Possible Prognostic Value of Leukotriene B₄ in Acute Respiratory Distress Syndrome

Joan R Masclans MD PhD, Joan Sabater MD, Judit Sacanell MD, Pilar Chacon MD PhD, Pilar Sabin MD PhD, Oriol Roca MD, and Mercè Planas MD PhD

OBJECTIVE: To study the major eicosanoids implicated in the pathophysiology of acute respiratory distress syndrome (ARDS) in order to estimate their relative prognostic values. METHODS: We conducted a prospective study in a consecutive series of patients with ARDS admitted to a university hospital intensive care unit. We measured the plasma concentrations of 3 inflammatory mediators (thromboxane B₂, 6-keto prostaglandin F₁α, and leukotriene B₄) in peripheral arterial and mixed venous plasma samples. RESULTS: We studied 16 patients with ARDS, who had a mean ± SD baseline ratio of PₐO₂ to fraction of inspired oxygen (PₐO₂/F(IO₂)) of 147 ± 37 mm Hg and a mean ± SD baseline lung injury score of 2.9 ± 0.37. The plasma concentrations of thromboxane B₂, 6-keto prostaglandin F₁α, and leukotriene B₄ were greater than the general-population reference levels in both arterial and mixed venous plasma, but only leukotriene B₄ was higher in arterial plasma than in mixed venous plasma (401 ± 297 pg/mL vs 316 ± 206 pg/mL, p = 0.04). When we correlated the eicosanoid concentrations with specific indicators of clinical severity, we found correlations only between the baseline PₐO₂/F(IO₂) and the arterial thromboxane B₂ level (r = −0.57, p = 0.02), the arterial leukotriene B₄ level (r = −0.59, p = 0.01), and the transpulmonary gradient of leukotriene B₄ level (r = −0.59, p = 0.01). We also found a correlation between the transpulmonary gradient of leukotriene B₄ and the lung injury score (r = 0.51, p = 0.04). The thromboxane B₂ concentration in arterial plasma and the leukotriene B₄ concentration in both arterial and mixed venous plasma were the only baseline plasma eicosanoid concentrations that predicted significant differences in outcome. When looking at the transpulmonary gradient of the eicosanoids studied, we found that only the gradient of leukotriene B₄ showed significant differences of clinical interest. Among survivors we observed practically no gradient (~4.9%), whereas among nonsurvivors we found a substantial positive gradient of 41.6% for the elevated arterial (post-pulmonary) values, compared with the pulmonary-artery (pre-pulmonary) values, and this difference was statistically significant (p = 0.02). CONCLUSIONS: The pro-inflammatory eicosanoid leukotriene B₄ showed the best correlation with lung-injury severity and outcome in patients with ARDS. Key words: acute respiratory distress syndrome, ARDS, leukotriene B₄, thromboxane B₂, prostacyclin, prognostic value, outcome. [Respir Care 2007;52(12):1695–1700. © 2007 Daedalus Enterprises]

Introduction

Acute respiratory distress syndrome (ARDS) is characterized by severe acute respiratory insufficiency, and each year it may affect as many as 200,000 people in the United States alone. Despite recent advances in mechanical ventilation, ARDS is associated with great (co)morbidity and
greater than 40% mortality. A large number of factors have been implicated in the development of ARDS. Examples include various vasoactive substances and agents that promote cell aggregation and modify permeability, such as histamine, serotonin, the cytokine system, and some lipid mediators (prostaglandins, leukotrienes, and platelet-activating factor). The use of biological markers to establish prognostic indicators in ARDS should improve outcome prediction and optimize therapeutic decisions.

Arachidonic acid is a polyunsaturated essential fatty acid that is converted to various biologically active derivatives, including lipid mediators, also known as eicosanoids. The cyclooxygenase enzyme adds 2 oxygen molecules to form endoperoxide G2, the common precursors of eicosanoids, known as prostanoids. These constitute a chemical family of cyclic compounds (prostaglandins and thromboxanes). If one molecule of oxygen is added, the lipoxygenase enzyme facilitates the synthesis of other metabolically active eicosanoids called leukotrienes.

