Eicosanoids as Risk and Prognostic Factors for Acute Respiratory Distress Syndrome in Sepsis Patients

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Abstract

Although a number of studies have reported elevated levels of eicosanoids in acute lung injury with sepsis, the possibility that eicosanoids may act as risk and prognostic factors for sepsis patients who develop acute respiratory distress syndrome (ARDS) remains poorly studied. To clarify this aspect, we measured the levels of eicosanoids and used logistic regression analysis and receiver operating characteristic (ROC) curves to investigate whether eicosanoids could act as risk and prognostic factors for sepsis patients who develop ARDS. We conducted a case-control study comparing 13 sepsis patients with ARDS and 23 sepsis patients without ARDS. The plasma levels of leukotriene B4 (LTB4), 6-keto-prostaglandin F1α (6-keto-PGF1α) and thromboxane B2 (TXB2) were measured by radioimmunoassays as substitutes for the plasma levels of PGI2 and TXA2, which are unstable. The levels of eicosanoids in sepsis patients with ARDS were significantly higher than those in sepsis patients without ARDS. Logistic regression analysis revealed that LTB4 was the only risk factor for sepsis patients with ARDS (odds ratio, 1.10; P=0.02). The area under the ROC curve values for all eicosanoids were significantly greater than 0.5 (P<0.001), and the likelihood value for the TXB2 levels was higher than those of the other eicosanoids. We conclude that LTB4 may be an important risk factor for sepsis patients with ARDS, while TXA2 may be an important prognostic factor for sepsis patients with ARDS.

Keywords: Leukotriene B4; Thromboxane A2; Prostaglandin I2; Lung injury; Logistic regression analysis; Receiver operating characteristic curve

Introduction

Acute respiratory distress syndrome (ARDS) is known to be associated with systemic inflammatory response syndrome (SIRS) through sepsis, and the inflammatory response is produced by inflammatory cells and/or endotoxins. Various inflammatory mediators lead to a critical event in the development of organ injury in the microvessels of organs, especially the lungs. Leukotrienes, thromboxanes and prostanoids, collectively referred to as eicosanoids, have been reported to be involved in sepsis [1-4], burns [5] and ARDS [6,7]. Leukotrienes contribute to pulmonary edema by inducing neutrophil influx and activation and increasing vascular permeability. Leukotriene B4 (LTB4), which is derived by 5-lipoxygenation of arachidonic acid, is synthesized by neutrophils, monocytes, macrophages and endothelial cells, and promotes neutrophil attachment to the vascular endothelium and lung injury [8]. Thromboxane A2 (TXA2) mediates the aggregation and release mechanism of platelets and is also a potent vasoconstrictor [9], while prostaglandin I2 (PGI2) is a potent endogenous inhibitor of platelet aggregation and a vasodilator [10]. Thus, eicosanoids are involved in the pathological process of lung injury. However, few studies have clinically and systematically investigated the pathophysiological changes in sepsis patients who develop ARDS. In the present study, we measured the plasma levels of the eicosanoids LTB4, TXB2 and 6-keto-PGF1α and used logistic regression analysis and receiver operating characteristic (ROC) curves to determine which eicosanoids act as risk and prognostic factors for sepsis patients who develop ARDS.

Materials and Methods

After obtaining informed consent from the patients or their families and approval to conduct the study from the Ethics Committee of our hospital, 36 adult sepsis patients were enrolled and assigned into 23 ARDS-free patients (14 males and 9 females; mean age, 57.78 years; age range, 51.08-64.48 years) and 13 ARDS patients (10 males and 3 females; mean age, 59.23 years; age range, 49.51-68.96 years). There were no significant differences between the groups regarding age or sex. The criteria for the diagnosis of ARDS were: PaO2/FiO2 (P/F ratio) ≤ 200 mmHg; absence of cardiac failure; pulmonary edema-like shadows on a chest X-ray; and pulmonary artery wedge pressure ≤ 18 mmHg when measured [11]. The criterion for the diagnosis of sepsis was a systemic response to infection, manifested by two or more of the following conditions: body temperature >38°C or <36°C; heart rate>90 beats/minute; respiratory rate>20 breaths/minute or PaCO2<32 mmHg; and white blood cell count>12,000/mm3 or<4000/mm3; or >10% immature (band) forms [12]. All the patients in the ARDS group had been endotracheally intubated and were on mechanical ventilation in the synchronized intermittent mandatory ventilation mode under intravenous sedation with midazolam 3-4 mg•h-1 and buprenorphine 0.03 mg•h-1.

