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Plasma lipid peroxides and antioxidants in human septic shock

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Abstract. In order to assess if an oxidant/antioxidant imbalance is involved in human septic shock and its outcome, we measured plasma levels of the lipid peroxides malondialdehyde – as thiobarbituric acid reactive substance – conjugated dienes and fluorescent products, together with the antioxidants alpha-tocopherol, glutathione peroxidase activity and selenium in 12 patients with septic shock and compared them with values of normal controls. At first measurements, malondialdehyde (median 3.9 μmol/l; range 2–38.8) and fluorescent products (median 21.2%; range 9.4–134) were elevated ($p < 0.05$), alpha-tocopherol (median 15 μmol/l; range 7–25) and selenium (median 0.76 μg/ml; range 0.49–1.09) were depressed ($p < 0.05$). Conjugated dienes and glutathione peroxidase activity were in the normal range. In non-survivors ($n = 5$) initial levels of malondialdehyde and fluorescent products (median 11 versus 3.1 μmol/l; 74 versus 13% respectively) were higher than in survivors ($p < 0.05$) and initial selenium levels were lower (median 0.58 versus 0.92 μg/l; $p < 0.05$). These results are consistent with the concept that an oxidant/antioxidant imbalance – as indicated by elevated plasma lipid peroxides and depressed antioxidants – is involved in human septic shock and a fatal outcome.

Key words: Septic shock – Lipid peroxidation – Alpha-tocopherol – Selenium – Oxygen free radicals – Outcome

Amongst the mediators involved in the pathophysiology of septic shock and subsequent tissue injury in animal models, oxygen free radicals may play a central role [1–11], even though this is not beyond debate [12, 13]. In human septic shock, an oxidant/antioxidant imbalance is also thought to be involved [14, 15]. In a broad spectrum of diseases which may relate to septic shock in man, such as the adult respiratory distress syndrome (ARDS) and multiple systems organ failure [16–21], evidence for oxygen free radical activity has been found. Oxygen free radical activity has been assessed indirectly by measurement of lipid peroxides [16, 19–21]. The antioxidant alpha-tocopherol (AT) has been shown to be depressed during critical illness [16] and ARDS [19, 22].

As none of these studies addressed the specific question whether oxygen free radicals are involved in human septic shock and ultimate outcome, we studied prospectively plasma levels of lipid peroxides and antioxidants in 12 patients with septic shock and determined the relationship of these variables to the severity of disease and outcome. We measured the following lipid peroxides [1, 3–6, 8, 11, 16, 19, 21]: malondialdehyde (MDA), conjugated dienes (CD), fluorescent products (FP), and the antioxidants [1, 6–8, 10, 11, 13, 15, 16, 19, 23, 24]: AT, glutathione peroxidase (GSH-Px) activity and its co-factor selenium (Se). Arterial blood lactate was measured as a marker for tissue ischaemia [25]. APACHE II scores were obtained as a measure of the severity of illness [26].

Materials and methods

We studied 12 patients (11 men, 1 woman) after their admission to the medical intensive care unit, from the moment they fulfilled the criteria for septic shock. Sepsis was diagnosed on the basis of: clinical evidence of infection, fever (>38.5 °C) or hypothermia (<36 °C), and tachycardia (>90 beats/min). Shock was defined as a decrease in systolic blood pressure to less than 90 mmHg or a drop of 40 mmHg from baseline (or the necessity to use vasopressor drugs to maintain systolic blood pressure) for at least one hour and at least one or more manifestations of inadequate organ perfusion (altered mentation, elevated lactate >1.8 mmol/l, or oliguria, <30 ml for at least 1 h). A definite diagnosis of septic shock was made, when local and/or blood cultures yielded pathogenic microorganisms. The patients who died during the septic shock episode were considered as non-survivors, the others as survivors. Patients received conventional therapy, including antibiotics, fluid resuscitation, vasopressor and/or inotropic agents, mechanical ventilation, and total parenteral nutrition (which contained lipid emulsion twice weekly, but no supplementation of AT or Se), when needed on clinical grounds. Plasma samples were taken during 72 h after the patients were entered into the study, at intervals of 24 h and in the first 24 h at intervals of 6 h. Arterial blood samples were taken in EDTA tubes, immediately centrifugated, and stored at −20 °C. Just before processing, they were thawed. MDA was measured as the thiobarbituric

