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# Metabolic resuscitation in sepsis : could antioxidants be the answer?

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## INTRODUCTION

Antioxidants are molecules that inhibit oxidation which under certain conditions leads to the production of free radicals, highly reactive species characterized by an unpaired electron which enter into further chain reactions that lead to cell damage. (1) In biological systems these include reactive oxygen species (ROS) which include the hydroxyl radical (OH.), hydrogen peroxide (H2O2) and the superoxide anion (O2.-) among others. The generation of such species may trigger a variety of pathological responses and any disequilibrium between production of ROS and the ability to attenuate the damage that such species may incur is referred to as oxidative stress. Oxidative stress may result in damage to any component of the cell and may result in DNA damage through base damage as well as strand breaks and also some ROS may act as cellular messengers causing disruption in cellular signaling. Cellular protection against oxidative stress may be through chelation of trace metals involved in free radical generation or through the actions of antioxidants. Antioxidants are broadly classified into two groups, depending on whether they are soluble in water (hydrophilic), such as vitamin C or fat soluble such as Vitamin E (lipophilic). Hydrophilic antioxidants are thought to predominantly react with oxidants in the cell cytosol and plasma whereas lipophilic antioxidants protect cell membranes from oxidation: a process termed lipid peroxidation. (2) The synergism between different antioxidant systems is complex. Indeed, both vitamin C and vitamin E were shown to have a direct interaction with vitamin C "repairing" the α-tocopherol radical with rates approaching diffusion limited outlining the reactivity of these species. (3)

One of the areas that has attracted considerable interest with regard to the role of oxidative stress is the host response to sepsis. (4) Sepsis remains a major cause of death worldwide affecting over 18 million people annually with a mortality rate approaching 80% in those individuals with multi-organ failure and in the US hospital costs total over $24 billion dollars. (5, 6) Therapy for severe sepsis is predominantly supportive with the relatively recent introduction of care bundles including antibiotic therapy being introduced. However, the precise pathogenesis of sepsis-induced organ failure remains elusive and although likely multifactorial in nature certainly microvascular dysfunction appears to be central to the process. (7) Microvascular dysfunction involves impairment of arteriolar reactivity, derangement of endothelial barrier integrity and microthombi induced plugging of the capillaries thus any therapy that addresses these issues may translate into improved outcomes.

Key words: sepsis, antioxidants, resuscitation

## THE CASE FOR ANTIOXIDANT SUPPLEMENTATION?

Given the putative role of oxidative stress in the pathogenesis of sepsis it follows that antioxidant supplementation may be of potential benefit. Numerous candidate molecules exist but the most studied is that of ascorbic acid (Vitamin C). The almost ubiquitous nature of ascorbic acid throughout the animal kingdom is of interest not least because only humans and guinea pigs fail to synthesize it due to a lack of the enzyme L-Gulonolactone Oxidase which catalyzes the conversion of L-Gulonolactone to L-Ascorbic acid therefore it is only obtained through diet. Ascorbic acid is vital for numerous cellular processes as outlined in Table 1. The pivotal role for vitamin C is in its antioxidant capacity as outlined in Figure 1. Certainly preclinical studies have demonstrated that high-dose ascorbate administration can
prevent or restore microcirculatory flow impairment through inhibition of nicotinamide adenine dinucleotide phosphate-oxidase as well as inducible nitric oxide synthase, augmenting tetrahydrobiopterin. This may prevent uncoupling of oxidative

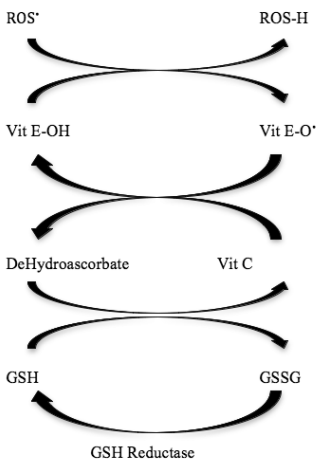


*Figure 1. Schematic representation of the potential antioxidant cascade involving water soluble (Vitamin C) and fat soluble (Vitamin E) antioxidants.*

phosphorylation leading to a potential reduction in ROS including superoxide and peroxynitrite. Moreover, vascular responsiveness to vasoconstrictors may be restored through vitamin C administration and also it may preserve the endothelial barrier through preventing apoptosis. Fur-

