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REVIEW

Antioxidant therapy in sepsis

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Abstract. **Oxidative stress due to an overwhelming release of reactive nitrogen / oxygen species (R(N)OS) is held largely responsible for sepsis-induced organ failure and mortality. Excessive R(N)OS production is neutralized by endogenous antioxidants (reduced glutathione (GSH), enzymes such as GSH peroxidase, and antioxidant vitamins) but this antioxidant potential becomes dramatically reduced in sepsis. Exogenous antioxidant supplementation may aid to restore antioxidant capacity.**

The literature on antioxidant therapy in sepsis is reviewed. Particular attention is given to clinical application. Selected articles focus on N-acetylcysteine (NAC), selenium, the antioxidant vitamins C and E, and NO synthase inhibition. In general, these substances display potent antioxidant activity in association with improved haemodynamic and organ function in animal experiments and in small clinical trials. Differences in dosing, time to start, and duration of treatment (NAC and selenium), the impossibility to judge its intrinsic effect because of concomitant treatment with other anti-inflammatory or immune-enhancing agents (vitamins C and E)), and even worsening of cardiac function and outcome (NAC and non-selective NO synthase inhibition) question the potential role of these agents in sepsis treatment.

Keywords: sepsis, severe sepsis, septic shock, organ failure, oxidative stress, antioxidant therapy, lipid peroxidation, free radicals, reactive oxygen species, vitamin C, ascorbic acid, vitamin E, alpha-tocopherol, beta-carotene, selenium, N-acetylcysteine, glutathione

Introduction

Free radicals and secondarily formed molecules that are either oxygen-centred (reactive oxygen species; ROS), or nitrogen-centred (reactive nitrogen species; RNS) are increasingly recognized as key mediators in the pathogenesis of sepsis. Several mechanisms are involved in the release of R(N)OS during sepsis [1]. Ischaemia / reperfusion processes generate oxyradicals from eicosanoid metabolic pathways, and by activation of xanthine oxidase. Continuous leakage of electrons from a dysfunctional mitochrondrial respiratory electron transport chain enhances oxyradical formation from incomplete reduction of oxygen. By far the most important source of R(N)OS, however, are the neutrophils (Figure 1). ROS produced by these cells are essential for killing bacteria but, if released excessively, they become detrimental to endothelium and tissues. ROS then attack proteins, polysaccharides, polyunsaturated fatty acids, and nucleic acids, resulting in structural, functional, or genetic cell damage [2]. A "basal" release of nitric oxide (NO) modulated by endothelial constitutive NO synthase (eNOS) sustains cardiac output and nutritive blood flow. In contrast, inducible NO synthase (iNOS) is held responsible for an excessive and long-lasting generation of NO that increases microvascular permeability and causes systemic vasodilatation and refractory shock [3].

R(N)OS also act as second messengers in cellular signal transduction and gene expression, probably through activation of NFκB [4]. As such, they may trigger the release of various pro-inflammatory cytokines and increase the expression of adhesion molecules. This enhances interactions between neutrophils and endothelial cells and promotes accumulation of neutrophils in blood vessels which results in more R(N)OS generation amplifying and/or perpetuating inflammation and subsequent tissue injury.

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Humans possess a complex endogenous antioxidant defence system that either directly inhibits the production of R(N)OS or aborts their deleterious actions. This system has both non-enzymatic and enzymatic components. Non-enzymatic antioxidants include vitamins (C, E, and β-carotene), albumin, ubiquinone, uric acid, and sulphydryl group donors (e.g. reduced glutathione). Major enzymatic compounds are glutathione peroxidase, superoxide dismutase,

