Antioxidant therapy in critical care—Is the microcirculation the primary target?

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This review presents the rationale for the therapeutic use of antioxidants in treating critically ill patients; it is not a systematic review of the clinical evidence that has been assessed recently by others. Clinical and nonclinical evidence is presented to support the notion that natural antioxidants are of therapeutic value in treating cardiovascular shock. Oxidative stress is a major promoter and mediator of the systemic inflammatory response. The microcirculation is particularly susceptible to oxidative stress that causes hemodynamic instability, leading to multiple organ failure due to systemic inflammatory response syndrome. Vitamin C is the antioxidant used experimentally to demonstrate oxidative stress as a key pathophysiologic factor in septic shock. Pharmacologic studies reveal that vitamin C (as ascorbate), at supraphysiologic doses, significantly affects the bioavailability of nitric oxide during acute inflammation, including inhibiting nitric oxide synthetase induction. Parenteral high-dose vitamin C inhibits endotoxin-induced endothelial dysfunction and vasohyporeactivity in humans and reverses sepsis-induced suppression of microcirculatory control in rodents. In severe burn injury, in both animals and patients, parenteral high-dose vitamin C significantly reduces resuscitation fluid volumes. Therefore, a significant body of pharmacologic evidence and sound preliminary clinical evidence supports the biological feasibility of using the exemplary antioxidant, vitamin C, in the treatment of the critically ill. (Crit Care Med 2007; 35[Suppl.]:S577–S583)

KEY WORDS: systemic inflammatory response syndrome; oxidative stress; antioxidant therapy; parenteral vitamin C; ascorbate; endothelial dysfunction; severe burn; multiple organ failure

Severe tissue trauma and sepsis drastically disturb systemic metabolic and regulatory processes (1–3). These disturbances are a direct consequence of the initial and sustained activation of the innate immune system, which evokes oxidative stress, further stimulates inflammatory processes, and is a key physiologic factor that drives the systemic inflammatory response syndrome (SIRS) and the consequent multiple organ failure (4, 5). Oxidative stress refers to the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in amounts that exceed the antioxidant protective capacity of the body (6–8). Following severe trauma and during sepsis, ROS are generated by the primarily activated inflammatory and immune cells as well as, along with RNS, within the systemic inflamed and ischemic tissues. The antioxidant capacity is drastically decreased in the face of sustained and excessive production of ROS and RNS. Oxidative stress is not considered an epiphenomenon in the critically ill patient but a central pathophysiologic factor particularly in driving the systemic inflammatory response that can lead to multiple organ failure in patients following severe trauma and sepsis (6–8).

As recently described by Heyland and colleagues (8), following trauma or during sepsis there is a progressive decline in immune function accompanied by a generally sustained systemic inflammatory response. Oxidative stress is involved in both these processes, and the larger the degree of oxidative stress, the poorer is the morbidity and higher is the mortality (9–11). Therein lies the rationale for using antioxidant therapy in the severely critically ill patient. In particular, there is clinical evidence that oxidative stress is a vital factor in septic shock and that antioxidants are of therapeutic potential in treating shock and preventing multiple organ failure (11, 12). The therapeutic use of antioxidants is now considered a serious option for severely critically ill patients. Favorable evidence accumulating in recent years from a variety of small clinical studies has been evaluated in recent reviews (6–8). There is now a solid consensus in favor of further addressing the use of antioxidant therapy in large multicenter randomized trials (8). Normal protection of the body from the toxic effects of ROS and RNS is dependent on a complex interacting mixture of endogenous and exogenous (dietary) antioxidants. Antioxidant therapy in the context of the critically ill patient has been referred to as immune nutrition with the aim of restoring normal redox conditions by supplementation with mixtures of antioxidants and antioxidant precursors. However, important questions remain open, such as which antioxidants to use. There is an intuitive notion that administration of a single antioxidant may disturb the redox balance and evoke prooxidative effects, but there is no evidence of this. The question of dose is obviously vital—do recommended daily allowances provide any guide? This would seem to be unlikely (6) given the fact that recommended daily allowances aim at prophylaxis whereas the critically ill patient is

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experiencing acute severe oxidative stress that needs to be rapidly counteracted.

