 mL of Toluene-Scintillator (Packard) was added, samples were mixed, and radioactivity was measured 48 hours later. In this way, ~70% of the ³H-BPAP metabolite ³H-benzoyl-Phe (³H-BPhe) and <10% of the parent ³H-BPAP were extracted in the organic phase of the mixture (ie, toluene). The precise values were calculated by identical processing of separate tubes containing substrate or previously synthesized product.

**Enzyme Activity Indices**

PCEB-ACE activity was estimated as υ (³H-BPAP transpulmonary hydrolysis) and A_max/K_m.¹⁶

\[
υ = \frac{v}{c} \times \frac{k_{cat}}{K_m}
\]

\[
A_{max}/K_m = \frac{\text{total enzyme mass} \times k_{cat}}{K_m}.¹⁶,²⁰
\]

Pulmonary plasma flow and cardiac output (CO) were calculated as previously described.²⁰

Under normal lung conditions, A_max/K_m is an index of PCEB-ACE mass and, for ACE that is evenly distributed along the luminal endothelial surface area,⁹ an index of DPCSA in animals⁷,¹⁰,¹¹ and humans.¹⁶ Under toxic conditions (alterations of enzyme expression and/or kinetic constants), A_max/K_m should be considered an index of functional capillary surface area (FCSA),¹⁶ related to enzyme quantity available for reaction (the product of DPCSA and the enzyme mass expressed on the endothelial surface) and to enzyme kinetic constants. In this case, A_max/K_m may not be used as a DPCSA index.

**Statistical Analysis**

Data are presented as mean±SEM and individual values. Paired and Student’s t test, ANOVA followed by the Newman-Keuls’ test, ANCOVA for repeated measures, regression analysis, and Fisher’s exact test were used as appropriate. Pearson’s correlation coefficient (r) and Spearman’s coefficient (rs) are reported where appropriate. Differences were considered significant at a value of P<0.05.
Results

Single/First PCEB-ACE Activity Estimation

Patients’ characteristics, diagnosis, hematocrit, hemodynamics, arterial blood gases, and days of ventilatory failure at PCEB-ACE activity determination are presented in the Table. Ventilatory failure duration was not related to v (r = −0.080, P = 0.657), A\textsubscript{max}/K\textsubscript{m} (r = −0.119, P = 0.509), or any other disease-related parameter (LIS, LIS components, APACHE II, or CO; data not shown). Consequently, ventilatory failure duration did not affect the obtained data.

Arterial outflow concentration curves of total \textsuperscript{3}H and the surviving \textsuperscript{3}H-BPAP through a single transpulmonary pass and the corresponding hydrolysis (v) of \textsuperscript{3}H-BPAP by PCEB-ACE of 2 representative patients are shown in Figure 1. The patient with mLIs (LIS = 1.7) exhibits higher cumulative v (0.63) than the sLI patient (LIS = 2.7; v = 0.2).

PCEB-ACE activity parameters of the 33 patients, grouped according to the American-European Consensus Criteria, \textsuperscript{1} are presented in Figure 2 (top). The only patient who met the criteria for ALI was added to the ARDS group. Both v and A\textsubscript{max}/K\textsubscript{m} were decreased in ALI/ARDS (n = 20) compared with NoALI (n = 13) (0.4 ± 0.05 versus 1.15 ± 0.06, P < 0.01, and 2184 ± 497 versus 6128 ± 693 mL/min, P < 0.01, respectively, by Student’s t test). Similar decreases were present in the 19 ARDS patients (0.39 ± 0.05 and 2226 ± 522 mL/min, P < 0.01 versus the NoALI group).

PCEB-ACE activity values of the 33 patients grouped according to LIS \textsuperscript{17} are presented in Figure 2 (bottom). As LIS increased from 0 (nLI; n = 7) to >2.5 (sLI; n = 8), v and A\textsubscript{max}/K\textsubscript{m} decreased from 1.11 ± 0.09 to 0.3 ± 0.06, P < 0.001, and 6596 ± 768 to 1332 ± 266 mL/min, P < 0.005, respectively, by ANOVA. Significant v differences were observed among all 3 groups, whereas there were no differences in

![Image of Figure 2](http://circ.ahajournals.org/)

![Image of Figure 3](http://circ.ahajournals.org/)
A_{max}/K_m between mLI (n = 18; LIS = 0.1 to 2.5) and sLI (Newman-Keuls’ test).