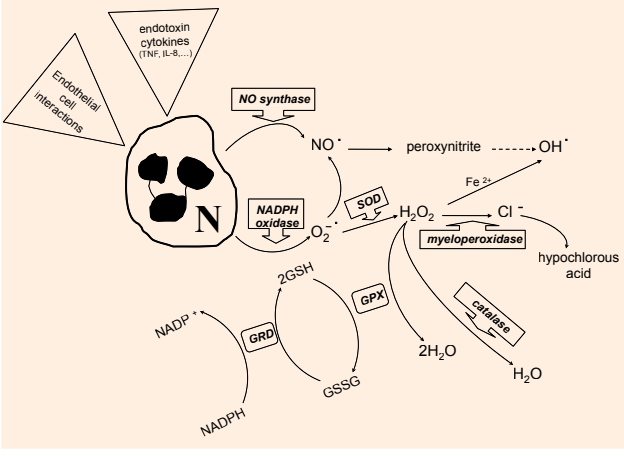

Figure 1. As part of the respiratory burst, initiated by activation of membrane-bound NADPH oxidase, activated neutrophils (N) release the highly reactive superoxide anion ($O_2^{-\bullet}$).The superoxide dismutase (SOD) enzyme, present in cytosol, mitochondria and extracellular fluids, converts superoxide to hydrogen peroxide (H_2O_2). H_2O_2 is capable to cross cell membranes and, within cells, reacts with metal ions (usually iron (Fe^{2+})) to form the highly toxic hydroxyl radical ($OH^{\bullet}$). Catalase decomposes H_2O_2 into water and O_2 whereas the selenium-dependent enzyme glutathione peroxidase (GPX) catalyses the reduction of H_2O_2 via oxidation of reduced glutathione (GSH). The resulting oxidised glutathion (GSSG) is then regenerated by glutathion reductase (GRD) using NADPH from the pentose phosphate pathway.

Interaction between neutrophils and endothelial cells incites the latter to produce large amounts of nitric oxide ($NO^{\bullet}$). $NO^{\bullet}$ reacts avidly with superoxide to form peroxynitrite, another powerful oxidant, that eventually decomposes to $OH^{\bullet}$.

Table 1. Studies on N-acetylcysteine in sepsis

Author [ref]	Patients (n)		Shock / mean APACHE II score†	Start of treatment	NAC		Main results
	NAC	control			bolus*	CI	
Spies [19]	29	29	yes / 19	< 72 hrs	yes	12.5 mg/hr for 90 min	Transiently improved tissue oxygenation; higher survival in "responders"
Rank [20]	30	30	yes / 18	< 24 hrs	yes	12.5 mg/hr for 90 min	Increased hepatic blood flow; improved liver function
Spapen [21]	12	10	yes / 23	< 4 hrs	yes	50 mg/kg/4hrs	Improved oxygenation and static lung compliance; reduced length of ventilatory support and ICU stay; lower plasma IL-8 levels
Ortolani [22]	10	10	yes / 20.5	< 24 hrs	no	75 mg/kg/day for 5 days (+ GSH 70 mg/kg/day)	Decreased lipoperoxidation; less organ failure at day 10
Peake [23]	10	10	yes / 33	< 24 hrs	yes	50 mg/kg/4hrs + 100 mg/kg/24hrs for 44 hrs	Cardiovascular depression; no improvement of oxygenation, oxygen transport parameters, and organ failure
Emet [24]	27	26	no / 13	< 4 hrs	yes	12.5 mg/kg/hr for 6 hrs	No effect on haemodynamics, oxygenation, inflammatory response, and incidence of organ failure.

† in NAC-treated patients; * bolus = 150 mg/kg intravenously
NAC, N-acetylcysteine; APACHE, Acute Physiology And Chronic Health Evaluation; CI, continuous infusion; IL, interleukin; GSH, reduced glutathione; ICU, intensive care unit

catalase, and the heme oxygenase system with their cofactors selenium, zinc, magnesium, and iron.

Oxidative stress is clearly implicated in human sepsis as shown by increased plasma lipid peroxidation products (malondialdehyde, thiobarbituric acid reactant substances), and depleted antioxidant resources [5,6]. A high level of oxidative stress that surpasses antioxidant defence capacity is associated with a higher incidence of multiorgan failure and increased mortality [6,7].