acid reactive substance, assayed by a spectrophotometrical method [27], without the high pressure chromatography separation step. CD were also assayed by a spectrophotometrical method [28] and FP by a spectrofluorometrical method [28]. AT was measured with a high pressure liquid chromatography technique [29]. GSH-Px activity and Se were measured by spectrophotometrical methods [30, 31]. Lactate was measured spectrophotometrically (Analytical Chemistry Analyzer, Du Pont, Wilmington, Delaware). Routine laboratory measurements were done every 24 h. The APACHE II score was obtained at 24 h intervals. Nine healthy adults (hospital personnel) served as controls.

Statistical analysis

Group data are expressed as median and range. Values are reported for the first, highest, lowest, and last measurements, since they showed considerable fluctuations during the study period. Changes were determined as percentage changes from baseline values. The Wilcoxon signed rank test or the Wilcoxon rank-sum test were used for analysis. Correlation coefficients were determined according to Spearman's rank correlation method. A two-tailed p value <0.05 was considered statistically significant.

Results

Patients characteristics are shown in Table 1; there were 7 survivors. There were no major differences between survivors and nonsurvivors in age, sex, underlying disease, median duration of septic shock before study entrance (9 h; range 3–24 vs. 14; range 6–29), causative microorganisms or positive blood cultures (2/7 vs. 2/5), highest temperature (median 39.7 °C, range 37.4–40.1 vs. 39.3; range 38.3–40.4), systolic blood pressure (median 95 mmHg; range 85–106 vs. 94; range 80–127), vasopressor medication and mechanical ventilation. In 3 surviving patients no pathogenic microorganisms were found, partly due to prior treatment with antibiotics. Survivors had less frequent renal insufficiency (2/7 vs. 3/5 with serum creatinine levels >200 µmol/l) and hepatic dysfunction (1/7 vs. 2/5 with total bilirubin levels >20 µmol/l) compared to non-survivors. There were more blood samples obtained from survivors (5 vs. 7/patient) because of early death in the non-survivor group (2 patients died within 15 h, 1 within 52 h after study entrance). Of the patients who survived the septic shock episode, 4 were discharged from the intensive care and 3 died of multiple organ failure in the unit.

A. Group as a whole

At the initial measurements the median plasma levels of MDA (3.9 µmol/l; range 2–38.8) and FP (21.2%; range 9.4–134) were elevated ($p<0.05$), but the median CD level (168 µmol/l; range 54–239) was in the normal range. The median AT level (15 µmol/l; range 7–25) was depressed ($p<0.05$). The median GSH-Px activity level (485 U/l; range 339–1057) was in the normal range, but the median Se level (0.76 µg/l; range 0.49–1.09) was depressed ($p<0.05$). The lactate level was not significantly elevated (median 2 mmol/l; range 0.6–4). The APACHE II score was high (median 25.5; range 16–35).

From the variables studied, the MDA level increased between first and last measurement (to median 6.5 µmol/l; range 2.1–48.6, $p<0.05$), the FP level remained elevated and the AT and Se levels depressed. Although the GSH-Px activity level declined between the first and the last measurements (to median 430 U/l; range 216–883, $p<0.05$), it did not differ from control. The MDA and FP levels correlated with each other at the first ($r=0.86$, $p=0.01$) and at the last measurement ($r=0.83$,