The lungs are a major site of eicosanoid synthesis. Prostaglandin I2 (also known as prostacyclin) and thromboxane A2 are considered the most important arachidonic acid derivatives in the lungs. Thromboxane A2 increases pulmonary vascular resistance, whereas prostacyclin dilates the pulmonary vascular bed. Leukotriene B4 is the major arachidonic acid derivative produced via 5-lipoxygenase, and it is released by alveolar macrophages and neutrophils. Unlike with other compounds produced via similar metabolic pathways (leukotrienes C4, D4, and E4), which are potent bronchoconstrictors, the main effect of leukotriene B4 is related to the inflammatory response, and it facilitates capillary extravasation. Antonelli et al suggested that leukotrienes may play an important role in the pathophysiological chain of events that starts with a cellular lesion and may constitute a key factor in the development of ARDS.

The pulmonary hypertension observed in ARDS could be due to the action of various vasoconstrictor lipid mediators (thromboxane A2 in particular) and to blockade of vasodilator prostaglandins, with an apparent role as modulators of pulmonary hypertension (prostaglandin I2 in particular). These conclusions are based on observations in animals and humans. Blockade or modulation of these arachidonic acid derivatives might have a role in the management of ARDS. Clinical studies have looked at the effects of blockade of the formation of vasoconstrictor prostaglandins at various points in the inflammatory pathway—mostly aiming to block phospholipase A and cyclooxygenase—and at the effects of stimulating an increase in the concentrations of vasodilator prostaglandins. However, clinical efficacy results were inconclusive. Our study group has worked on this topic before, studying the possible modulation of eicosanoids as a function of fatty acid intake in parenteral nutrition, as well as the potential hemodynamic effects such fat emulsions may have in the presence of an imbalance between those mediators. Although the prognostic values of baseline eicosanoid concentrations for outcome prediction in ARDS previously observed by us are weak, there is clearly a tendency for such correlations.

The objective of the present study was to determine the plasma concentrations of major arachidonic acid derivatives potentially involved in the pathophysiology of ARDS, and to estimate the prognostic value of these mediators for outcome prediction in ARDS.

**Methods**

**Patients**

We conducted a prospective study in a consecutive series of 16 patients with ARDS admitted to the ICU of the Hospital Universitari Vall d’Hebron, Barcelona, Spain, within the first 48 hours that they met the diagnostic criteria of ARDS of the American-European Consensus Conference on ARDS. The study was approved by the appropriate ethics committee.

**Outcome Measures**

The following variables were recorded/calculated: baseline ratio of PaO2 to fraction of inspired oxygen (PaO2/FIO2) at the time the patient met the ARDS diagnostic criteria, lung injury score, Acute Physiology and Chronic Health Evaluation (APACHE II) score, number of days on mechanical ventilation, duration of ICU stay, and survival.

**Eicosanoid Assays**

We determined thromboxane B2, a stable metabolite of the potent vasoconstrictor thromboxane A2, and 6-keto prostaglandin F1α, a stable metabolite of the vasodilator prostacyclin, as typical arachidonic acid derivatives of the cyclooxygenase pathway, and leukotriene B4, a potent pro-inflammatory compound, as an arachidonic acid derivative of the lipoxygenase pathway.

These eicosanoids were determined in arterial plasma (post-pulmonary) and in mixed venous plasma (pre-pulmonary) by electroimmunoassay (Amersham International, Buckinghamshire, United Kingdom). We used EDTA (ethylenediaminetetraacetic acid) as an anticoagulant, to avoid “in-plasma” activation of leukocytes by indomethacin.
For each study eicosanoid we calculated the arterial/mixed-venous (transpulmonary) gradient to provide a non-invasive measure of any specific intrapulmonary behavior of these compounds. This gradient was calculated by subtracting the eicosanoid concentration in mixed venous plasma from that in arterial plasma.

The arterial plasma samples were drawn from a peripheral arterial catheter while the mixed venous plasma samples were obtained from a Swan-Ganz catheter.

The reference ranges used were those determined in healthy individuals by the same laboratory in our institution.

Statistical Analysis

The data were imported from a database (Access, Microsoft, Redmond, Washington) to statistics software (SPSS, SPSS, Chicago, Illinois). The following statistical variables were calculated for numerical variables: mean, standard deviation, and range (where appropriate). Differences between the means were analyzed via the Mann-Whitney U test for independent samples and the Wilcoxon signed rank test for related samples. Correlations were analyzed via Spearman’s correlation coefficient. The level of statistical significance was defined as \( p < 0.05 \).