In this review, the recent antioxidant clinical studies will be only briefly reviewed since there currently exist many up-to-date reviews (6–8). This review will focus on a single antioxidant, vitamin C, and discuss the experimental as well as clinical evidence indicating that a high dose of a single antioxidant may provide clinical benefit in the treatment of the severely critically ill patient. This discussion will particularly deal with the rationale for high doses of antioxidant to protect and stabilize the microvasculature and so prevent circulatory shock. The aim here is to highlight the peripheral vasculature as the target for antioxidant therapy and so provide a means of preventing multiple organ failure. There is increasing experimental evidence that oxidative stress is an early mediator of inflammation-induced endothelial dysfunction and vasohyporeactivity. These findings are vital to a rational assessment of the therapeutic potential of antioxidant administration for the treatment of SIRS and the prevention of multiple organ failure in the critically ill patient. Following severe trauma and sepsis, acute endothelial dysfunction contributes significantly to the hemodynamic instability, compromised microcirculatory function, and inadequate tissue perfusion that lead to severe edema and multiple organ failure (13, 14). The evidence that antioxidants, and in particular vitamin C, are of therapeutic potential in treating the hemodynamic instability in the critically ill patient is reviewed here.

Evidence of the Clinical Benefit of Antioxidant Therapy in Critical Care

The recently published reviews of the clinical benefit of antioxidants in treating the critically ill (6–8) provide a consensus that further studies are warranted since current evidence is encouraging. Nearly all clinical studies have so far tested natural antioxidants or precursors of natural antioxidants, and usually administration of only single agents has been tested. In their review, Crimi and colleagues (7) considered only randomized clinical trials, finding that the number of good quality studies is limited and that a variety of antioxidants have been tested. This means that a thorough systematic review is not possible. N-acetylcysteine is the most extensively studied antioxidant in critically ill patients, and the trials provide inconsistent outcomes. The reviewers suggested that larger trials are warranted because early administration of the antioxidant may be critical for success. They speculated that only specific populations of critically ill patients may obtain benefit from N-acetylcysteine. The reviewers found only one trial of selenium that met their quality criteria, but the positive outcome of this small study did appear promising in terms of resolving organ dysfunction and reducing the incidence of acute renal failure. Trials of the antioxidant vitamins C and E were included in the review and also provided encouraging results. These two vitamins were often tested in combination and most often as enteral supplements. However, there is good evidence to indicate that in the case of vitamin C, parenteral high-dose administration is required to attain therapeutic concentrations (15). Berger (6) also emphasized that antioxidants need to be used at supraphysiologic doses to combat the severe acute oxidative stress occurring in the critically ill patient and that recommended daily allowances are not relevant in determining dose. The critically ill patient experiences a severe decline in the level of antioxidant micronutrients that serve a variety of metabolic functions, and these micronutrients need replenishing. However, there is a drastic pathophysiologic decline in antioxidant status that needs to be considered as an additional, separate aspect of nutritional supplementation.

Vitamin C, Oxidative Stress, Inflammation, and the Cardiovascular System

The cardiovascular system, and specifically the endothelium, is especially susceptible to oxidative stress, which is a major factor in many cardiovascular diseases (16, 17). Patients with many other chronic diseases, such as arthritis (18), diabetes (19), and heavy smoking (20), that are associated with oxidative stress exhibit a risk of developing hypertension that is greater than that exhibited by the general population. The high rate of hypertension in such patients has been shown not to be associated with any of the established cardiovascular disease risk factors (21) and may be directly associated with these patients' reduced antioxidant capacity and an underlying low-grade systemic inflammation. This is mirrored by the reduced plasma ascorbate levels recorded in these patients. There is considerable evidence that vitamin C can protect and restore cardiovascular function by acting on the cardiovascular endothelium (22, 23). Similarly, in severe acute systemic inflammation, such as that following severe tissue trauma (24), sepsis (25), respiratory distress syndrome (26), and acute pancreatitis (27), there is a drastic reduction of plasma levels of ascorbate that reflects oxidative stress and is associated with systemic endothelial dysfunction (impairment of the processes of vasodilation, antihemostasis, and antiatherogenesis and breakdown of endothelial barrier function) (Fig. 1).