Table 1. Clinical characteristics of 12 patients with septic shock

Age/Sex	Underlying condition	Source of sepsis	Mechanical ventilation/ vasopressor medication	Organism(s) isolated (source)	Outcome
33/M	Barbiturate intoxication	Aspiration pneumonia	+/+	*Haemophilus influenzae* (sputum)	Survived
68/M	Acute myocardial infarction	Pneumonia	+/+	*Moraxella* (sputum)	Survived
66/M	Cardiac arrest	Pneumonia	+/+	*Streptococcus pneumoniae* (blood, sputum)	Survived
69/M	Shock wave urolithotripsy	Urinary tract	−/−	*Enterococcus faecalis* *Proteus mirabilis* (blood, urine)	Survived
70/M	Immune suppression, rheumatoid arthritis	Aspiration pneumonia	+/+	−	Survived
69/M	Obstruction ileus	Fecal peritonitis	+/+	−	Survived
77/M	Alcohol abuse	Pneumonia	+/+	−	Survived
52/M	Legionella sepsis	Pneumonia	+/+	*Aspergillus fumigatus* (blood, sputum)	Died
75/M	Chronic renal insufficiency	Urinary tract	−/+	*Escherichia coli* (urine)	Died
88/M	Acute renal failure, prostatectomy	Catheter sepsis	+/+	*Streptococcus Lancefield group C* (insertion site abscess)	Died
55/M	Non-Hodgkin Lymphoma, allogenic bone marrow transplantation	Pneumonia	+/+	*Aspergillus fumigatus* (sputum)	Died
20/F	Barbiturate intoxication	Pneumonia	+/+	*Enterococcus faecalis* (blood)	Died

+ or − denotes presence or absence of mechanical ventilation or vasopressor medication

$p < 0.01$). The MDA level correlated with the serum bilirubin level at first ($r = 0.91$, $p < 0.01$) and at last measurement ($r = 0.92$, $p < 0.01$), with the serum alkaline phosphatase level ($r = 0.59$, ns. and $r = 0.77$, $p < 0.05$ resp.) and the serum with creatinine level ($r = 0.6$, ns. and $r = 0.83$, $p < 0.05$ resp.). At first and last measurements, FP level correlated with bilirubin ($r = 0.75$, $p < 0.05$, and $r = 0.75$, $p < 0.05$ resp.), with alkaline phosphatase ($r = 0.61$, ns. and $r = 0.75$, $p < 0.05$ resp.) and with creatinine ($r = 0.67$, $p < 0.05$, and $r = 0.87$, $p < 0.005$ resp.).

B. Survivors versus non-survivors

Table 2 describes the study parameters for survivors and non-survivors separately. In the non-survivors ($n = 5$) initial MDA and FP levels were higher than in survivors even though these parameters were elevated in the latter. The initial MDA level correlated with the APACHE II scores in non-survivors ($r = 0.80$, $p = 0.05$). As opposed to survivors, the last measured GSH-Px activity level in non-survivors was depressed in comparison with controls. The initial Se level was low in survivors, but even more decreased in non-survivors. The L level rose in non-survivors, but declined in survivors, whereas initial levels were similar. The changes in APACHE II score (−30%; range −36 to −6) in survivors, differed significantly ($p < 0.05$) from those in non-survivors (0%; range −13 to +74).

Discussion

The main finding of this study is, that the plasma lipid peroxides malondialdehyde and fluorescent products were elevated early in septic shock, and that the plasma antioxidant alpha-tocopherol was depressed. The levels of conjugated dienes and glutathione peroxidase activity did not differ from normal. The plasma level of selenium was depressed. The initial malondialdehyde and fluorescent products levels of patients who died in septic shock were higher and the initial selenium level was lower than those in survivors.

Oxygen free radicals are increasingly recognized as final mediators of tissue injury in inflammation [14, 23]. Oxygen free radicals are released extracellularly by activated leukocytes at a focus of inflammation [14, 15, 23, 28]. Oxygen free radicals can also be generated during hypoxia or ischemia; they are then formed within the cell via the xanthine-xanthine oxidase reaction [15] amongst others. Oxygen free radicals react with all biological substances. Most susceptible, however, are polyunsaturated fatty acids. Reactions with these cell membrane constituents lead to lipid peroxidation – with the formation of reactive fatty acid radicals – with the formation of reactive fatty acid radicals – and ultimately to membrane disintegration. In the termination phase of the lipid peroxidation process, the lipid peroxides malondialdehyde, conjugated dienes and high molecular weight fluorescent products are formed, together with volatile hydrocarbons as ethane and pentane [23]. α-tocopherol functions as a free radical scavenger – especially in cell membranes – where it terminates lipid peroxidation [15, 23]. Selenium-dependent glutathione peroxidase acts as an antioxidant by inhibiting both the primary and the secondary initiation phases of lipid peroxidation [15, 23] and by detoxifying hydrogen peroxide to water [23].