Table 1. Major Roles of Ascorbic Acid (Vitamin C)

| Antioxidant Effect | Major water soluble antioxidant |
|---|---|
| Collagen & Bone Formation | Vitamin C-dependant Proline and Lysine hydroxylases convert procollagen to collagen |
| | Necessary for Osteoblast function |
| Amino Acid Formation & Catecholamine Synthesis | Tryptophan and Tyrosine Metabolism |
| | Dopamine Hydroxylase is Vitamin C dependant |
| Folic Acid Metabolism | Essential for folate reduction to tetrahydrofolate |
| Hormone Synthesis | Co-factor in the synthesis of aldosterone, corticosteroids and some peptide hormones |
| Drug Detoxification | Component of the mixed-function oxidase system |

thermore it has been proposed that high-dose vitamin C may augment the host response against infection such as protection against overwhelming oxidative stress due to ischemia/reperfusion, sepsis or burns. This may of course be in combination with other antioxidants. (8)

Many questions remain unanswered with regard to the role of antioxidant supplementation in sepsis. What is clear, however, is that several studies have shown that sepsis depletes endogenous plasma ascorbate which is not corrected by parenteral nutrition containing a moderate amount of ascorbate (200 mg/day). Levels do return to normal upon resolution of the inflammatory illness which may reflect accelerated destruction of ascorbate. (9) Interestingly, parenteral supplementation may restore plasma ascorbate levels in critically ill patients if the administered dose is adequate. For example, parenteral ascorbate at a dose of 300 mg/day fails, but 1000 mg/day can be effective. (10) However, the response to vitamin C repletion is somewhat variable in our critically ill patients. After injection of a mixture of antioxidants containing a nominal dose of 1000 mg ascorbate serial blood sampling demonstrated a raised plasma ascorbate in some individuals but not in others. (11) Such variability may reflect different rates of extracellular oxidation of ascorbate or cellular uptake of ascorbate or indeed differences in the rates of urinary excretion of ascorbate. Until recently results of trials involving antioxidant supplementation have been mixed. For example, administration of ascorbate (1500 mg/day) in combination with other antioxidants (vitamin E, beta carotene, zinc, and selenium) failed to decrease mortality in critically ill adults. (12) Two small, randomized clinical trials have given parenteral ascorbate at high doses as an adjuvant therapy to patients at high risk of becoming septic. In burns patients at high risk of sepsis demonstrated that infusion of ascorbate decreased oedema and improved respiratory function. (13) In a study on surgical patients (91% trauma) a combination of ascorbate (3000 mg/day i.v. for up to 28 days) and vitamin E was administered. (14) Multiple organ failure was significantly less likely to occur in patients receiving antioxidants than in patients receiving standard care, with a relative risk of 0.43 but with wide confidence limits (95% confidence interval 0.19-0.96). Patients randomized to antioxidant supplementation also had a shorter duration of mechanical ventilation and length of ICU stay. Despite these mixed results the potential of antioxidant supplementation continues to attract attention.

## RECENT EVIDENCE

A recent study has regenerated interest in the use of ascorbic acid in the critically ill particularly in sepsis following publication of a before-after study. (15) Patients were considered to have sepsis coupled with a procalcitonin level of >2 ng/ml underwent treatment.
Patients were treated with a combination of thiamine 200mg iv 12 hourly, as well as 6 hourly ascorbic acid 1.5 G and hydrocortisone 50 mg.

47 patients were in each group which, although not randomized, were well matched in terms of illness severity. Around 50% of each group were mechanically ventilated, 46% on vasopressors, over 60% had evidence of renal dysfunction and approaching 30% had positive blood cultures. The primary endpoint was mortality. Data was analysed using logistic multivariate analysis and also propensity adjusted outcomes. Regardless of statistical model the outcome was the same: a dramatic reduction in mortality in those receiving the vitamin "cocktail" ($p<0.001$). Moreover, the predicted mortality in the control group was as expected from APACHE IV data of 40% whereas that in the treated group was 10%. Furthermore the rate of renal replacement therapy fell from 33 to 10% in the treatment group. As the authors point out "No patient in the treatment group developed progressive organ failure and the four deaths in this group were related to the patients underlying disease; these patients did not die from sepsis related complications". An astonishing observation.