Search strategy

Both authors independently searched MEDLINE and EMBASE data bases. Keywords used included : sepsis, severe sepsis, septic shock, organ failure, oxidative stress, antioxidant therapy, lipid peroxidation, free radicals, reactive oxygen species, vitamin C, ascorbic acid, vitamin E, alpha-tocopherol, beta-carotene, selenium, N-acetylcysteine, glutathione.

Only peer-reviewed, prospective, randomized, and controlled trials in septic patients were accepted upon mutual agreement. Studies of specific or mixed ICU populations were also taken into consideration if at least half of the patients enrolled were diagnosed at inclusion as having sepsis or infection, and had received study medication. A total of 51 relevant citations were identified from the computerized bibliographic databases, of which 15 met the acceptance criteria.

Summary of findings

N-acetylcysteine

The water-soluble thiol glutathione is one of the cornerstones of the organism's antioxidant defence. Under conditions of oxidant stress, reduced glutathione (GSH) acts as a substrate for the enzyme glutathione peroxidase (GPX) which is essential for the detoxification of hydrogen and lipid peroxide. Accordingly, plasma GSH levels can become severely depleted in sepsis [8,9]. Following administration, N-acetylcysteine (NAC) is converted into L-cysteine, one of the constituents of GSH. Thus, NAC is considered to act as a cysteine donor replenishing the intracellular GSH stores. NAC has also intrinsic antioxidant capacities. It scavenges hypochlorous acid, reacts with hydroxyl radicals and hydrogen peroxide [10] , increases cytoplasmic superoxide dismutase activity, and slows down autocatalytic lipid peroxidation. NAC can suppress the activation of neutrophils and macrophages [11], attenuates endotoxin-induced leukocyte-endothelial cell adhesion and reduces capillary leakage [12]. Pretreatment with NAC in fluid-resuscitated endotoxic dogs improved cardiac function, attenuated pulmonary hypertension, increased global and regional blood flow, and enhanced tissue oxygen extraction capabilities [13,14]. However, when administered after 12 hours of endotoxaemia in volume-resuscitated pigs, NAC failed to improve gas exchange, systemic and pulmonary haemodynamics, and hepatosplanchnic perfusion [15].

High doses of NAC significantly affected human leukocyte function. NAC reduced neutrophil chemotaxis and oxidative burst activity whilst enhancing phagocytosis [16]. NAC also blocked the release of tumour necrosis factor alpha and interleukin-8, probably by modulating the gene expression of those mediators at the transcriptional level [17]. In healthy humans exposed to endotoxin, NAC mitigated the inflammatory response, improved systemic and renal haemodynamics and restored the systemic pressure response to noradrenalin [18].

Six studies have investigated adjuvant treatment with NAC in sepsis (Table 1) [19-24]. A total of 233 patients were randomized, of whom 118 received NAC. The majority of studies included septic shock patients with considerable variation in baseline severity of illness. Most patients were included within 24 hours after onset of sepsis. In all but one study, patients received a 150 mg/kg NAC bolus, followed by a continuous infusion varying in duration from 90 min to 5 days, or an NAC dose (ranging from 18.75 mg to 375 mg/kg). None of the studies was sufficiently powered to show an effect on mortality. The two studies using a short-term (90 min) infusion of NAC found a significant increase in cardiac output, tissue oxygenation, and hepatosplanchnic perfusion along with a