Results

Of the 16 patients studied, 14 were men (87.5%). Their mean age was 55 ± 16 y. At the time of meeting the diagnostic criteria for ARDS, the patients had a mean ± SD PaO2/FIO2 of 147 ± 37 mm Hg, a mean ± SD lung injury score of 2.9 ± 0.37, and a mean ± SD APACHE II score of 14 ± 3. Seventy-five percent of the patients had ARDS for both types of plasma sample. The thromboxane B2 concentration in both arterial and mixed venous plasma was significantly higher than that in pulmonary artery (mixed venous) plasma (401 ± 297 pg/mL vs 316 ± 206 pg/mL, \( p = 0.04 \)).

When we correlated the eicosanoid concentrations with specific indicators of clinical severity (both mixed venous and arterial), we found correlations only between the baseline arterial thromboxane B2 concentration (\( r = -0.57, p = 0.02 \)), arterial leukotriene B4 concentration (\( r = -0.59, p = 0.01 \)) (Fig. 1), and the transpulmonary gradient of leukotriene B4 (\( r = -0.59, p = 0.01 \)). We also found a correlation between the transpulmonary gradient of leukotriene B4 and the lung injury score (\( r = 0.51, p = 0.04 \)).

Figures 2, 3, and 4 show the mean baseline concentrations of the 3 study eicosanoids as a function of outcome for both types of plasma sample. The thromboxane B2 concentration in arterial plasma and the leukotriene B4 concentration in both arterial and mixed venous plasma were the only baseline plasma eicosanoids to predict significant differences in outcome.

When looking at the transpulmonary gradient of the eicosanoids studied, we found that only the transpulmonary gradient of leukotriene B4 showed significant differ-

Table 1. Plasma Eicosanoid Concentrations

<table>
<thead>
<tr>
<th>Eicosanoid</th>
<th>Plasma Concentration (mean ± SD pg/mL)</th>
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<tbody>
<tr>
<td>Thromboxane B2</td>
<td>&lt;150</td>
</tr>
<tr>
<td>Leukotriene B4</td>
<td>&lt;75</td>
</tr>
<tr>
<td>6-Keto Prostaglandin F1α</td>
<td>&lt;50</td>
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<table>
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<tr>
<th>Reference (normal) value</th>
<th>Baseline arterial</th>
<th>Baseline mixed venous</th>
<th>p for baseline arterial versus mixed venous plasma</th>
<th>p for arterial mixed venous versus reference values</th>
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<tr>
<td></td>
<td>284 ± 127</td>
<td>401 ± 297</td>
<td>0.06</td>
<td>0.02</td>
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<tr>
<td></td>
<td>329 ± 139</td>
<td>316 ± 206</td>
<td>0.04</td>
<td>0.01</td>
</tr>
<tr>
<td>p</td>
<td>0.06</td>
<td>0.04</td>
<td>0.36</td>
<td>0.001</td>
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ences of clinical interest (Fig. 5). Among survivors we observed practically no gradient (−4.9%), whereas among nonsurvivors we found a substantial positive gradient (41.6%) for the elevated arterial values compared with the mixed venous values, and this difference was statistically significant at p = 0.02.

**Discussion**

We studied a series of relatively young patients (mean age 55 y) with severe acute lung injury, as demonstrated by a mean lung injury score of 2.9 ± 0.37 and a baseline $P_{A}O_{2}/F_{I}O_{2}$ of 147 ± 37 mm Hg, whereas mortality was similar to that of series with a better prognosis.

Our results show that, though the plasma concentrations of all study eicosanoids were higher than those in healthy controls, only the plasma levels of thromboxane B$_2$ and leukotriene B$_4$ were correlated with lung injury severity, and may have predictive value for outcome in patients with ARDS.

Apart from their basic gas-exchange function, the lungs are also a site where metabolic processes take place. In fact, the lungs are involved in both the synthesis and breakdown of various inflammatory mediators, such as the phospholipid derivatives commonly known as eicosanoids. The arachidonic acid derivatives (eicosanoids) of the cyclooxygenase pathway include thromboxanes and prostaglandins (prostacyclin), whereas leukotrienes are arachidonic acid derivatives of the lipoxygenase pathway. Platelet-activating factor is another compound that is derived from membrane phospholipids and might also have a role in the pathophysiology of inflammation, but its metabolism uses a different enzymatic pathway.

