Oxidative stress has been implicated in the manifestations of critical illnesses, including ischemia and reperfusion injury and systemic inflammatory states. This review describes the evidence for increased oxidative stress in critically ill patients and explores the data regarding antioxidant therapy for these conditions. Antioxidant therapies reviewed include N-acetylcysteine, selenium, vitamins E and C, superoxide dismutase, catalase, lazaroïds, and allopurinol. We focus on the results of these interventions in animal models and human trials, when available.


Increasing evidence supports the role of systemic oxidative stress in the development and manifestation of critical illness. Oxidative stress is defined as a state in which the level of toxic reactive oxygen intermediates (ROI) overcomes the endogenous antioxidant defenses of the host. Oxidative stress can result, therefore, from either an excess in oxidant production, or depletion of antioxidant defenses. Reactive oxygen intermediates are produced as a result of normal physiologic processes, including leakage of electrons from cellular electron transfer chains, and as byproducts of membrane lipid metabolism (Figure 1). During illness, ROI are produced by phagocytic cells as a mechanism to kill invading microorganisms. When inflammation becomes systemic, however, as in sepsis or the systemic inflammatory response syndrome, loss of control of ROI production may lead to nondiscriminatory bystander injury in the host. Reactive oxygen intermediates cause direct cellular injury by oxidative injury to cellular proteins and nucleic acids, and by inducing lipid peroxidation, which leads to the destruction of the cell membrane.

In addition to causing direct cytotoxicity, ROI also play a role as second messengers in the intracellular signaling pathways of inflammatory cells. In particular, the activation of the critical nuclear transcription factor, nuclear factor κB (NF-κB), has been induced by hydrogen peroxide and blocked by several antioxidants, including vitamin E.1,2 Nuclear factor κB is a central transcription factor involved in the regulation of numerous proinflammatory genes, including many cytokines (tumor necrosis factor, interleukin [IL]-1, IL-6, IL-8, IL-2), hematopoietic growth factors (granulocyte-macrophage colony-stimulating factor, macrophage colony-stimulating factor, granulocyte colony-stimulating factor), cell adhesion molecules (CAM) (intercellular CAM-1, endothelial-leukocyte adhesion molecule 1, vascular CAM-1) and nitric oxide synthase (iNOS).3 Nuclear factor κB has been demonstrated as an important mediator in the signal transduction for both endotoxin and inflammatory cytokine-induced activation.3 A second major transcription factor, activator protein 1 (AP-1), also seems to be regulated by changes in the redox state of the cell and can be activated by both oxidants and antioxidants depending on the cell type and on intracellular conditions.4-6 In addition, several inflammatory genes have pro-motor sites for AP-1, although its role in inflammatory signaling remains less well documented than NF-κB.4 Thus, altering the redox state of the cell may contribute to the ongoing inflammatory cytokine production and progression of systemic inflammation, leading to organ injury. This

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may be manifest by the development of the acute respiratory distress syndrome (ARDS) or multiple organ failure syndrome.

In addition to states of systemic inflammation, oxidative stress has been implicated in the manifestations of another common cause of critical illness: ischemia and reperfusion injury. Ischemia of tissue beds followed by reperfusion with oxygenated blood, during resuscitation, leads to significant production of ROI. This is primed by the increased activity of xanthine oxidase and increased production of hypoxanthine due to loss of adenosine triphosphate; XO, xanthine oxidase. The small square bullet denotes a free radical.

The enzymatic antioxidants include superoxide dismutase, which catalyzes the conversion of O$_2^-$ to H$_2$O$_2$ and H$_2$O; catalase, which then converts H$_2$O$_2$ to H$_2$O and O$_2$; and glutathione peroxidase, which reduces H$_2$O$_2$ to H$_2$O by oxidizing glutathione (GSH). Re-reduction of the oxidized form of glutathione (glutathione disulfide) is then catalyzed by glutathione reductase. These enzymes also require trace metal cofactors for maximal efficiency, including selenium for glutathione peroxidase; copper, zinc, or manganese for superoxide dismutase; and iron for catalase.