Table 2. Studies on selenium supplementation in sepsis

Author [ref]	Patients (n)		Pathology	Selenium dose		Main results
	Se+	control		bolus*	CI	
Zimmermann [33]	20	20	Sepsis + organ failure	1 mg	1 mg/day for 15 days	25% reduction of mortality rate; decreased inflammatory response
Angstwurm [34]	21	21	Sepsis, septic shock	-	535µg/day, 3 days 285µg/day, 3 days 155µg/day, 3 days	Reduced incidence of acute renal failure, requiring RRT; trend towards lower mortality in Se+ group (p=0.13)
Mishra [35]	18	22	Septic shock	-	474µg/day, 3 days 316µg/day, 3 days 158µg/day, 3 days	No effect
Forceville [37]	31	29	Septic shock	-	4mg on day 1, then 1mg/day for 9 days	No effect
Angstwurm [36]	92	97 †	SIRS, sepsis, septic shock	1 mg for 14 days	1mg/day	14,3% reduction in 28-day mortality (p=0.05)

† per protocol analysed patients; * bolus = sodium selenite
SIRS, systemic inflammatory response syndrome; Se+, Selenium supplemented; CI, continuous infusion; RRT, renal replacement therapy

systemic vasodilatation [19,20]. Haemodynamic changes, however, were transient and appeared to be limited to NAC "responders" (i.e. patients increasing VO_2 by more than 10%) [19]. Given the low total dose of NAC infused, this observation probably reflects only the volume effect of the NAC bolus and its associated high plasma NAC concentrations. Two studies included patients with early septic shock and comparable disease severity [21,22]. They demonstrated significant *in vivo* biological (decreased peroxidative stress, lower plasma interleukin-8 levels) and clinical (improved lung function, less organ failure over time) actions of NAC. However, both studies enrolled only a few patients, very different NAC infusion regimens were used, and one study [22] was not blinded. Peake et al applied a 48-hour infusion with NAC in septic shock patients with significant comorbidity and upfront compromised cardiac function. On comparison with controls, NAC-treated patients experienced a progressive deterioration in cardiovascular performance [23]. Septic patients with underlying heart disease may be particularly prone to develop myocardial dysfunction during NAC treatment. Insufficient metabolization of NAC at the endothelial cell level with resulting high plasma levels and hence excess NO supply, may explain this detrimental effect [25]. Finally, the only study that included septic patients without shock and with relatively low APACHE II scores, did not show any effect of early NAC infusion on haemodynamic, oxygenation, and inflammatory parameters [24].

It is important to notice that the administration of NAC late in the course of sepsis may be hazardous. Patients who did not "respond" to the NAC bolus received the drug approximately 1 day later than the "responders" and had a significantly lower survival [19]. Also, Molnar et al found that NAC (bolus, followed by 12 mg/kg/hr for 3 to 5 days) did not prevent organ failure in a mixed population of ICU patients, half of whom had infection or sepsis [26]. Patients who received NAC more than 24 hours after hospital admission even had an increased mortality rate and also needed more inotropic support, suggesting impaired cardiac function.

Selenium

Selenium (Se) plays an important role in antioxidant defence as it is incorporated as selenocysteine at the active centre of GPX. Se deficiency is associated with a decrease in GPX activity which leads to accumulation of lipid peroxides. Se compounds, such as sodium selenite, also behave like pro-oxidants [27]. Deliberate administration of pro-oxidant Se doses in humans promotes apoptosis of pro-inflammatory cells [28] and transiently inhibits NFκB binding to DNA [29]. Finally, Se *in se* can modulate inflammatory reactions during sepsis by impairing monocyte adhesion [30]. Se levels become markedly decreased in sepsis and septic shock and the degree of deficiency correlates with severity of disease, organ failure, and incidence of mortality [31,32].

Five studies investigated the effect of selenium supplementation (Se+) in critically ill septic patients [33-37]. Study details are depicted in Table 2. None of the trials reported deleterious effects of Se administration. Blood Se concentrations and GPX activity increased significantly during Se treatment whereas they remained low in the control groups [34,36].

