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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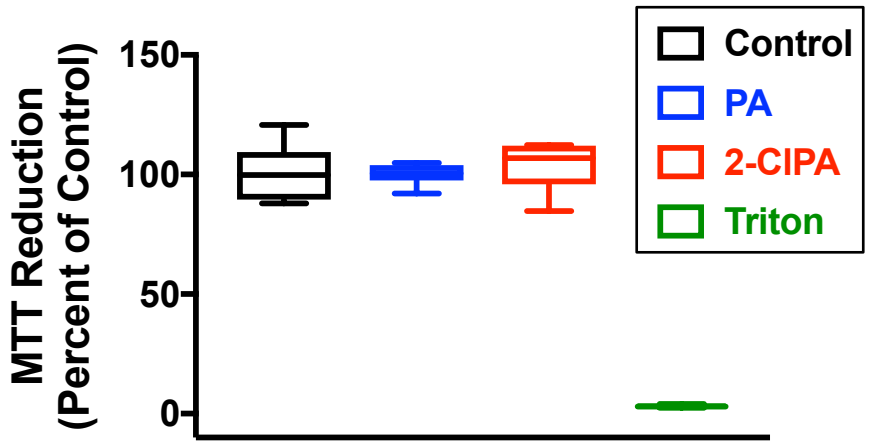

Fig. S1. 2-ClPA does not alter HMVEC-L metabolic activity. Cells were treated with BSA-conjugated lipids (10 μM) for 30 min. Metabolic activity of HMVEC-L was measured using an MTT assay following manufacturer's protocol. MTT reduction is expressed as percent of control MTT reduction (BSA only, designated 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).

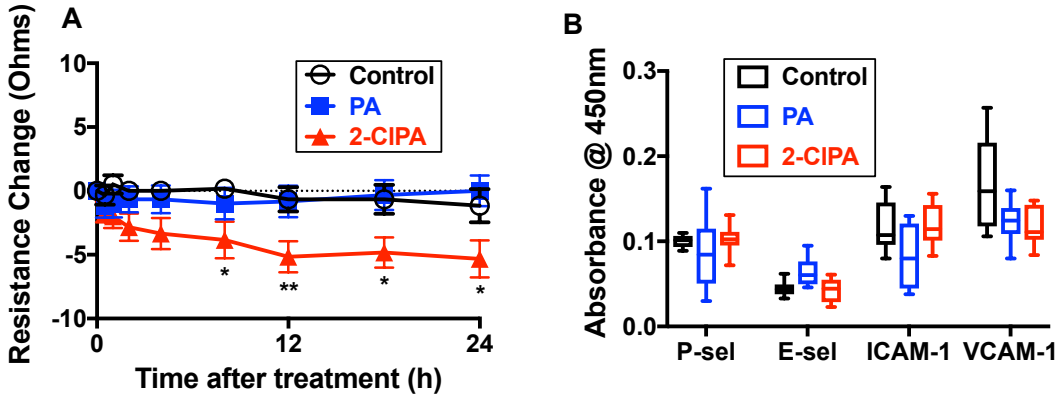

Fig. S2. 2-ClPA effects on human renal glomerular microvascular endothelial cell. 2-ClPA modestly increases human renal glomerular microvascular endothelial cell barrier permeability (A; n=6 for each data point; mean ± SEM) with no effect on surface adhesion molecule expression (B, n=8 for each adhesion 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.

	ARDS (n=100)	Non-ARDS (n=98)	p-value
Age	60.2 ± 14.2	61.7 ± 16.3	0.486
Female gender	38 (38%)	46 (47%)	0.181
APACHE III	81.5 (65 – 94)	69 (56 – 83)	< 0.001
Neutropenic day 0	13 (13%)	18 (19%)	0.312
Race, proxy-stated			
Caucasian	66 (66%)	55 (55%)	0.188
African American	26 (26%)	37 (%)	
Asian	4 (4%)	3 (%)	
Other or > 1	4 (4%)	1 (%)	
Infectious Source			
GU	5 (5%)	19 (20%)	< 0.001
GI	12 (12%)	13 (13%)	
Pulmonary	57 (57%)	25 (26%)	
Head/Neck	1 (1%)	4 (4%)	
Blood	9 (9%)	11 (11%)	
Bone / soft tissue	5 (5%)	4 (4%)	
Unclear	11 (11%)	21 (22%)	
Infectious Etiology			
Gram positive	36 (36%)	19 (19%)	0.060
Gram negative	9 (9%)	20 (20%)	
Mixed	21 (21%)	19 (19%)	
Viral	1 (1%)	0	
Fungal	2 (2%)	1 (1%)	
Culture negative	30 (30%)	32 (33%)	
30 day mortality	74 (75%)	34 (34%)	< 0.001

Table S2. Associations of Total and Free Plasma 2-ClFA Molecular Species with ARDS. Median and (interquartile range) are shown, and groups are compared by the Wilcoxon Rank sum test.