Table 2. Plasma levels of lipid peroxides, antioxidants, lactate, and APACHE II scores of patients with septic shock (median; range)

	Survivors ($n = 7$)				Non-survivors ($n = 5$)				Controls ($n = 9$)
	First	Lowest	Highest	Last	First	Lowest	Highest	Last	
Malondialdehyde (μmol/l)	3.1[a] 2–6.6	2.7[a] 1.8–6.6	4.2[a,b] 2.7–13.7	3.7[a,b] 2.1–13.7	11[a,b] 2.3–38.8	9.3[a] 2–38.8	11[a] 3.5–48.6	9.3[a] 3.5–48.6	1.6 1.3–1.9
Conjugated dienes (μmol/l)	144 79–219	144 59–215	219 90–357	150 3–357	192 54–239	191 54–239	274 82–366	217 80–366	139 88–160
Fluorescence products (%*)	13[a] 9–72	9 4–59	27[a] 12.2–176	27[a] 4–166	74[a,b] 16–134	61.5[a,c] 10.1–134	105[a] 25.3–190	105[a] 10–190	6.5 5.2–9.9
α-Tocopherol (μmol/l)	15[a] 12–18	12[a] 6–18	15[a] 12–28	12.5[a] 6–16	18 7–25	9[a] 7–16	18 8–25	11[a] 7–16	>20**
Glutathione Peroxidase activity (U/l)	485 387–1057	425[b] 184–883	581 422–1057	425[b] 216–883	447 339–813	378[a] 263–676	527 340–822	435[a] 340–676	648 435–758
Selenium (μg/l)	0.92[a] 0.66–1.09	0.52[a] 0.38–0.86	0.89[a] 0.7–0.09	0.7[a] 0.6–1.04	0.58[a,b] 0.49–0.85	0.58[a] 0.2–0.83	0.99[a] 0.58–1.07	0.59[a] 0.35–1.07	1.13 0.95–1.71
Lactate (mmol/l)	2.2 0.6–2.9	0.8 0.5–2.3	2.2 0.7–4.8	0.9[b] 0.8–2.4	1.9 1.1–3.3	1.8[c] 1.1–3.3	2.7 1.2–5.6	3.15[d] 1.7–5.6	<1.8**
APACHE II score	23[a] 16–34	20[a] 13–29	23[a] 16–34	20[a,b] 3–29	28[a] 19–35	28[a] 20–35	33[a] 22–35	30[a,d] 20–35	0

* arbitrary units, ** normal laboratory values.
[a] $p < 0.05$ compared to controls;
[b] $p < 0.05$ compared to survivors on admission;
[c] $p < 0.05$ compared to survivors lowest level;
[d] $p < 0.05$ compared to survivors at last measurement

The enhanced lipid peroxidation as found in our patient group, is in line with studies in animal models of septic shock, in which elevated lipid peroxides were demonstrated in plasma, liver, brain, and lung [1, 3, 5, 6] and with studies in patients with diseases which may relate to septic shock [16, 19, 20]. Since direct detection of oxygen free radicals in vivo is difficult, many studies on tissue injury in inflammation, have relied on a beneficial response to treatment with scavengers of oxygen free radicals or on elevated products of the action of oxygen free radicals, including lipid peroxides, as indirect evidence that oxygen free radicals are involved [14, 23]. Lipid peroxides can be used as in vivo indicators of oxygen free radical activity during septic shock, since pretreatment with antioxidants, including α-tocopherol in animal models, depressed lipid peroxidation [1, 3, 4, 8, 11] and improved survival [1, 2, 7, 9, 10]. The difference in the evolution of the plasma levels of conjugated dienes versus malondialdehyde or fluorescent products in our patients, is in line with an animal model of lung injury evoked by complement activation [28], where the elevation of plasma conjugated dienes was only transient but the levels of malondialdehyde and fluorescent products were persistently increased.