The nonenzymatic antioxidants include the lipidsoluble vitamins (vitamin E, and vitamin A or β-carotene) and the water-soluble vitamins (vitamin C and glutathione). Vitamin E has been described as the major chain-breaking antioxidant in humans. Vitamin E is a generic term encompassing a collection of tocopherols and tocotrienols obtained from plant oils. The most biologically active form is α-tocopherol. Because of its lipid solubility, vitamin E is located in cell membranes where it interrupts lipid peroxidation and plays a role in modulating intracellular signaling pathways that rely on ROI. Vitamin C can also directly quench ROI, including O$_2^-$, HO, and 1O$_2$. Vitamin A is a term encompassing a collection of retinols obtained in the diet primarily from dairy products, eggs, liver, and fortified cereals. β-Carotene is found in a variety of fruits and vegetables, and it provides approximately 25% of the vitamin A in Western diets. Dietary β-carotene is converted to retinol at the level of the intestinal mucosa, and it functions as a chain-breaking antioxidant.

Vitamin C (ascorbic acid), obtained primarily from citrus fruits, functions as a water-soluble antioxidant capable of broadly scavenging ROI, including the major neutrophil oxidants: HO, H$_2$O$_2$, and hypochlorous acid. Under certain circumstances, vitamin C has been shown to have pro-oxidant properties as well. For example, when combined with iron, it has been shown to accelerate lipid peroxidation, which leads to cellular membrane damage. Finally, GSH, which is synthesized intracellularly from cysteine, glycine, and glutamate, is capable of either directly scavenging ROI, or enzymatically doing so via glutathione peroxidase (Figure 2). In addition, GSH is crucial to the maintenance of enzymes and other cellular components in a reduced state. The majority of GSH is synthesized in the liver, and approximately 40% is secreted in the bile.

The enzymatic and nonenzymatic antioxidant systems are intimately linked to one another, as illustrated in Figure 2. Both vitamin C and GSH have been implicated in the recycling of α-tocopherol radicals. In addition, the trace elements selenium, manganese, copper, and zinc play important roles as nutritional antioxidant cofactors. Selenium is a cofactor for the enzyme glutathione peroxidase; and manganese, copper, and zinc are cofactors for superoxide dismutase. Zinc also acts to stabilize the cellular metallothionein pool, which has direct free radical quenching ability. The complex interactions of these different antioxidant systems may imply that successful therapeutic strategies will depend on the use of a combination of various antioxidants rather than a single agent.

### Table

**Antioxidant Defenses**

<table>
<thead>
<tr>
<th>Enzymatic</th>
<th>Nonenzymatic</th>
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<td>Superoxide dismutase (O$_2^-$ → H$_2$O$_2$)</td>
<td>Vitamin E</td>
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<tr>
<td>Catalase (H$_2$O$_2$ → H$_2$O + O$_2$)</td>
<td>Vitamin C</td>
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<td>Glutathione peroxidase</td>
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<td>(GSH + H$_2$O$_2$ → GSSG + H$_2$O)</td>
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EVIDENCE OF OXIDATIVE STRESS IN CRITICAL ILLNESS

Numerous investigators have evaluated the systemic oxidant state of critically ill and injured patients. Surrogate by-products of membrane lipid peroxidation are elevated in the serum of several critically ill patient populations.\textsuperscript{13-19} In addition, there is evidence of increased oxidant activity in the lungs of patients with the acute respiratory distress syndrome (ARDS) as manifest by increased myeloperoxidase activity and products of lipid peroxidation detected in the bronchoalveolar lavage fluid.\textsuperscript{17} Measurement of antioxidant defenses has consistently demonstrated depressed plasma levels of vitamins E and C in patients with sepsis and ARDS.\textsuperscript{12,17,20-23} Low plasma vitamin C levels have also been shown to be predictive of the development of multiple organ failure syndrome in populations at risk.\textsuperscript{24} Similarly, glutathione levels are depressed in the plasma of patients with hepatic failure, in polytrauma patients, and in the bronchoalveolar lavage fluid of those with ARDS.\textsuperscript{19,25-27}

A recently developed assay measuring total serum antioxidant status has also been applied to several populations of critically ill patients.\textsuperscript{28,32} This assay is based on the inhibition by serum antioxidants of the absorbance of the radical cation 2,2’-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS). These studies have demonstrated mixed results; however, on the whole they support the presence of increased systemic oxidative stress and the depletion of antioxidant defenses during critical illness. As a result, several investigators have sought to evaluate the usefulness of antioxidant therapy for these patients.

