Supplementary Materials (2 Figures and 6 Tables)

Myeloperoxidase-derived 2-chlorofatty acids contribute to human sepsis mortality via acute respiratory distress syndrome

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Fig. S1. 2-CIPA does not alter HMVEC-L metabolic activity. Cells were treated with BSA-conjugated lipids (10 µM) for 30 min. Metabolic activity of HMVEC-L was measured using an MTT assay following manufacturer’s protocol. MTT reduction is expressed as percent of control MTT reduction (BSA only, designated as 100%). Triton treatment (0.1%) is a positive control in these experiments. Box plots show median and 25th and 75th percentile, and whiskers show minima and maxima for each condition (n=6).
Fig. S2. 2-ClPA effects on human renal glomerular microvascular endothelial cell. 2-ClPA modestly increases human renal glomerular microvascular endothelial cell barrier permeability (A; n=6 for each data point; mean ± SEM) with no effect on surface adhesion molecule expression (B, n=8 for each adhesion molecule). For all panels the lipid addition was at 10 µM. Statistical analysis in Panel A by ANOVA with Tukey post hoc test. * and ** p<0.05 and p<0.01, respectively, when compared to untreated controls. Box plots show median and 25th and 75th percentile, and whiskers show minima and maxima for each condition.
**TABLE S1. Characteristics of study population.** Continuous data are compared by Student’s T or Wilcoxon Rank sum test as appropriate. Categorical data are compared by the Chi square test.

<table>
<thead>
<tr>
<th></th>
<th>ARDS (n=100)</th>
<th>Non-ARDS (n=98)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>60.2 ± 14.2</td>
<td>61.7 ± 16.3</td>
<td>0.486</td>
</tr>
<tr>
<td>Female gender</td>
<td>38 (38%)</td>
<td>46 (47%)</td>
<td>0.181</td>
</tr>
<tr>
<td>APACHE III</td>
<td>81.5 (65 – 94)</td>
<td>69 (56 – 83)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Neutropenic day 0</td>
<td>13 (13%)</td>
<td>18 (19%)</td>
<td>0.312</td>
</tr>
<tr>
<td>Race, proxy-stated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>66 (66%)</td>
<td>55 (55%)</td>
<td>0.188</td>
</tr>
<tr>
<td>African American</td>
<td>26 (26%)</td>
<td>37 (%)</td>
<td></td>
</tr>
<tr>
<td>Asian</td>
<td>4 (4%)</td>
<td>3 (%)</td>
<td></td>
</tr>
<tr>
<td>Other or &gt; 1</td>
<td>4 (4%)</td>
<td>1 (%)</td>
<td></td>
</tr>
<tr>
<td>Infectious Source</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GU</td>
<td>5 (5%)</td>
<td>19 (20%)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>GI</td>
<td>12 (12%)</td>
<td>13 (13%)</td>
<td></td>
</tr>
<tr>
<td>Pulmonary</td>
<td>57 (57%)</td>
<td>25 (26%)</td>
<td></td>
</tr>
<tr>
<td>Head/Neck</td>
<td>1 (1%)</td>
<td>4 (4%)</td>
<td></td>
</tr>
<tr>
<td>Blood</td>
<td>9 (9%)</td>
<td>11 (11%)</td>
<td></td>
</tr>
<tr>
<td>Bone / soft tissue</td>
<td>5 (5%)</td>
<td>4 (4%)</td>
<td></td>
</tr>
<tr>
<td>Unclear</td>
<td>11 (11%)</td>
<td>21 (22%)</td>
<td></td>
</tr>
<tr>
<td>Infectious Etiology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gram positive</td>
<td>36 (36%)</td>
<td>19 (19%)</td>
<td>0.060</td>
</tr>
<tr>
<td>Gram negative</td>
<td>9 (9%)</td>
<td>20 (20%)</td>
<td></td>
</tr>
<tr>
<td>Mixed</td>
<td>21 (21%)</td>
<td>19 (19%)</td>
<td></td>
</tr>
<tr>
<td>Viral</td>
<td>1 (1%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Fungal</td>
<td>2 (2%)</td>
<td>1 (1%)</td>
<td></td>
</tr>
<tr>
<td>Culture negative</td>
<td>30 (30%)</td>
<td>32 (33%)</td>
<td></td>
</tr>
<tr>
<td>30 day mortality</td>
<td>74 (75%)</td>
<td>34 (34%)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>
Table S2. Associations of Total and Free Plasma 2-CIFA Molecular Species with ARDS.
Median and (interquartile range) are shown, and groups are compared by the Wilcoxon Rank sum test.