Zimmermann et al showed a non-significant reduction in mortality rate in Se+ patients with sepsis and organ failure [33]. In a population of predominantly septic shock patients, Angstwurm et al observed less acute kidney injury requiring renal replacement therapy, and a tendency towards improved clinical outcome in the Se+ group [34]. In contrast, Mishra et al, adopting a similar Se treatment protocol, found no effect of Se supplementation on organ failure and mortality rate [35]. Moreover, due to an unexpectedly low consent rate, this study had to be curtailed after recruiting only 40 of the intended 80 patients. These three studies are fraught with significant bias. All were single-centred and enrolled only a limited number of patients. In two studies [33,34] Se was open label provided which raises doubt about concealment of treatment allocation. All were underpowered to show a distinctive effect on important endpoints such as mortality, need and duration of mechanical ventilation, reversal of shock, and time spent in the ICU or in the hospital. The exact number of patients with infectious SIRS and the precise timing of intervention for each treatment arm cannot be determined from the Zimmermann study [33]. Patients who did not receive Se supplementation in the Angstwurm and Mishra studies had higher baseline severity of disease [34,35] and either more documented [35] or more severe [34] infection. Finally, patients were not treated according to the

Table 3. Studies on vitamin supplementation in sepsis.

Author [ref]	Patients (n)		Pathology	Relevant compounds in treatment group	Route	Main results
	treatment	control				
Galley [51]	16	14	Septic shock	- NAC, 150 mg/kg bolus + 20 mg/kg for 1 hr - Vitamin C 1g bolus - Vitamin E 400mg bolus	IV	Transient increase in heart rate and cardiac index; decrease in systemic vascular resistance index
Gadek [52]	51 27	47 † 23	ARDS Sepsis/ pneumonia	- EPA / GLA - Vitamin C 844 mg/L - Vitamin E 317 IU/L - β-carotene 5 mg/L - Selenium 77 µg/L for 4-7 days	Enteral	Improved oxygenation; reduced length of ventilatory support and ICU stay; less new organ failure
Pontes-Arruda [53]	55	48 †	Severe sepsis and septic shock, mechanically ventilated	- EPA / GLA / DHA - Vitamin C 840 mg/L - Vitamin E 320 IU/L - β-carotene 670 mg/L - Selenium 130 µg/L for at least 4 days	Enteral	Improved oxygenation, reduced length of ventilatory support and ICU stay less new organ failure; reduced mortality
Beale [54]	27	28	Sepsis	At inclusion : - Tributyrin - Vitamin C 3 g/L - Vitamin E 1 g/L TE - β-carotene 20 mg/L - Selenium 600 µg/L From day 2: - Selenium, + 130 µg/L - EPA /DHA for 10 days	Enteral	Faster recovery from organ failure

† per protocol analysed patients.
ARDS, adult respiratory distress syndrome; NAC, N-acetylcysteine; IV, intravenously; EPA, eicosapentaenoic acid; GLA, γ-linolenic acid; DHA, docosahexaenoic acid; TE, total equivalent; IU, international units; ICU, intensive care unit.

Surviving Sepsis Campaign guidelines that were published in 2004 [38].

Two large multicentre studies, investigating Se supplementation in a double-blinded fashion, were recently published [36,37]. Angstwurm et al conducted a study in 11 German ICUs, enrolling 249 patients with documented SIRS, sepsis, and septic shock and an APACHE III score > 70 [36]. The number of protocol violations being remarkably high (20%), 189 (92 Se+; 97 placebo) patients were finally included in the per-protocol analysis. Se+ patients received Se intravenously as a 1 mg sodium selenite bolus, followed by a daily continuous infusion of 1 mg for 2 weeks. The most salient finding of this study was a 14% absolute decrease in 28-day mortality in the Se+ group (42% versus 56%, p=0.05; OR, 0.56; 95% CI, 0.32-1.00). Subgroup analysis indicated that mortality was most reduced in patients suffering septic shock with disseminated intravascular coagulation, and in patients with APACHE III scores above 102 or with more than three organ failures. It is noteworthy that this study failed to demonstrate any benefit of Se on kidney function as was previously observed by the same principal investigator [34]. Also, it is difficult to explain why Se did not affect organ failure in general but nonetheless lowered mortality. Forceville et al studied 60 patients with septic shock in 7 French ICUs [37]. Patients were randomized to receive placebo or a 10-day continuous infusion, starting with 4 mg sodium selenite on day 1, and 1 mg/day thereafter. The study failed to show any difference between Se+ and placebo patients for primary (time to resolution of shock) and secondary (duration of mechanical ventilation, ICU and hospital length of stay, and mortality) endpoints.