Vitamin C Protects and Restores Endothelial Function

A reduced response to pharmacologic activation of endothelium-dependent vasodilatation has been shown to be a prognostic marker of cardiovascular risk (28, 29). Human coronary and peripheral arteries show endothelial dysfunction in a variety of conditions, including atherosclerosis, hypercholesterolemia, smoking, and hypertension. Acute endothelial dysfunction occurs in a number of critical conditions in which local tissue trauma or infection leads to a breakdown of the regulation of systemic microcirculation endothelial function that may lead to organ failure and a state of shock (13, 14).

Endothelial dysfunction is pharmacologically manifest as a loss of endothelium-dependent vasodilation in response to acetylcholine infusion and is associated with decreased generation of nitric oxide (NO) by the endothelium. Defective vasodilatory function is considered as a marker for endothelial dysfunction, but endothelial dysfunction also involves the impairment of the endothelium's antithrombotic and antiatherogenic properties, which also relate to the bioavailability of NO. Furthermore, on exposure to oxidative stress, the endothelial barrier function can become compromised as proinflammatory genes become induced in the endothelium.

It has been established that complete restoration of NO bioavailability requires millimolar concentrations of ascorbate (15, 21, 30, 31) that are only possible to achieve by parenteral vitamin C administration (32). This very likely explains the failure of many studies of oral vitamin C to show any clinical benefit in cardiovascular disease. Recent pharmacologic studies further emphasize the need for

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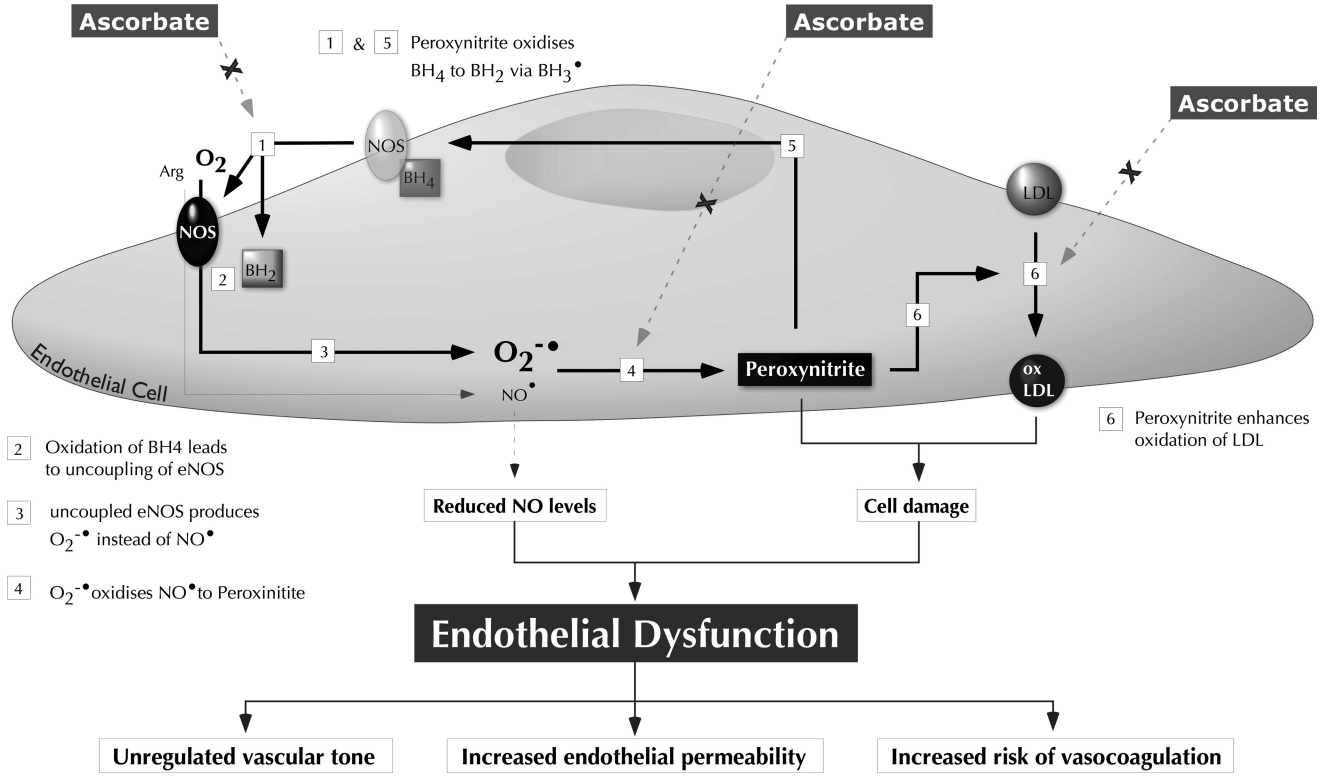