The methods for measuring the plasma eicosanoid levels were reported previously [5]. Briefly, blood samples were obtained from the ARDS patients within 24 h of ARDS onset and from the ARDS-free patients on the day of admission to the intensive care unit. All blood samples were collected into tubes containing indomethacin-supplemented 4.5 mM ethylenediaminetetraacetic acid (EDTA) and centrifuged at 3000 rpm for 15 min to obtain the plasma. For the LTB4 assay, the plasma was mixed with a mixture ofethyl acetate and methanol (2:1) and centrifuged at 3000 rpm for 15 min to obtain the supernatant. All plasma samples were stored at -80°C until analysis. LTB4, 6-keto-PGF1α and TXB2 were measured by radioimmunoassays (MEN Research Products, DuPont, Boston, MA). The normal ranges...
for the levels of LTB₄, 6-keto-PGF₁α, and TXB₂ were 77.0 ± 15.7, 12-33 and 14-50 pg/ml, respectively. ROC curves for the eicosanoids were constructed for the sepsis patients with ARDS. We plotted the true-positive rate (sensitivity) on the vertical axis of the graph against the false-positive rate (1-specificity) on the horizontal axis for each threshold. An ideal test should have high sensitivity and low (1-specificity), and therefore produce a curve close to the upper left corner of the graph. The best cut-off points for eicosanoids as prognostic factors were identified as those values that simultaneously maximized the sensitivity and specificity. The likelihood ratios (LRs), defined as the ratio of the frequency of a finding among the diseased patients (true-positive rate) to the frequency of the finding among the non-diseased patients (false-positive rate), and the sensitivity/(1-specificity) ratios were also calculated. A true diagnostic test usually has an LR>10, while an exclusion test has an LR<1. The data are reported as means and 95% confidence intervals (CIs). Mann-Whitney tests were used for comparisons of the eicosanoid levels between ARDS and ARDS-free patients. All analysis were performed using the StatView J4.5 software (Abacus Concepts, Berkeley, CA). The ROC curves and logistic regressions were analyzed using SPSS (Chicago, IL). Values of P<0.05 were considered statistically significant.

Results

Table 1 compares the eicosanoid levels in the ARDS and ARDS-free patients. The levels of all three factors were significantly higher in the ARDS patients than in the ARDS-free patients (LTB₄, P<0.0001; TXB₂, P<0.0001; 6-keto-PGF₁α, P<0.0001). Table 2 shows the logistic regression analysis for the eicosanoids in the ARDS patients. The values of the odds ratios for LTB₄, TXB₂, and 6-keto-PGF₁α were 1.10 (95% CI, 1.01-1.19; P=0.02), 1.04 (95% CI, 0.98-1.11; P=0.17) and 0.99 (95% CI, 0.83-1.19; P=0.95), respectively. Table 3 and Figure 1 show the ROC curves for the eicosanoids generated for the ARDS patients. The area under the ROC curve values for LTB₄, TXB₂ and 6-keto-PGF₁α were 0.933*, 0.905* and 0.878*, respectively. *P<0.001 for AUC vs. 0.50. Table 4 shows the best cut-off points, sensitivities, specificities and LR values. The LR values for LTB₄, TXB₂, and 6-keto-PGF₁α were 7.95, 14.30 and 12.37, respectively.

![Figure 1: Receiver operating characteristic (ROC) curves for sepsis patients with ARDS. The area under the ROC curve (AUC) values for LTB₄, TXB₂ and 6-keto-PGF₁α are 0.933*, 0.905* and 0.878*, respectively. *P<0.001 for AUC vs. 50.](image)

Discussion

We measured the plasma levels of three eicosanoids in sepsis patients, and determined whether any of these eicosanoids could act as risk and prognostic factors for sepsis patients who develop ARDS. Our study had four main findings: 1) the levels of all three eicosanoids were significantly higher in the ARDS patients than in the ARDS-free patients; 2) only LTB₄ was a risk factor for sepsis patients with ARDS; 3) all three eicosanoids were specific factors for ARDS; and 4) the likelihood value of the TXB₂ levels was higher than those of the other eicosanoids.

The present study provides information that will be useful for elucidating the process and mechanism of lung injury and for establishing therapeutic strategies in the future. Receptor antagonist and/or synthetase inhibitor analysis have been carried out in patients with ARDS. Thromboxane synthesis inhibitors and/or antagonists improved the acute cardiopulmonary effects of bolus endotoxin [13-15] and increased patient survival [16-18]. The thromboxane A₂ synthesis inhibitor ketoconazole was found to significantly reduce the rate of ARDS development in sepsis patients and decrease their mortality rate [19]. In contrast, ketoconazole was not found to reduce the mortality or duration of mechanical ventilation in a randomized trial [20]. The beneficial effects of ketoconazole may be attributed to the inhibition of leukotriene formation as well as thromboxane A₂ synthesis [7]. The results of the present study revealed that the likelihood value of the TXB₂ levels was higher than those of the other eicosanoids examined.