Baseline patient characteristics were very much the same in both studies, except for a higher incidence of diabetes and chronic renal failure in the German trial. 28-day mortality rate in the placebo group of both studies was also similar. How then are the differences between these studies explained? The French study included fewer patients and was thus underpowered to evaluate a survival benefit. Differences in outcome may have been related to a different approach of sepsis resuscitation since both trials finished enrollment in 2004. From diagnosis, patients were included within 24 hours in the German study and within 48 hrs in the French study. This could imply that patients enrolled in the Forceville study received Se supplementation later in the course of sepsis, probably attenuating the beneficial effect. Another, but difficult to prove, explanation might be an incipient toxicity of sodium selenite counterbalancing any moderate beneficial effect related to subsequent Se infusion. Still, the most plausible reason for the observed difference is the use of the high Se bolus dose. The only studies that found a mortality benefit of Se supplementation in critically-ill patients also used a bolus injection of sodium selenite at the start of treatment [33,39]. In contrast with continuous administration, a bolus injection might rapidly raise blood selenium concentrations to levels at which the *in vivo* beneficial Se effect is likely to occur. *In vitro* studies suggest that such effect requires Se concentrations > 5 µmol/L [40]. As recently shown in an ovine model of septic shock, such blood levels were indeed obtained by bolus but not by continuous infusion of Se [41].

Both the French and the German study also have significant shortcomings. Patient enrollment was laborious and prolonged (about 4 patients per year in each participating centre!), which suggests presence of selection bias. More importantly, both studies did not control for confounding variables (gender, severity of illness, degree of organ failure, glycaemic control). Additional research on Se supplementation in sepsis should focus on determination of the

optimal dose, and on identifying those patients who are most likely to benefit from this treatment. In a meta-analysis of 186 critically ill patients, Heyland et al showed that only those studies using a Se dose above 500 µg/day were associated with a trend towards a lower mortality [42]. The same authors, using escalating doses of Se in patients with septic or cardiogenic shock, concluded that administration of 800 µg Se (in combination with other antioxidants) was effective and safe [43]. While awaiting the results of an ongoing large multicentre trial to evaluate whether this dose has a positive effect on mortality, it actually seems that a Se dose less than 800 µg may lack efficacy in septic patients whilst doses exceeding 1000 µg might be harmful.

Antioxidant vitamins

Vitamins C and E are biologically the most important antioxidant vitamins. They act synergistically both *in vitro* and *in vivo*. Vitamin C is a powerful electron donor, reacting with superoxide and hydroxyl radicals. Vitamin E protects against lipid peroxidation resulting in the production of α-tocopherol radicals which are, in turn, recycled to vitamin E by interference of vitamin C. Oxidized vitamin C then is regenerated by GSH. Parenteral high-dose vitamin C reverses sepsis-induced suppression of microcirculatory control in rodents [44], and completely restores the endotoxin-induced vascular hyporeactivity [45] and impaired endothelial vasodilatation [46] in humans. High-dose enteral vitamin E modulates the monocyte/macrophage response to endotoxin [47]. Plasma concentrations of both vitamins are markedly depleted in patients with severe sepsis and septic shock [5,48].

Two large controlled studies showed striking protective effects of enteral supplementation of vitamins C and E in critically ill patients [49,50]. In a large cohort of 595 critically ill, predominantly male, trauma patients, the incidence of organ failure was reduced and ICU stay considerably shortened in subjects treated with vitamin C (3g/day, intravenously) and vitamin E (3000 IU/day, enterally) [49]. In a population of 216 medicosurgical and coronary care patients, those who received supplementation with vitamin C (500 mg/day) and vitamin E (400 IU/day) for 10 days had reduced 28-day mortality [50]. Unfortunately, these studies included a negligible number of septic patients.