The spectrophotometrically determined products of lipid peroxidation thus indirectly reflect oxygen free radical activity in vivo [1, 3–6, 8, 11, 16, 19, 21, 28]. However, there is a possibility of a non-specific elevation of malondialdehyde and fluorescent products due to the assaying process itself [27, 32], or to a possible overlap between the optical spectra of haemoglobin or haem pigment and the lipid peroxides [27, 33]. Although, in our study, the correlations of malondialdehyde and fluorescent products with bilirubin and with creatinine could indicate a non-specific elevation, neither bilirubin nor creatinine interfere with the assay method for malondialdehyde [27]. The correlations of malondialdehyde and fluorescent products levels with alkaline phosphatase levels, as observed in animal models [5] and in critically ill patients [16] also, makes a nonspecific elevation less probable. As all three patients with an initially normal plasma creatinine (< 100 µmol/l) had elevated levels of malondialdehyde and fluorescent products, renal dysfunction during septic shock could not have been the sole reason of elevated lipid peroxides. Furthermore, lipid peroxides directly correlated with the APACHE II score in non-survivors. Hence, our data suggest that elevated lipid peroxides, independently from the assaying process itself, related to tissue injury in septic shock.

The depression of antioxidants in our study is comparable to that seen in animal models of septic shock [5, 6] and in patients with diseases, which may relate to septic shock [16, 19, 22]. The fall in α-tocopherol in our patients can be attributed to an inadequate intake and/or to an increased consumption [19, 22]. Since we did not measure plasma total lipids, we cannot exclude however, that low α-tocopherol levels did not reflect a real deficiency state. The low selenium level in our patients can be attributed to inadequate intake or to redistribution from the plasma pool into the tissues as defense mechanism against oxidative processes, as recently suggested by a study in critical ill patients [34]. The low plasma selenium level might be involved in the decreased plasma glutathione peroxidase activity in non-survivors, since glutathione peroxidase activity direc relates to the selenium level in healthy adults [24]. Since plasma and erythrocyte selenium-glutathione peroxidase activities correlate only weakly with selenium-glutathione peroxidase activity in liver and platelets of healthy adults [24], it is possible that plasma glutathione peroxidase activities reflected the status of this enzyme in blood. Hence, we cannot exclude that the low final plasma glutathione peroxidase activity in non-survivors, may not be indicative of a deficiency of tissue enzyme activity.

The enhanced lipid peroxidation products together with depression of antioxidants in our patients, reflected cell membrane damage due to oxygen free radicals. These oxygen free radicals were presumably generated by activated leukocytes as part of the inflammatory responses during early septic shock. It seems less likely that the radicals were generated in ischemic tissues due to hypoperfusion during the shock state, since the initial lactate level was still in the normal range. One may speculate that the plasma lipid peroxides originated from damaged membranes of blood cells and endothelial cells at the sites of leukocyte sequestration in the capillaries [23]. Although we did not find a relationship between lipid peroxides and indicators of tissue cell damage like lactate dehydrogenase, the relations of the lipid peroxide levels with bilirubin and creatinine suggest that tissue damage in liver and kidney contributed to a rise in plasma lipid peroxides. Fatty acid radicals and fatty acid peroxy-radicals released during lipid peroxidation, propagate further peroxidation [23], so that enhanced lipid peroxidation early in septic shock may not have been an effect of injury only, but may have caused additional tissue damage.

In conclusion, our results indicate that oxygen free radicals are formed in human septic shock, and that an oxidant/antioxidant imbalance is involved in tissue injury and outcome. Antioxidant therapy, such as vitamin E supplementation, might prevent lipid peroxidation and tissue injury during sepsis and septic shock.

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