<table>
<thead>
<tr>
<th></th>
<th>ARDS (n=100)</th>
<th>Non-ARDS (n=98)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total 2-CIPA (nM)</td>
<td>2.01 (1.10 – 3.86)</td>
<td>1.47 (0.62 – 2.15)</td>
<td>0.0011</td>
</tr>
<tr>
<td>Free 2-CIPA (nM)</td>
<td>0.73 (0.35 – 1.62)</td>
<td>0.39 (0.17 – 0.71)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Total 2-CISA (nM)</td>
<td>2.96 (1.30 – 5.60)</td>
<td>1.59 (0.82 – 3.38)</td>
<td>0.0008</td>
</tr>
<tr>
<td>Free 2-CISA (nM)</td>
<td>1.12 (0.48 – 2.54)</td>
<td>0.43 (0.22 – 0.98)</td>
<td>0.0001</td>
</tr>
<tr>
<td>MPO (pM) N=110*</td>
<td>1384.3 (551 – 2794)</td>
<td>1257.2 (559 – 2199)</td>
<td>0.28</td>
</tr>
<tr>
<td></td>
<td>n=54</td>
<td>n=56</td>
<td></td>
</tr>
</tbody>
</table>
Table S3: Comparison of Plasma 2-CIFA Concentrations between Healthy Controls and Septic Subjects. Median and (interquartile range) and shown. Groups are compared by non-parametric test of trend and demonstrate an increase in plasma 2-CIFA concentrations with increasing sepsis severity.

<table>
<thead>
<tr>
<th></th>
<th>Controls (n=4)</th>
<th>Sepsis Survivors (n=91)</th>
<th>Sepsis Non-survivors (n=109)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total 2-CIPA (nM)</td>
<td>0.79 (0.45 - 1.05)</td>
<td>1.56 (0.77 - 2.27)</td>
<td>1.92 (0.99 - 3.83)</td>
<td>0.007</td>
</tr>
<tr>
<td>Free 2-CIPA (nM)</td>
<td>0.20 (0.18 - 0.27)</td>
<td>0.42 (0.18 - 0.88)</td>
<td>0.62 (0.30 - 1.40)</td>
<td>0.003</td>
</tr>
<tr>
<td>Total 2-CISA (nM)</td>
<td>0.83 (0.78 - 1.19)</td>
<td>2.03 (0.90 - 3.77)</td>
<td>2.71 (1.04 - 4.61)</td>
<td>0.114</td>
</tr>
<tr>
<td>Free 2-CISA (nM)</td>
<td>0.24 (0.15 - 0.31)</td>
<td>0.50 (0.25 - 1.22)</td>
<td>0.83 (0.42 - 2.37)</td>
<td>0.001</td>
</tr>
</tbody>
</table>
Table S4: Characteristics of plasma myeloperoxidase (MPO) subgroup. Due to sample volume remaining after mass spectroscopy, only 55% of the population had MPO assayed.

Characteristics of the MPO subgroup are compared to those who did not have MPO measured by the Student’s T test, Wilcoxon ranksum test, or by chi square test as appropriate.