Four studies that evaluated the effect of antioxidant vitamin supplementation in sepsis were identified (Table 3) [51-54]. A uniform characteristic of these studies is the concomitant use of other agents with intrinsic anti-inflammatory, antioxidant, or immune-enhancing potential. In an open-label study including 30 patients with septic shock, the treatment group received an intravenous "cocktail" consisting of NAC (bolus + 1 hr infusion) in association with single boluses of vitamins C and E [51]. Treatment did not affect total plasma antioxidant capacity as determined by enhanced chemiluminescence (median 1120 (520-2360) at baseline versus 1150 (460-2160) µmol/L at 6h) but increased plasma nitrite levels. Initially elevated lipid peroxide and malondialdehyde concentrations did not further increase. Clinically, a transient rise in heart rate and cardiac output and a decreased systemic vascular resistance were observed. Although not considered as such by the authors, the latter effect is likely to be NAC-driven. Indeed, a similar haemodynamic pattern is commonly observed when the initial 150mg/kg NAC bolus is administered in patients with septic shock [19-21]. These changes appear to be short-lived and probably merely reflect both a volume and a concentration dependent vasorelaxant effect of the bolus infusion.

Gadek et al used for 4 to 7 days a special formula containing vitamins C and E but also β-carotene, selenium, eicosapentaenoic acid (EPA, fish oil) and γ-linolenic acid (GLA, borage acid) in patients with ARDS [52]. From the 146 patients enrolled, ninety-eight were evaluable, of whom 50 had pneumonia or sepsis. Patients fed the enriched enteral diet (n = 51; 27 with pneumonia or sepsis) had improved oxygenation, reduced length of ventilatory support and ICU stay, and less organ failure. These results were recently confirmed by Pontes-Arruda et al using a similar high-fat enteral formula containing an oil blend of EPA/GLA in association with antioxidants in mechanically ventilated patients with severe sepsis and septic shock [53]. The solution contained higher amounts of β-carotene and selenium. A total of 165 patients were enrolled of whom 103 subjects were deemed evaluable. Compared with controls, patients who received the study diet had better oxygenation parameters and more ventilator-free days. They spent fewer days in the ICU and developed less new organ failure. Most importantly, there were 18 deaths in the group that received the study diet compared with 25 deaths in the control group, resulting in an absolute risk reduction for mortality of 19.4% (95% confidence interval 0.3-36.7). A major drawback of the Gadek and Pontes-Arruda studies is the impossibility to determine to what extent the anti-inflammatory (EPA/GLA) and the antioxidant (vitamins, selenium) compounds of the diet contributed each to the observed clinical success. Two retrospective analyses of the Gadek study tried to elucidate possible mechanisms underlying the observed remarkable benefit [55,56]. In patients fed the special diet, plasma levels of β-carotene and vitamin E were restored whereas oxidant stress did not diminish [55]. In a subgroup of 43 patients (30 with sepsis or pneumonia, of whom 15 received the diet), bronchoalveolar lavage showed decreased levels of interleukin-8 and leukotriene B4 in association with a reduction of neutrophils and protein leakage [56].

Beale et al administered an enteral formula containing glutamine dipeptide, tributyrine and a high load of vitamins C and E, β-carotene and selenium to patients with sepsis [54]. This pharmaconutritive supplement was started within 24 hours of ICU admission and continued for up to 10 days. From day 2, this group additionally received an immune-enhancing feed (glutamin/arginine/glycine plus EPA/docosahexaenoic acid). This complex trial was stopped prematurely after 55 patients had been enrolled because subjects receiving the combined pharmaco- and immunonutrition formula (n = 27, 17 completing the protocol) experienced a faster recovery from organ failure than those who were fed the control diet. No significant treatment-related differences in antioxidant and inflammatory parameters were observed. Vitamin C, vitamin E, and β-carotene levels rose rapidly in the intervention group whilst Se levels (despite daily doses exceeding 300µg in many patients!) remained similar and relatively low throughout. Again, this study does not allow outlining of the individual contribution of the antioxidant vitamins in achieving the observed beneficial clinical outcome.