Clearly this study has several limitations. It is single centre and adopts a before/after non-blinded design and as such will be open to the usual statistical criticisms. It also examined three simultaneous interventions and as therefore ascribing benefit to one particular agent is impossible. However the effect is dramatic especially as studies to-date have not showed any near such response from any of the single agents. Is there evidence that patients with sepsis treated with Thiamine 200mg iv 12 hourly show a 30% fall in observed mortality when used in isolation ? No there is not. Is there evidence that patients with sepsis treated with 6 hourly Ascorbic acid 1.5 G show a 30% fall in observed mortality when used in isolation ? No there is not. And hydrocortisone 50 mg. Any evidence of a 30% fall in observed mortality when used in isolation ? No there despite the numerous sepsis trials. So why should such synergy exist and is it biologically plausible?

## BIOLOGICAL PLAUSIBILITY

The combination of ascorbate plus steroid was used as they are considered synergistic

particularly in terms of endothelial integrity. However it seems implausible that such a dramatic effect is seen when each component in isolation has little or no effect on outcomes in sepsis. The explanation as to why this combination appeared to have a marked effect on the course of sepsis is proposed to be related to the multiple and overlapping effects of all three agents with vitamin C and corticosteroids acting synergistically. (16) The latter is explained by the observation that ascorbic acid has been demonstrated to reverse oxidation of cysteine thiol groups of the glucocorticoid receptor affecting ligand binding and the efficacy of glucocorticoids. However this was in cloned cellular models and not in critically ill patients. (17) Also the hypothesis that glucocorticoids may increase expression of vitamin C transporters has again only been observed in cellular systems. (18) Nevertheless the observations are remarkable prompting the authors to state "We believe that the results of our study provide sufficient information for the design of an adequately powered, high quality pragmatic trial to confirm the findings of our study". Certainly the antioxidant role of ascorbate under such conditions, in particular as a "metabolic resuscitator" in terms of oxidative stress, is somewhat fanciful. Evidence seems to suggest that marked oxidative stress as a result of sepsis initiates changes in mitochondrial function. Under normal conditions a complex system of interacting antioxidant defences is able to combat oxidative stress and prevents mitochondrial damage but there is still little conclusive evidence of any attenuation of such damage with systemic antioxidant supplementation. (4) It may be that studies on antioxidant supplementation to-date have not specifically targeted the mitochondria. Therefore reliable delivery and activity of a targeted antioxidant to the mitochondria may be necessary and such an approach may be plausible where delivery of the antioxidant is achieved using a carrier molecule or through administration of an antioxidant which naturally acts or accumulates in mitochondria. Alternative approaches may include augmenting mitochondrial antioxidant defences by pharmacological means or by increasing endogenous expression of antioxidant enzymes genetically. Certainly there is no evidence that vitamin C loading in the critically ill in anyway influences the antioxidant status of the mitochondria.

## SAFETY OF VITAMIN C

In general ascorbate is not a dangerous intervention for most individuals although there are concerns that high-dose ascorbate may induce pro-oxidant effects in patients. (19) This may occur through interaction with transition metals such as iron facilitating the Fenton reaction with hydrogen peroxide to generate ROS. However, this pro-oxidant effect is not supported by observations in most of the subjects and patients who have been studied. For example, repeated i.v. injection of 750–7500 mg/day of vitamin C for 6 days in healthy volunteers did not induce a pro-oxidant change in plasma markers. (19) However, high-dose ascorbate increases the risk of intravascular haemolysis in patients with glucose-6-phosphate dehydrogenase deficiency. (20) The other concern is that formation of calcium oxalate stones in the kidneys is a potential adverse effect of long-term administration of high-dose ascorbate. (21)

## THE FUTURE?

Despite their being many proponents of vitamin C therapy several questions remain to be solved, including optimal dose, timing and combination of vitamin C with other antioxidants. (8, 22) High-dose vitamin C is cheap and relatively well tolerated. The study by Marik, no matter what one thinks of the results, will no doubt stimulate much debate and the only robust rebuttal will be data from a multi-centre randomized controlled trial. Of course, if this is negative we will then be faced with the argument that it may be centre specific! It does seem implausible that such a dramatic effect will be replicated however we will have to wait and see. In the meantime will you be supplementing your patients antioxidants?

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