The negative correlations of individual v and A_{max}/K_m values versus the corresponding LIS are shown in Figure 3: As LIS increased from 0 to 3.7, v decreased linearly from 1.56 to 0.08 (r = -0.827, P < 0.001), and A_{max}/K_m decreased linearly from 10 149 to 385 mL/min (r = -0.684, P < 0.001). The relations of v and A_{max}/K_m with the 3 LIS components are presented in Figure 4: Both v and A_{max}/K_m correlated inversely with chest roentgenogram score (r_s = -0.775, P < 0.001 and r_s = -0.687, P < 0.005, respectively) and PEEP (r_s = -0.646, P < 0.001 and r_s = -0.566, P < 0.001, respectively), whereas they increased linearly with increasing PaO_2/FIO_2 (r = 0.712, P < 0.001 and r = 0.703, P < 0.001, respectively).

An inverse correlation was noted between v, A_{max}/K_m, and APACHE II score (Figure 5; n = 29; r = -0.672, P < 0.001 and r = -0.704, P < 0.001, respectively).

Individual v and A_{max}/K_m values plotted against CO are shown in Figure 6 (left). Hydrolysis (v) appeared to be independent of CO (r = 0.228), whereas a positive linear relationship was observed between A_{max}/K_m and CO (r = 0.731, P < 0.001). However, a multiple linear regression analysis including both enzyme activity parameters revealed that v was associated positively with A_{max}/K_m (P < 0.001) and negatively with CO (P < 0.001).

When A_{max}/K_m values are divided above and below the observed mean (3738 mL/min), they form a high group (13 patients; A_{max}/K_m range, 4378 to 10 149 mL/min) and a low group (20 patients; A_{max}/K_m range, 385 to 3144 mL/min). The relationship of v versus CO, when divided into these groups, is presented in the top right of Figure 6: Regression analysis, adjusting for groups and the group-CO interaction, showed a negative relationship between v and CO (P < 0.01). The low-group slope (b_{low} = -0.093) was steeper but not significantly different from the high-group slope (b_{high} = -0.048). A similar analysis revealed a significantly steeper positive slope for the high group in A_{max}/K_m (Figure 6, bottom right; P < 0.05). When each group was examined separately, v correlated inversely with CO in the low (r = -0.484, P < 0.05) and approached significance in the high group (r = -0.361 and r = -0.101, respectively). Hydrolysis (v) and A_{max}/K_m correlated inversely with chest roentgenogram score in the low group (r_s = -0.477, P < 0.05 and r_s = -0.449, P < 0.05, respectively) and were unrelated to it in the high group (r_s = -0.430 and r_s = 0.311, respectively).
Fourteen patients survived and 19 did not. Twelve survivors belonged in the high group (12/13) and 2 in the low group (2/20), denoting a higher survival rate in the former (Figures 6 and 7; \( P, 0.001 \) with Fisher’s exact test).

Multiple \((n=3)\) PCEB-ACE Activity Estimations
In 11 subjects, as LIS increased significantly from 0.85\(\pm\)0.24 to 1.27\(\pm\)0.29 \((P<0.05)\), \(v\) decreased from 0.86\(\pm\)0.11 to 0.66\(\pm\)0.10 and \(A_{\text{max}}/K_m\) decreased from 5077\(\pm\)970 to 3715\(\pm\)581 mL/min. Hydrolysis \((v)\) correlated inversely to LIS \((P<0.01)\); there was no correlation between LIS and \(A_{\text{max}}/K_m\) (ANCOVA repeated measures).

Technique Reproducibility
In 7 patients with LIS=0, \(v\) and \(A_{\text{max}}/K_m\) values were similar in the first and second PCEB-ACE activity estimation (1.14\(\pm\)0.18 versus 1.08\(\pm\)0.15 and 4450\(\pm\)788 versus 4104\(\pm\)639 mL/min, respectively, by paired \(t\) test), with tight repeatability in each patient (6\% and 8\%, respectively).

Figure 6. Left, Relationship of BPAP hydrolysis \((v; \text{top})\) and positive correlation of \(A_{\text{max}}/K_m\) values (bottom) with CO. Right, Relationships of BPAP hydrolysis \((v; \text{top})\) and \(A_{\text{max}}/K_m\) values (bottom) with CO after data division in high and low groups. Circles indicate high group subjects; inverted triangles, low group subjects; open symbols, nonsurvivors; and solid symbols, survivors. Linear curves represent least-squares lines.