Nitric oxide synthase inhibition

Excessive and sustained production of NO during sepsis is associated with the development of peripheral vasodilatation, capillary leakage, and altered cellular oxygen utilization. Clinically, this has been linked to the occurrence of hypotension, decreased responsiveness to vasopressors, and development of organ failure [57]. Intravenous infusion of the nonselective NO synthase inhibitor N^G-methyl-L-arginine hydrochloride for up to 72 hours in patients with early septic

shock lowered elevated plasma nitrate concentrations, increased vascular tone, reduced cardiac index and oxygen delivery, and promoted faster resolution of shock [58,59]. However, a subsequent phase III randomized, double-blind, placebo-controlled trial in nearly 800 patients with septic shock was prematurely discontinued due to a significant increase in overall mortality in the NO synthase inhibitor group (59% vs. 49% ; $p < 0.001$) [60]. The reason for this higher mortality rate is unclear. Interestingly, a greater proportion of patients receiving the investigational drug experienced serious cardiovascular events (i.e. decreased cardiac output, heart failure, pulmonary and systemic arterial hypertension, cardiogenic shock). Subgroup analysis revealed that patients who received a lower maximum dose rate of the study drug (≤ 5 mg/kg/h) had an improved survival rate as compared with the placebo group, whereas the outcome of patients administered more than 5 mg/kg/h was worse than in the placebo group [60]. However, a possible dose-related effect on mortality remains speculative given the low number of patients receiving the lower dose of study drug.

Since profound and sustained production of NO is considered to be derived from iNOS, a more selective inhibition of this enzyme whilst leaving eNOS activity intact may have potential benefit on vascular dysfunction in sepsis. The selective iNOS inhibitors S-methylisothiourea sulfate [61] and aminoguanidine [62], have been shown to improve or to prolong survival in rodent models of endotoxic shock. In contrast, other iNOS inhibitors such as mercaptoethylguanidine and ONO-1714 either failed to decrease mortality [63] or improved survival only when used at the appropriate dose and time points [64] in rats subjected to caecal ligation and puncture. No clinical trials using selective iNOS inhibition have been published.

Conclusion

Upfront distortion of the pro-oxidant / antioxidant balance is relevant to the development of sepsis-induced microvascular and organ failure. Early antioxidant supplementation thus seems to be a logical way to protect septic patients. However, proof of the benefit of this approach is not convincing. Clinical trials investigating antioxidant treatment were mostly performed on small-sized, heterogeneous, or specific patient groups, limiting extrapolation of results to a large population. The timing of administration is of concern. Antioxidants apparently should be given during early resuscitation of sepsis. If administered during evolving sepsis, in particular when organ failure has already developed, they may either lack efficacy or become harmful.

Another important issue is the correct dose to use. In fact, dosing guidelines are not clearly defined, neither for intravenous (NAC, selenium) nor for enteral (vitamins C and E) administration. Also, the pharmacologic behaviour of these agents (metabolization, dose-response, toxicity, and drug interactions) are only partly documented or unknown. Some trials investigated the effects of co-administration of different antioxidants or a combination of antioxidants with other anti-inflammatory and/or immune-enhancing compounds. Although a combination of drugs with different protective properties could theoretically perform better than monotherapy, this approach thwarts the interpretation of study results since the proportional impact of each individual agent cannot be estimated. Finally, oxidative stress is only one of the many pathways within the complex pathophysiology of sepsis. It is unlikely that antioxidant therapy alone will entangle the many intertwined inflammatory and non-inflammatory sepsis-induced processes.

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