ANTIOXIDANT THERAPY

\textbf{N-Acetylcysteine}

The most widely used antioxidant in experimental and clinical models is N-Acetylcysteine (NAC). Nacetylcysteine is converted, in vivo, to L-cysteine, which is used to replete intracellular stores of glutathione. The thiol group on the NAC molecule affords it direct antioxidant activity as well.\textsuperscript{33} N-Acetylcysteine is an attractive agent for clinical trials, as it has been safely used in humans for several years for the treatment of acetaminophen overdose, and as a mucolytic agent in patients with obstructive pulmonary disease.\textsuperscript{34} N-Acetylcysteine can be administered orally, intravenously, or as an inhalation agent. The oral administration of NAC increases GSH levels in the liver, plasma, and bronchoalveolar lavage fluid, suggesting a widespread systemic effect.\textsuperscript{35}

Use of NAC in animal models of ischemia and reperfusion injury and ARDS has demonstrated encouraging results.\textsuperscript{36-45} In models of acute lung injury, based on the intratracheal administration of lipopolysaccharide or IL-1, there was attenuation of pulmonary injury and a significant reduction in lung permeability and lipid peroxide production, even when NAC was administered up to 2 hours after endotoxin or IL-1 challenge.\textsuperscript{37,42} A more recent study has demonstrated that liposomal encapsulation of NAC, administered intratracheally, leads to a prolonged protective effect in a rat model of acute lung injury.\textsuperscript{46}

Based on the encouraging results in animal studies, several human trials of NAC for the treatment of ARDS have been completed.\textsuperscript{46,47,49} Recent studies of patients with ARDS have confirmed the ability of parenteral NAC administration to increase GSH levels in the bronchoalveolar lavage fluid and within pulmonary granulocytes.\textsuperscript{50,51} Clinical trials to demonstrate benefit in patients with ARDS, however, have had equivocal results. Jepsen et al,\textsuperscript{47} in a prospective, randomized, double-blinded trial of NAC vs a placebo in patients with established ARDS, were unable to show any difference in the PaO$_2$/FiO$_2$ ratio or survival between the groups. Similarly, Domenighetti et al,\textsuperscript{49} in a similar group of patients, demonstrated improved oxygenation and a decreased need for ventilatory support in the NAC-treated group. Bernard et al,\textsuperscript{48} in a prospective, randomized, double-blinded trial of NAC, or pro-cysteine vs placebo, were able to show an increase in red blood cell GSH levels, suggesting that the drugs were active and that the number of days of acute lung injury were significantly reduced. There was no difference in mortality in any of these studies, but all had relatively small sample sizes. Further trials are needed to determine whether patients at an earlier stage in the disease process, or preferably, those at risk for the development of ARDS, will benefit from NAC treatment.

Trials of NAC for other critically ill patient populations have also had mixed results. No overall outcome benefit was seen in a mixed population of patients in an intensive care unit.\textsuperscript{52} Two studies of NAC administration to patients undergoing a liver transplantation have demonstrated contradictory results, with one showing no benefit, and the other showing improved liver function and better graft survival in the NAC-treated group.\textsuperscript{53,54}
A study of the hemodynamic effects of NAC administration in patients with sepsis, revealed that 45% of patients given NAC demonstrated an increase in oxygen consumption, which was associated with an increase in gastric mucosal pH.\textsuperscript{55} These NAC responders had a better survival rate than nonresponders. Similarly, a more recent study of NAC administration to patients with sepsis demonstrated attenuation of oxidative stress and improvement in clinical scores for these patients.\textsuperscript{56} Lastly, NAC administration has demonstrated significant benefit in the treatment of fulminant hepatic failure secondary to acetaminophen toxicity, and it is widely used for this indication.\textsuperscript{27,58}

Additional clinical trials with larger numbers of patients are needed to better define which population of critically ill patients may benefit from NAC therapy. In addition, it will be important to define the appropriate timing for intervention in each disease process. As suggested by the results of the ARDS trial, patients with established disease may not benefit, as the oxidant damage has been done. It may be more appropriate to target patients early in the inflammatory process or at the time of reperfusion following ischemic insults.