Figure 1. Reduced ascorbate levels/oxidative stress. *BH*, hydrobiopterin; *Arg*, arginine; *NO*, nitric oxide; *NOS*, nitric oxide synthase; *eNOS*, endothelial nitric oxide synthase; *LDL*, low-density lipoprotein.

supraphysiologic doses of ascorbate to restore endothelial function (33, 34). Doses must be sufficient to achieve millimolar concentrations of plasma ascorbate (34), and simply restoring physiologic ascorbate levels (70–100 μM) is inadequate. This is because high doses are necessary to compete with the superoxide radical (35), which is generated during oxidative stress, interacts with NO and abolishes its biological activity, and is the major cause of endothelial dysfunction (36, 37).

Several mechanisms have been considered to explain the ability of ascorbate to preserve NO. These include ascorbate-induced decreases in low-density lipoprotein oxidation, scavenging of intracellular superoxide, release of NO from circulating or tissue S-nitrosothiols, direct reduction of nitrite to NO, and enhancement of endothelial NO synthase (NOS) activity. The protective and restorative actions of vitamin C on the endothelial function are explained, in part, by its ability, at millimolar concentrations, to maintain or restore endothelial NOS activity (23) and simultaneously prevent oxidative scavenging of NO (33, 38). Another crucial pharmacologic property of vitamin C has been revealed in a rat sepsis model (39, 40). A single high-dose (200 mg/kg) bolus injection of vitamin C prevented the sepsis-induced systemic vascular hyporeactivity and inflammation-induced expression of the inducible form of NOS (41). Induction of inducible NOS in phagocytes, vascular smooth muscle, and endothelial cells is a recognized pathophysiologic factor in the development of sepsis-induced vascular hyporeactivity and multiple organ failure (39, 42) (Fig. 2).

Vitamin C in Trauma and Sepsis

Several studies indicate that patients with sepsis, or following severe trauma, experience a drastic reduction in circulating ascorbate levels and a general depletion of antioxidant capacity. It was reported many years ago and confirmed more recently that trauma and surgical patients have drastically reduced blood ascorbate levels (19, 43, 44) and that supplementation with high doses of vitamin C is required to restore ascorbate levels to normal (19, 24, 43, 44). In critically ill patients, both clinical and pharmacologic studies indicate that supraphysiologic doses of ascorbate, in the gram range, are necessary to achieve therapeutic benefit (44, 45). Long and colleagues (24) showed that 3000 mg/day for 2 days administered parenterally to critically ill patients restored plasma levels to just within the normal range in some but not all patients.

Failure to restore a normal plasma antioxidant potential is strongly associated with an unfavorable outcome following severe sepsis (11, 46). The clinical value of high-dose vitamin C repletion was indicated by the findings of a randomized prospective study of critically ill surgical patients (25). Nathens and colleagues (25) demonstrated that 3000 mg of vitamin C per day (in combination with 2000 IU of α-tocopherol per day) reduced the risk of pulmonary morbidity and multiple organ failure and the duration of mechanical ventilation and intensive care.

In the critically ill patient, drastic depletion of ascorbate is due to the acute oxidative stress that is intrinsic to the systemic inflammatory response and is a causative factor in the microvascular endothelial dysfunction that underlies shock and risk of multiple organ failure (26). There is a reduction in the concentrations of ascorbate in the circulation (18) partly due to the accumulation of ascorbate within inflamed tissue by means of uptake of oxidized ascorbate (dehydroascorbate) via glucose transport-