	ARDS (n=100)	Non-ARDS (n=98)	p-value
Total 2-ClPA (nM)	2.01 (1.10 – 3.86)	1.47 (0.62 – 2.15)	0.0011
Free 2-ClPA (nM)	0.73 (0.35 – 1.62)	0.39 (0.17 – 0.71)	0.0001
Total 2-ClSA (nM)	2.96 (1.30 – 5.60)	1.59 (0.82 – 3.38)	0.0008
Free 2-ClSA (nM)	1.12 (0.48 – 2.54)	0.43 (0.22 – 0.98)	0.0001
MPO (pM) **N=110***	1384.3 (551 - 2794) n=54	1257.2 (559 – 2199) n=56	0.28

Table S3: Comparison of Plasma 2-ClFA 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-ClFA concentrations with increasing sepsis severity.

	Controls (n=4)	**Sepsis Survivors (n=91)**	**Sepsis Non-survivors (n=109)**	**p-value**
Total 2-ClPA (nM)	0.79 (0.45 - 1.05)	1.56 (0.77 - 2.27)	1.92 (0.99 - 3.83)	0.007
Free 2-ClPA (nM)	0.20 (0.18 - 0.27)	0.42 (0.18 - 0.88)	0.62 (0.30 - 1.40)	0.003
Total 2-ClSA (nM)	0.83 (0.78 - 1.19)	2.03 (0.90 - 3.77)	2.71 (1.04 - 4.61)	0.114
Free 2-ClSA (nM)	0.24 (0.15 - 0.31)	0.50 (0.25 - 1.22)	0.83 (0.42 - 2.37)	0.001

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.

	MPO measured (n=110)	MPO not measured (n=88)	p-value
Age	60.5 ± 15	61.3 ± 16	0.68
Female gender	52 (47%)	31 (36%)	0.10
APACHE III	78 (64 – 92)	71 (57 – 87)	0.04
Neutropenic day 0	20 (18%)	11 (12%)	0.29
Race, proxy-stated			
Caucasian	69 (63%)	52 (59%)	0.44
African American	36 (33%)	27 (31%)	
Asian	4 (4%)	3 (3%)	
Other or > 1	1 (1%)	4 (4%)	
Infectious Source			
Non-pulmonary	59 (54%)	49 (56%)	0.71
Pulmonary	51 (46%)	38 (44%)	
Infectious Etiology			
Gram positive	50 (45%)	45 (52%)	0.47
Gram negative	29 (26%)	34 (39%)	0.07
Culture negative	35 (32%)	27 (31%)	0.91
30 day mortality	63 (57%)	45 (52%)	0.44

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.

	All Neutropenic Sepsis (n=31)	**Neutropenic, Developed ARDS (n=13)**	**Neutropenic, Non ARDS (n=18)**	**p-value**
Free 2-ClPA (nM)	0.31 (0.16 – 0.61)	0.55 (0.21 – 1.05)	0.25 (0.16 – 0.40)	0.139
Free 2-ClSA (nM)	0.24 (0.06 – 0.70)	0.48 (0.06 – 1.12)	0.12 (0.07 – 0.38)	0.215

Table S6. No statistically significant difference in plasma 2-ClFA 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.

	Gram negative Sepsis (n=63)	**Non-GN Sepsis** (n=137)	**p-value**
Total 2-ClPA (nM)	1.53 (0.62 – 2.32)	1.75 (0.94 -3.30)	0.249
Free 2-ClPA (nM)	0.43 (0.21 – 0.77)	0.55 (0.26 – 1.30)	0.080
Total 2-ClSA (nM)	2.07 (0.86 – 3.31)	2.55 (1.01 – 4.61)	0.082
Free 2-ClSA (nM)	0.58 (0.33 – 1.25)	0.72 (0.28 – 1.83)	0.399