**Selenium**

Another strategy to indirectly alter the antioxidant balance is the repletion of the trace element selenium. Selenium is a critical cofactor for the function of the enzyme glutathione peroxidase, which is involved in the oxidation of glutathione. One study has evaluated selenium supplementation in patients with systemic inflammatory response syndrome, in which all patients had low serum selenium levels at the onset of the study.\textsuperscript{59} These authors demonstrate a lower frequency of renal failure, a more rapid resolution of organ dysfunction, and a trend toward a decreased mortality rate for patients receiving selenium supplementation. Further study is needed to fully elucidate the mechanism of benefit and clinical usefulness of this approach in different patient populations.

**Vitamin E**

Serum and tissue α-tocopherol levels fall steadily and dramatically in the first 24 hours following endotoxin infusion or cecal ligation and puncture.\textsuperscript{60,61} Several investigators have demonstrated improved survival following α-tocopherol treatment in these animal models of sepsis.\textsuperscript{62,64} In addition, α-tocopherol treatment in animals with sepsis has been shown to decrease hepatic lipid peroxidation, attenuate disseminated intravascular coagulation, and reduce plasma lactate levels.\textsuperscript{65,66,68} Additional models of excessive inflammation in which α-tocopherol has been shown to have beneficial effects include a murine hepatic ischemia–reperfusion model, a rat renal ischemia–reperfusion model and in pulmonary inflammation following zymosan-induced peritonitis in rats.\textsuperscript{67,69} In the liver ischemia–reperfusion study, the α-tocopherol–treated group demonstrated decreased lipid peroxidation, enhanced adenosine triphosphate generation, increased survival, and attenuation of hepatic damage.\textsuperscript{68} In a model of renal warm ischemia, α-tocopherol pretreatment had protective effects on the kidney, as evidenced by enhanced adenosine triphosphate levels during reperfusion and lower serum creatinine levels. Increased survival was also noted in ischemic rats following treatment with α-tocopherol.\textsuperscript{69} In the case of zymosan-induced peritonitis, administration of α-tocopherol immediately following intraperitoneal zymosan injection lead to a decrease in production of pulmonary lipid peroxidation by-products, and attenuation of pulmonary tissue damage when compared with controls.\textsuperscript{97} This attenuation of pulmonary injury may be due to the marked inhibition of the alveolar macrophage proinflammatory response, which we have demonstrated following enteral α-tocopherol supplementation.\textsuperscript{70}

A recent study has examined the effect of oral vitamin E supplementation on human monocyte function in healthy volunteers, who were given 1200 IU of α-tocopherol for 8 weeks.\textsuperscript{71} Their monocytes were then harvested and found to have significantly suppressed responses to endotoxin, including decreased ROI production during the respiratory burst, decreased IL-1β production, and inhibition of monocyte-endothelial adhesion.

Despite encouraging results in animal studies and the several reports of decreased levels of vitamin E in critically ill patients, there has been only 1 clinical trial. This is likely owing to the lack of an intravenous preparation. As a result, studies are limited to the oral route, which may lead to impaired drug absorption in this patient population. One study has involved enteral vitamin E supplementation in patients with ARDS.\textsuperscript{72} In this study, serum α-tocopherol levels, following 1-g/d supplementation, were not increased in the ARDS patients to the same degree as controls. However, it is unclear whether this was due to excessive consumption of vitamin E in these patients, or malabsorption due to severity of illness. Clearly, more well-controlled, randomized, prospective studies are needed. In addition, supplementation with higher doses of vitamin E, comparable to the efficacious animal studies, may be necessary to document a protective effect.