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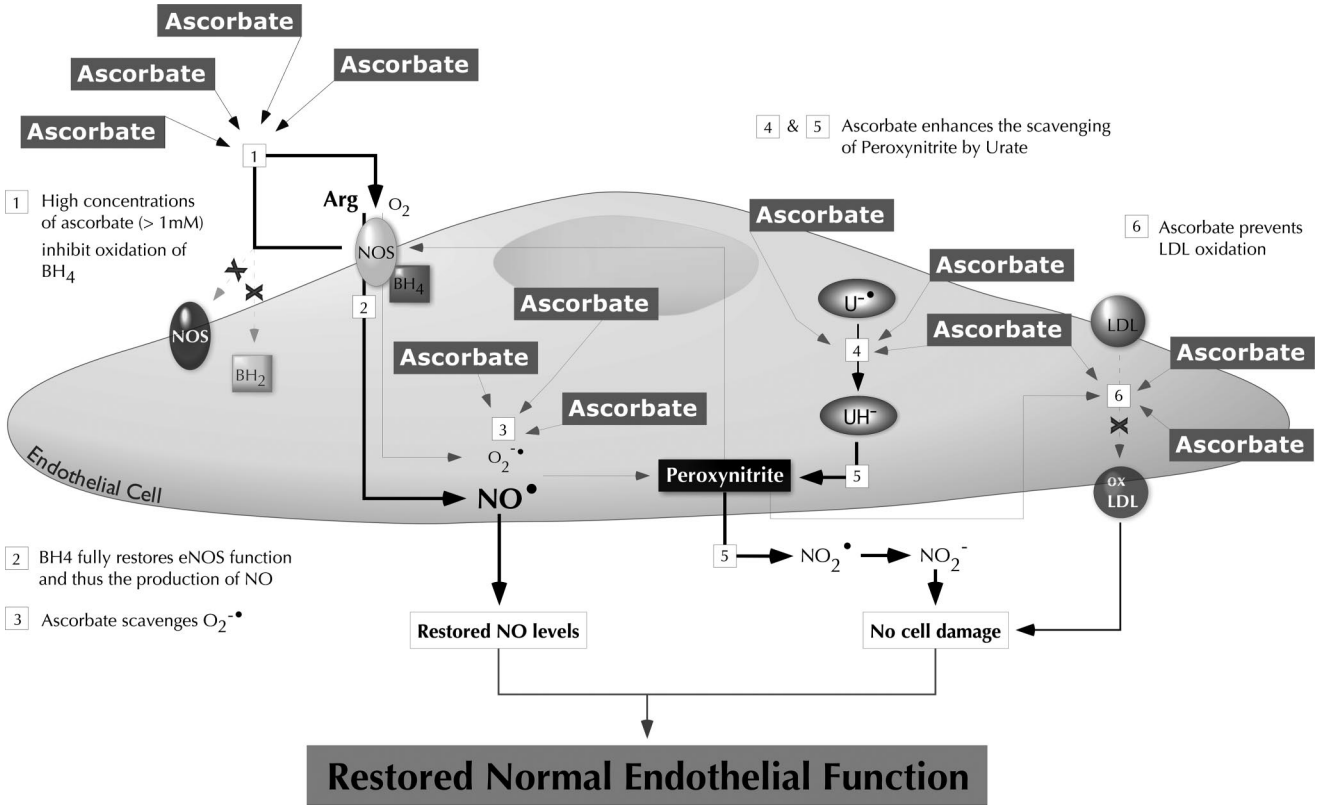

Figure 2. Therapeutic levels of ascorbate/oxidative stress. *BH*, hydrobiopterin; *Arg*, arginine; *NO*, nitric oxide; *NOS*, nitric oxide synthase; *eNOS*, endothelial nitric oxide synthase; *LDL*, low-density lipoprotein.

ers (41) and partly due to metabolism and breakdown of oxidized ascorbate before intracellular reductive recycling. Due to the limited bioavailability of oral vitamin C (15), parenteral administration is required to achieve a therapeutic restoration of normal body levels of ascorbate and combat the oxidative stress.