Figure 7. Relationships of BPAP hydrolysis \((v; \text{top})\) and \(A_{\text{max}}/K_m\) values (bottom) with \(Pao_2/Fio_2\) (left) and chest roentgenogram scores (right) after data division in high and low groups. Circles indicate high group subjects; inverted triangles, low group subjects; open symbols, nonsurvivors; and solid symbols, survivors. Linear curves represent least-squares lines.
Discussion

Pulmonary endothelium possesses important metabolic activities, which may be altered early in ALI and may contribute to its progression. Numerous investigations of endothelium-related mediators of ALI in animals and humans have provided insights into the pathogenesis of the syndrome and have examined potential predictors of either ARDS development in high-risk patients or outcome. In this respect, pulmonary endothelium-mediated extraction of serotonin correlated with ARDS severity, whereas pulmonary propranolol extraction was decreased in patients at risk. More recently, the net balance between pulmonary clearance and release of endothelin-1, which is produced by endothelial cells and extracted by pulmonary endothelium, was decreased early in ALI, whereas it reversed in patients who recovered. Other markers of endothelial dysfunction in ALI/ARDS include plasma levels of von Willebrand factor, soluble endothelium-derived adhesion molecules (E- and P-selectins, intracellular adhesion molecule-1), and plasma soluble-ACE activity. The endothelial markers thus far identified, however, have not been useful to predict ARDS development in multiple at-risk patients. Consequently, the need for new methods of directly assessing pulmonary endothelial function in humans still exists.

In this study, using indicator-dilution techniques, we have estimated PCEB-ACE activity in vivo in critically ill patients. Animal studies have shown that PCEB-ACE dysfunction is an early and sensitive index of ALI induced by insults such as bleomycin, oxygen toxicity, phorbol esters, and chest irradiation. In humans without lung disease, there is evidence for homogeneous PCEB-ACE concentrations and \( t_c \) in both lungs. In that study, BPAP hydrolysis appeared to be independent of CI, within the normal CI range, indicative of similar \( t_c \) among subjects. PCEB-ACE parameters showed a relatively broad range of values among subjects, with tight reproducibility in each individual. A similar pattern was obtained in our 7 patients with LIS = 0, further confirming the repeatability of the technique.

Two typical determinations of the single-pass transpulmonary BPAP hydrolysis in 1 mL patient and 1 sLI patient are given in Figure 1. The former exhibited a moderate decrease of \( v \); the latter, a profound depression of PCEB-ACE activity. Sample-to-sample hydrolysis (instantaneous \( v \)) appears to fluctuate in both patients. Instantaneous \( v \) should reflect ACE activity within individual groups of capillaries and may be influenced by substrate transit time, enzyme concentration, and kinetic constants. The pattern in both patients may reflect the presence of a mild heterogeneity, possibly related to pathway alterations, including flow alterations, in remodeled microvessels. Alternatively, different capillary groups in the same patient might exhibit various degrees of kinetic constant or enzyme concentration alterations. The latter would be consistent with the nonhomogeneous lung pathology in ALI/ARDS.

Patients were grouped according to the American-European Consensus criteria to allow comparisons with other studies: PCEB-ACE activity parameters were decreased in patients with ALI/ARDS compared with NoALI patients (Figure 2), suggesting pulmonary endothelial dysfunction. BPAP \( v \) and \( A_{\text{max}}/K_m \) values in NoALI patients were similar to values reported in humans with no lung disease (1.29±0.14 and 4564±425 mL/min), possibly suggesting unaltered pulmonary endothelial function in these high-risk, mechanically ventilated patients.

The use of LIS introduced by Murray et al allows quantification of the clinical severity of lung injury. PCEB-ACE activity was already altered at the stage of mL (Figures 2 and 3), suggesting that pulmonary endothelial dysfunction might occur early during the ALI continuum. The negative correlation of \( v \) and \( A_{\text{max}}/K_m \) with increasing LIS denotes that the clinical severity of the syndrome is related to the degree of PCEB-ACE activity depression and consequently to the underlying pulmonary endothelial dysfunction. This is further supported by the sustained negative correlation of \( v \) with LIS over time in the subjects who underwent sequential studies. The lack of correlation between LIS and \( A_{\text{max}}/K_m \) should be related to variations of FCSA during the course of the disease.