**Vitamin C**

Despite demonstration of depressed vitamin C levels in critically ill patients, supplementation with vitamin C alone has not been studied.\textsuperscript{21,23,24} This may be because of the appropriate concern that under conditions of severe oxidant stress, vitamin C can function as a pro-oxidant by promoting iron-catalyzed reactions as an electron donor.\textsuperscript{73} Infusion of vitamin C in patients with sepsis results in rapid consumption, due to either the promotion of redox cycling of iron or as a result of radical scavenging. There seems to be a differential handling of infused vitamin C in patients with sepsis vs healthy subjects, and further studies are needed to elucidate the relative antioxidant and pro-oxidant mechanisms potentially involved.\textsuperscript{73}

**Superoxide Dismutase and Catalase**

Results of superoxide dismutase administration in animal models of sepsis have been variable. In general, su-
peroxide dismutase is effective when administered before the onset of sepsis, but when administered after sepsis, it has been established that it may be harmful. Superoxide dismutase scavenges superoxide but produces hydrogen peroxide, which requires clearance by catalase. If hydrogen peroxide is not effectively cleared, levels of the highly reactive hydroxyl radical may increase. Therefore, in this situation, superoxide dismutase may act predominantly as a pro-oxidant. Thus, it seems logical that use of superoxide dismutase therapy must include the addition of catalase administration. A potential limiting factor for both agents is their distribution. Both are large molecules, and are restricted largely to the extracellular, nonmembrane-bound space. As such, their effectiveness may be limited. Use of the 2 agents in combination has been investigated in one study of dogs with endotoxemia, and demonstrated no benefit from the combined administration whether given before or after endotoxin challenge.

**Combination Therapy**

Based on the recognition that lipid-soluble and water-soluble antioxidants may act in a synergistic fashion, such as during the recycling of vitamin E by vitamin C, it has been suggested that a more appropriate clinical approach involves the replacement of a “cocktail” of antioxidants rather than a single agent. Two clinical trials have investigated this approach. Galley et al administered a combination of NAC, vitamin C, and α-tocopherol to patients in septic shock. They demonstrated a transient beneficial hemodynamic response, but did not assess the effect on outcome. A second study evaluated a supplemented enteric formulation with increased levels of vitamins E and C and β-carotene in patients with ARDS. Patients on this diet required less ventilatory support, had a shorter stay in the intensive care unit, and had a decrease in the development of organ failure when compared with control patients. However, this modified diet also had alterations in the lipid content, with selective increase in the proportion of ω-3 fatty acids. Thus, it is unclear whether the benefits seen in this study are due to an increase in antioxidant activity, or to the effects of altered lipid metabolism on inflammatory cells.

**Lazaroids**

Lazaroids are 21-aminosteroids, which are nonglucocorticoid analogs of methylprednisolone with multiple actions, including the scavenging of ROI, the attenuation of inflammation, and the stabilization of biological membranes. Lazaroids seem promising in animal models of endotoxia, inhalational injury, and acute lung injury. It is likely that human trials are forthcoming. Currently, it remains unclear whether their primary effect is due to the scavenging of ROI, or modulation of the inflammatory response via inhibition of cytokine production.

**Allopurinol**

During ischemia and reperfusion injury, up-regulation of xanthine oxidase contributes to increased ROI production (Figure 1). Allopurinol is an inhibitor of xanthine oxidase, which has been studied as a potential therapy to down-regulate this process. Allopurinol is effective at attenuating the damage from ischemia and reperfusion injury in a number of animal models and, however, the results in sepsis models have been variable. This suggests that the primary mechanism for free radical production in sepsis is not dependent on the xanthine oxidase pathway. Use of allopurinol in human trials has been confined to its preoperative administration to patients undergoing coronary bypass surgery, during which it has proven beneficial in attenuating the cardiac ischemia and reperfusion injury associated with this procedure.

**SUMMARY**

Toxic ROI play a role in the manifestations of critical illness due to both ischemia or reperfusion injury and systemic inflammation. Reactive oxygen intermediates clearly cause direct tissue injury, which can lead to organ failure. In addition, recent studies demonstrate their immunomodulatory role as second messengers within inflammatory cells. Supplemental antioxidant therapy seems promising in the regulation of the uncontrolled production of ROI in these situations. Prior to instituting this therapy, however, we must define the appropriate time points for intervention in each disease process. It seems that treatment becomes increasingly difficult as the inflammatory process and the damage induced becomes irreversible with time. In addition, we need to explore combinational therapy, as it is likely that repletion of both lipid-soluble and water-soluble antioxidants will be required. Lastly, the relatively inexpensive nature of these agents makes funding from industrial partners highly unlikely. A significant challenge lies in finding agencies willing to support encouraging therapeutics such as “simple antioxidants.”

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