There is experimental evidence that parenteral high-dose vitamin C prevents sepsis-induced vascular hyporeactivity in humans (47, 48). In the first of these two studies performed in human volunteers (47), prior parenteral administration of vitamin C (24 mg/min closed intra-arterial infusion) prevented endotoxin (systemic lipopolysaccharide)-induced endothelial dysfunction as measured by the decreased response to acetylcholine (locally infused) stimulated forearm blood flow without having an effect on normal blood flow. In the second study (48), vitamin C, applied in the same way and at the same dose as in the first study, prevented the endotoxin-induced reduced vasoconstrictor response, in human volunteers, to both noradrenalin and angiotensin II. Both these vasoconstrictors act independently of the endothelium, but their vasoconstrictor effects are inhibited under inflammatory conditions due to oxidative stress (49). Similar results have been obtained in animal models of sepsis with high-dose (200 mg/kg) parenteral vitamin C (39, 40). These findings indicate that oxidative stress is a causative factor in the sepsis-induced hyporeactivity of the vasculature and reveal that the therapeutic potential of antioxidants in stabilizing the circulation is due not only to a reversal of endothelial dysfunction.

More recent animal experiments (rat sepsis model) have demonstrated reversal of sepsis-induced dysregulation of microvascular function (50). Despite volume resuscitation, this sepsis model exhibited a maldistribution of capillary blood flow within 24 hrs and hypotension within 48 hrs. It was shown that this sepsis-induced microcirculatory dysfunction could be prevented by parenteral vitamin C (74-mg/kg bolus) administered 6–24 hrs after the septic insult. Thus, vitamin C can reverse microcirculatory dysfunction after the onset of sepsis. These findings are significant in revealing the potential of antioxidant therapy and, in particular, parenteral high-dose vitamin C as a serious therapeutic option in the treatment of shock in critically ill patients (51).

Vitamin C in Severe Burns

Severe burn is a form of trauma in which there is clinical and experimental evidence of the benefit of high doses of vitamin C in treating the breakdown of systemic microvascular function. Severe burn usually refers to patients with ≥30% of the body surface area burned and who have, characteristically, an extremely bad prognosis largely due to the development of SIRS and shock and to multiple organ failure that develops during the first 24–48 hrs postburn.

Severe burns evoke a systemic inflammatory response that leads to endothelial dysfunction and fluid and protein leakage from the intravascular space to the interstitial space (52, 53). This burn injury-induced hypovolemic shock is associated with oxidative stress (54–56). Current treatment strategies for severe burns focus on fluid resuscitation, but this fails to combat SIRS (57) and the consequent endothelial dysfunction and may even further increase edema. Following burn injury, due to the ensuing oxidative

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stress, there is an increased requirement for vitamin C as indicated by the reduced vitamin C blood levels seen in such patients (57).

In animal studies, high-dose vitamin C treatment attenuates shock and reduces the risk of organ failure (53, 58). Fujimoto and colleagues (58) indicated that high-dose vitamin C (1 g/hr), administered in third-degree burns affecting 70% of body surface area in guinea pigs, maintained adequate hemodynamic stability even in the presence of a reduced resuscitation fluid volume. For this effect to occur, vitamin C had to be given within 8 hrs following burn injury and for the duration of 24 hrs. The fluid volume could be reduced by up to 75% of the Parkland volume without depression in cardiac output. Fujimoto and colleagues also reported that high-dose vitamin C counteracts the negative interstitial fluid hydrostatic pressure and early edema development in thermally injured rats. They concluded that the mechanisms of the effectiveness of vitamin C in thermal injury had to be further elucidated. In the burn-injured sheep model, Dubick and colleagues (59) obtained similar findings to Fujimoto and colleagues. Dubick and colleagues showed a significant reduction in fluid resuscitation volumes (50% reduction by 48 hrs) following infusion of high doses of vitamin C (250 mg/kg in the initial 500 mL followed by 15 mg/kg/hr). This was associated with a significant increase in antioxidant status.