**Figure 1:** Receiver operating characteristic (ROC) curves for sepsis patients with ARDS. The area under the ROC curve (AUC) values for LTB₄, TXB₂ and 6-keto-PGF₁α are 0.933*, 0.905* and 0.878*, respectively. *P<0.001 for AUC vs. 50.

**Table 4:** Best cut-off points.

<table>
<thead>
<tr>
<th>Eicosanoids</th>
<th>Cut-off Point</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>LR</th>
</tr>
</thead>
<tbody>
<tr>
<td>LTB₄ (pg.ml⁻¹)</td>
<td>103.9</td>
<td>0.69</td>
<td>0.91</td>
<td>7.95</td>
</tr>
<tr>
<td>TXB₂ (pg.ml⁻¹)</td>
<td>83</td>
<td>0.82</td>
<td>0.96</td>
<td>14.3</td>
</tr>
<tr>
<td>6-keto-PGF₁α (pg.ml⁻¹)</td>
<td>35</td>
<td>0.54</td>
<td>0.96</td>
<td>12.37</td>
</tr>
</tbody>
</table>

**Table 3:** ROC curve analysis of eicosanoids for ARDS in sepsis patients.

**Table 2:** Logistic regression analysis of eicosanoids for ARDS in sepsis patients.

<table>
<thead>
<tr>
<th>Eicosanoids</th>
<th>Odds Ratio</th>
<th>95% Confidence Interval</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LTB₄</td>
<td>1.10</td>
<td>1.01-1.19</td>
<td>0.02</td>
</tr>
<tr>
<td>TXB₂</td>
<td>1.04</td>
<td>0.98-1.11</td>
<td>0.17</td>
</tr>
<tr>
<td>6-keto-PGF₁α</td>
<td>0.99</td>
<td>0.83-1.19</td>
<td>0.85</td>
</tr>
</tbody>
</table>

**Table 1:** Comparisons of eicosanoids in the ARDS and ARDS-free sepsis patients.

<table>
<thead>
<tr>
<th>Eicosanoids</th>
<th>ARDS (-)</th>
<th>ARDS (+)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LTB₄ (pg.ml⁻¹)</td>
<td>62.7 (52.0-73.4)</td>
<td>118.3 (101.8-134.6)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>TXB₂ (pg.ml⁻¹)</td>
<td>38.1 (26.5-49.7)</td>
<td>94.2 (71.0-117.3)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>6-keto-PGF₁α (pg.ml⁻¹)</td>
<td>15.6 (11.7-19.6)</td>
<td>32.2 (25.2-39.3)</td>
<td>&lt;0.0002</td>
</tr>
</tbody>
</table>

**Table 3:** ROC curve analysis of eicosanoids for ARDS in sepsis patients.
and that TXA₂ is a prognostic factor for sepsis patients with ARDS rather than a factor for treatments.

Amat et al. [7] analyzed the leukotrienes LTB₄, LTC₄, and LTD₄ and reported that only the plasma concentration of LTB₄ on day 1 was a prognostic marker for ARDS, and that LTB₄ and interleukin-8 together could be useful markers of the mortality rate. Leukotriene antagonists, such as ONO-1078 [21], LY255283 [22] and ICI 198,615 [23], seemed to ameliorate the clinical outcome in models of LPS-induced acute lung injury. These antagonists reduced pulmonary extravascular water contents and bronchoalveolar lavage fluid protein concentrations. Moreover, cytosolic phospholipase A₂ (cPLA₂) is important in the pathogenesis of sepsis-induced ARDS, and LTB₄ is probably the major mediator of polymorphonuclear neutrophil infiltration among the cPLA₂ products [24,25]. The results of the present study indicate that LTB₄ is a factor for ARDS treatments in sepsis patients rather than a prognostic factor. However, few randomized trials of LTB₄ antagonists have been carried out in sepsis patients with ARDS.

Conclusion

In conclusion, among the eicosanoids examined, LTB₄ may be an important risk factor for sepsis patients who develop ARDS, while TXA₂ may be an important prognostic factor for sepsis patients who develop ARDS. Molecular biological studies and large randomized trials are needed to confirm these findings.

References