ARDS is associated with a wide variety of pulmonary and nonpulmonary risk factors that may impact the development and outcome of the syndrome. Factors produced in the arachidonic acid cascade are involved in the pathophysiology of ARDS. Whereas thromboxane and prostacyclin have traditionally been thought to have key roles in lung-injury formation, recent evidence suggests that arachidonic acid derivatives of the lipoxygenase pathway may have a more central role here.

Early evidence that implicated eicosanoids in the pathophysiology of ARDS came from studies in which the cyclooxygenase pathway was blocked. Although there have been isolated reports that cyclooxygenase blockade is associated with a potential improvement in pulmonary gas exchange, no study could definitively establish the clinical efficacy of this approach. It has also been postulated that corticosteroid use in patients with ARDS, with the aim of blocking the inflammatory cascade by blocking the phospholipase enzyme, might improve outcomes in patients.
with ARDS, but this intervention also produced negative results. Using a different tack, a number of authors tried stimulating an increase in the plasma concentrations of vasodilator prostaglandins, but the clinical utility of this approach could not be demonstrated.

However, leukotrienes in general, and leukotriene B₄ in particular, play a major role in leukocyte chemotaxis in the lungs, which in turn might trigger the remainder of the inflammatory cascade. In fact, elevated leukotriene concentrations have been found in both the bronchoalveolar lavage fluid and the plasma of patients with lung injuries, as well as those at risk of developing lung injury. In a recent paper, Caironi et al reported a central role of lipoxygenase pathway derivatives in the development of acute lung injury.

Unlike other studies that looked only at one particular eicosanoid, we determined the pre-pulmonary and post-pulmonary concentrations of the major biologically active arachidonic acid derivatives produced along both major enzymatic pathways (cyclooxygenase and lipoxygenase). We could dispense with bronchoalveolar lavage (which frequently causes alterations, albeit usually transient ones) because we determined not only absolute plasma eicosanoid concentrations but also the transpulmonary gradient of eicosanoid levels, which is an indicator of potential prognostic value.

Our results show that, though the plasma concentrations of all study eicosanoids were above normal, significant differences between arterial and pulmonary-artery plasma levels were found only for leukotriene B₄.

When we correlated eicosanoid concentrations with specific indicators of ARDS severity, we found correlations between the degree of hypoxia (as measured by the baseline Pₐₐₒ₂/FICO₂) and arterial thromboxane B₂ concentration, arterial leukotriene B₄ concentration, and the transpulmonary gradient of leukotriene B₄ levels. We also found a correlation between the transpulmonary gradient of leukotriene B₄ and the lung injury score. In other words, greater arterial thromboxane B₂ and/or leukotriene B₄ levels are associated with a lower Pₐₐₒ₂/FICO₂ (ie, greater hypoxemia), and a greater transpulmonary gradient is associated with greater ARDS severity.

With regard to outcome prediction, we found greater mortality among patients with greater baseline arterial thromboxane B₂ and leukotriene B₄ concentrations, greater baseline leukotriene B₄ concentrations in mixed venous plasma, and a greater transpulmonary gradient of leukotriene B₄ levels.

Given the limitations of our small but homogeneous series of patients, we can thus conclude from our observations that prostacyclin probably has a very minor role, compared with other eicosanoids. Thromboxane B₂ would appear to play an intermediate role, whereas leukotriene B₄ probably is a pro-inflammatory substance that has a fundamental role in the inflammatory cascade implicated in the development and outcome of ARDS. Moreover, as emerges from Figure 5, the observed differences between pre-pulmonary and post-pulmonary (transpulmonary gradient) leukotriene B₄ concentrations suggest that the amount of leukotriene B₄ in the lungs at the time the diagnosis of ARDS is established is a marker of poor outcome.

Conclusions

In this study we found evidence that supports the predictive utility of the transpulmonary gradient of these arachidonic acid derivatives, compared with measurements obtained from bronchoalveolar washings, which, in clinical practice, may be difficult to obtain because of the patient’s critical condition. However, the exhaled-breath condensate method may be another interesting noninvasive technique in this difficult clinical situation.
It is interesting to note that more than 75% of our patients presented with an intrapulmonary lung injury as the ARDS cause. It is difficult to extrapolate these results to another population. Because this is an exploratory study, the specific knowledge of the clinical utility of leukotriene B₄ measurements could require a prospective study for validation. In this way, research into the possible modulation of the lipoxygenase pathway may also be a promising future approach to further improving the outcome of patients with ARDS.

REFERENCES