The relationships between PCEB-ACE activity and the individual LIS parameters studied may be indicative of the pulmonary endothelial contribution to the pathogenetic process (Figure 4): The negative correlation of PCEB-ACE activity with the chest roentgenogram score suggests that greater endothelial dysfunction might be related to increased vascular permeability and the subsequent pulmonary edema revealed on chest radiographs. The positive correlation of PCEB-ACE activity with \( P_aO_2/F_iO_2 \) may be related to endothelium-mediated edema formation, altered flow in remodeled microvessels, gas diffusion abnormalities, and metabolic alterations of vasoactive peptide synthesis or clearance, all of which promote intrapulmonary shunting. Hydrolysis \( (v) \) increases linearly with increasing \( P_aO_2/F_iO_2 \) and reaches a plateau after 300 mm Hg, which appears to represent the optimal substrate hydrolysis, in the absence of ALI/ARDS. The negative correlation of PCEB-ACE activity with PEEP might reflect the underlying clinical severity that necessitates high PEEP application. It might also reflect decreased capillary recruitment secondary to the increased thoracic pressure and the subsequent fall of right ventricular preload. However, this should have induced a fall in CO; the fact that CO was not related to PEEP \( (r = -0.244) \) makes this explanation less likely.

The APACHE II classification system validates disease severity and allows estimations of patient prognosis in a general ICU population. The negative correlation between \( v \), \( A_{\text{max}}/K_m \), and the APACHE II score (Figure 5) indicates the relationship between pulmonary endothelial dysfunction and the underlying pulmonary and extrapulmonary disease severity. How might pulmonary endothelium be related to extrapulmonary disease? Endothelial dysfunction may be part of a cytokine-induced panendothelial injury, which might lead to multiple organ failure. ALI would thus be the pulmonary manifestation of this generalized syndrome. Alternatively, pulmonary endothelial metabolic alterations may induce the presence of cytotoxic mediators in the systemic circulation, thus promoting extrapulmonary injury.

Under normal conditions, transpulmonary hydrolysis was independent of CO in animals and humans. Repeated PCEB-ACE activity determinations performed in brain-dead subjects with LIS = 0, at different cardiac outputs in each subject, showed unchanged \( v \) over a wide range of CO (3.3 to 14.1 L/min), whereas \( A_{\text{max}}/K_m \) increased linearly with flow. This implies that higher pulmonary blood volumes are accommo-
dated mainly through parallel recruitment of capillaries with similar enzyme concentrations and \( \tau \). In our ALI/ARDS patients, the apparent independence of \( v \) from CO occurs because \( A_{\text{max}}/K_m \) acts as a confounding factor (Figure 6). When both enzyme parameters were included in the analysis or when patients were divided into high and low groups, \( v \) correlated inversely with CO, suggesting decreasing \( \tau \) at higher CO. This probably reflects vascular loss occurring from occluded or obliterated vessels or metabolic alterations affecting vascular tone. When all accessible capillaries are recruited, increases in blood flow result in lower \( v \) and consequently lower \( v \).

The division into high and low groups, above and below the observed \( A_{\text{max}}/K_m \) mean, reveals 2 different profiles in the \( A_{\text{max}}/K_m \) versus CO relationship (Figure 6): In the high group, higher CO is related to higher \( A_{\text{max}}/K_m \). This might suggest that reserves of healthy or mildly injured vascular bed are present and blood volume increases are accommodated, at least in part, through capillary recruitment. In the low group, the absence of an \( A_{\text{max}}/K_m \) increase with higher CO suggests that higher blood volumes should be accommodated mainly through dysfunctional capillaries, whereas no FCAS reserves are available to be recruited. The fact that \( v \) has a slope that is steeper than, although not significantly different from, the high group does not contradict the above hypothesis and should reflect the expected greater dependence of \( v \) on \( \tau \).

The division into subgroups makes apparent significantly different profiles in \( v \) and \( A_{\text{max}}/K_m \) versus indices of clinical severity such as PAo/Fio2, and the chest roentgenogram score (Figure 7): In the low group, higher enzyme parameters are related to better values of both indices, confirming the relation of PCEB-ACE activity to the clinical severity; in the high group, the lack of a relationship might suggest that enzyme activity is close to optimal, revealing the healthier status of these patients.

In our population, 12 of 14 survivors belong in the high \( A_{\text{max}}/K_m \) group; 9 among the former have no ALI (Figures 6 and 7). These healthier survivors have fewer failing organs, a more mildly injured pulmonary vascular bed, and consequently more adequate pulmonary endothelial function, indicated by high \( A_{\text{max}}/K_m \) values.

In summary, our study demonstrates that assessing pulmonary endothelial ACE activity in ICU patients, at the bedside, by means of indicator-dilution type techniques provides a safe, direct, and quantifiable index of pulmonary endothelial dysfunction in ALI. PCEB-ACE activity, ie, pulmonary endothelial function, is altered at an early stage of ALI and correlates with the severity of both lung injury and the underlying disease. This study provides new insights into ALI/ARDS pathophysiology.

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