In a controlled clinical study, Tanaka and colleagues investigated patients with >30% burns and treated with a dose of vitamin C 66 mg/kg/hr for 24 hrs (60). This corresponds to 110 g of vitamin C in a patient weighing 70 kg. The aim of this prospective, randomized study was to assess whether high-dose vitamin C treatment attenuates postburn lipid peroxidation, resuscitation fluid volume requirements, and edema generation in severely burned patients (60). Thirty-seven patients with burns over >30% of their total body surface area, hospitalized within 2 hrs of the burn injury, were randomly divided into ascorbic acid and control groups. Fluid resuscitation was performed using Ringer's lactate solution to maintain stable hemodynamic measurements and adequate urine output (0.5–1.0 mL/kg/hr). In the ascorbic acid group (n = 19; mean burn size, 63% ± 26% total body surface area; mean burn index, 57 ± 26; inhalation injury, 15 of 19), ascorbic acid was infused during the initial 24-hr study period. In the control group (n = 18; mean burn size, 53% ± 17% total body surface area; mean burn index, 47 ± 13; inhalation injury, 12 of 18), no ascorbic acid was infused. Hemodynamic variables, respiratory function, lipid peroxidation, and fluid balance were assessed for 96 hrs after burn injury. Two-way analysis of variance and Tukey's test were used to analyze the data. Heart rate, mean arterial pressure, central venous pressure, arterial pH, base deficit, and urine outputs were equivalent in both groups. The 24-hr total fluid infusion volumes in the control and ascorbic acid groups were 5.5 ± 3.1 and 3.0 ± 1.7 mL/kg per percentage of burn area, respectively ($p < .01$). In the first 24 hrs, the ascorbic acid group gained 9.2% ± 8.2% of pretreatment weight; controls gained 17.8% ± 6.9%. Burned tissue water content was 6.1 ± 1.8 vs. 2.6 ± 1.7 mL/g of dry weight in the control and ascorbic acid groups, respectively ($p < .01$). Fluid retention in the second 24 hrs was also significantly reduced in the ascorbic acid group. In the control group, the Pao_2/Fio_2 ratio at 18, 24, 36, 48, and 72 hrs after injury was less than that of the ascorbic acid group ($p < .01$). The length of mechanical ventilation in the control and ascorbic acid groups was 21.3 ± 15.6 and 12.1 ± 8.8 days, respectively ($p < .05$). Serum malondialdehyde levels were lower in the ascorbic acid group at 18, 24, and 36 hrs after injury ($p < .05$). Administration of high-dose vitamin C during the first 24 hrs after thermal injury also significantly reduced resuscitation fluid volume requirements, body weight gain, and wound edema. A reduction in the severity of respiratory dysfunction was also apparent in these patients.

CONCLUSIONS

Antioxidants should be considered a rational option in treating the critically ill patient, although more definitive evidence from multicenter trials is still required. As well as potentially suppressing SIRS and its hemodynamic consequences, antioxidant therapy may be a vital part of what is considered immune nutrition. The suppression of the cellular immune function of the critically ill patient may be not only due to the effect of the overactivated anti-inflammatory factors (8) but directly due to oxidative stress. Immune cells require high intracellular antioxidant levels for optimal function, and oxidative stress can induce immune cell death (61). However, we have reviewed the growing evidence that the microcirculation is a major target of antioxidant treatment. The reviewed experimental data provide the rationale for using high-dose parenteral vitamin C to treat endothelial dysfunction and shock and to prevent multiple organ failure. The experimental data indicate protection or restoration of the bioavailability of NO as a specific molecular target in the antioxidant treatment of shock. This provides a rationale for further clinical investigations of high-dose parenteral vitamin C in the treatment of critically ill patients. It may be that other antioxidants, at a sufficiently high concentration, exert similar effects. However, the current experimental and clinical evidence is most compelling for vitamin C. Vitamin C in the form of ascorbate, at millimolar pharmacologic doses, seems to possess the ideal pharmacologic profile for treating critically ill patients for shock and preventing multiple organ failure. Vitamin C inhibits endothelial dysfunction, primarily by supporting endothelial NOS activity; protects NO from oxidative scavenging; inhibits inflammation-induced inducible NOS activity in endothelial cells and prevents inflammation-induced vasoreactivity; and thereby facilitates restoration of vascular stability by vasoconstrictors. We and others have reviewed elsewhere (62, 63) the evidence that, in the case of vitamin C, high doses appear not to exert paradoxic pro-oxidative effects due to a much postulated disturbance of the body's complex mix of protective antioxidants. It is also postulated that in severely burned patients, free iron is generated from hemolyzed erythrocytes and from traumatized tissue and that high doses of vitamin C will react with this free iron to generate ROS. However, Tanaka and colleagues (60) showed decreased plasma lipid peroxide levels after infusion of 66 mg/kg/min in burn patients, while Dubick and colleagues (59) demonstrated that high doses of parenteral vitamin C preserved tissue antioxidant status in the burn-injury sheep model. So there is no evidence that high-dose parenteral vitamin C exerts any prooxidant effects in the critically ill patient.

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