<table>
<thead>
<tr>
<th></th>
<th>MPO measured (n=110)</th>
<th>MPO not measured (n=88)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>60.5 ± 15</td>
<td>61.3 ± 16</td>
<td>0.68</td>
</tr>
<tr>
<td>Female gender</td>
<td>52 (47%)</td>
<td>31 (36%)</td>
<td>0.10</td>
</tr>
<tr>
<td>APACHE III</td>
<td>78 (64 – 92)</td>
<td>71 (57 – 87)</td>
<td>0.04</td>
</tr>
<tr>
<td>Neutropenic day 0</td>
<td>20 (18%)</td>
<td>11 (12%)</td>
<td>0.29</td>
</tr>
<tr>
<td>Race, proxy-stated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>69 (63%)</td>
<td>52 (59%)</td>
<td>0.44</td>
</tr>
<tr>
<td>African American</td>
<td>36 (33%)</td>
<td>27 (31%)</td>
<td></td>
</tr>
<tr>
<td>Asian</td>
<td>4 (4%)</td>
<td>3 (3%)</td>
<td></td>
</tr>
<tr>
<td>Other or &gt; 1</td>
<td>1 (1%)</td>
<td>4 (4%)</td>
<td></td>
</tr>
<tr>
<td>Infectious Source</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-pulmonary</td>
<td>59 (54%)</td>
<td>49 (56%)</td>
<td>0.71</td>
</tr>
<tr>
<td>Pulmonary</td>
<td>51 (46%)</td>
<td>38 (44%)</td>
<td></td>
</tr>
<tr>
<td>Infectious Etiology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gram positive</td>
<td>50 (45%)</td>
<td>45 (52%)</td>
<td>0.47</td>
</tr>
<tr>
<td>Gram negative</td>
<td>29 (26%)</td>
<td>34 (39%)</td>
<td>0.07</td>
</tr>
<tr>
<td>Culture negative</td>
<td>35 (32%)</td>
<td>27 (31%)</td>
<td>0.91</td>
</tr>
<tr>
<td>30 day mortality</td>
<td>63 (57%)</td>
<td>45 (52%)</td>
<td>0.44</td>
</tr>
</tbody>
</table>
Table S5. 2-Chlorofatty acid concentrations in neutropenic patients with sepsis do not vary by ARDS status. Data are presented as median (interquartile range) for all neutropenic MESSI subjects and stratified by ARDS status. Note the similarity between values observed for neutropenic septic subjects and healthy controls as displayed in Table S3. The p-values reflect Wilcoxon ranksum testing between ARDS and non-ARDS neutropenic subjects. With only 31 neutropenic subjects, the subgroup was not adequately powered to detect modest differences and we caution that subgroup analyses, with reduced power and high variance, can yield erroneous statistical testing.

<table>
<thead>
<tr>
<th></th>
<th>All Neutropenic Sepsis (n=31)</th>
<th>Neutropenic, Developed ARDS (n=13)</th>
<th>Neutropenic, Non ARDS (n=18)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Free 2-CIPA (nM)</strong></td>
<td>0.31 (0.16 – 0.61)</td>
<td>0.55 (0.21 – 1.05)</td>
<td>0.25 (0.16 – 0.40)</td>
<td>0.139</td>
</tr>
<tr>
<td><strong>Free 2-CISA (nM)</strong></td>
<td>0.24 (0.06 – 0.70)</td>
<td>0.48 (0.06 – 1.12)</td>
<td>0.12 (0.07 – 0.38)</td>
<td>0.215</td>
</tr>
</tbody>
</table>
Table S6. No statistically significant difference in plasma 2-CI:FA concentrations between confirmed gram negative sepsis and other infectious sources (gram positive, fungal, viral, or unclear source). Analyses were performed using the Wilcoxon rank sum test to compare gram negative and non-gram negative sepsis.

<table>
<thead>
<tr>
<th></th>
<th>Gram negative Sepsis (n=63)</th>
<th>Non-GN Sepsis (n=137)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total 2-CI:FA (nM)</td>
<td>1.53 (0.62 – 2.32)</td>
<td>1.75 (0.94 -3.30)</td>
<td>0.249</td>
</tr>
<tr>
<td>Free 2-CI:FA (nM)</td>
<td>0.43 (0.21 – 0.77)</td>
<td>0.55 (0.26 – 1.30)</td>
<td>0.080</td>
</tr>
<tr>
<td>Total 2-CIA (nM)</td>
<td>2.07 (0.86 – 3.31)</td>
<td>2.55 (1.01 – 4.61)</td>
<td>0.082</td>
</tr>
<tr>
<td>Free 2-CIA (nM)</td>
<td>0.58 (0.33 – 1.25)</td>
<td>0.72 (0.28 – 1.83)</td>
<td>0.399</td>
</tr>
</tbody>
</table>