

Review

Hydrocortisone, Ascorbic Acid and Thiamine (HAT Therapy) for the Treatment of Sepsis. Focus on Ascorbic Acid

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Received: 12 October 2018; Accepted: 8 November 2018; Published: 14 November 2018



Abstract: Sepsis is a devastating disease that carries an enormous toll in terms of human suffering and lives lost. Over 100 novel pharmacologic agents that targeted specific molecules or pathways have failed to improve the outcome of sepsis. Preliminary data suggests that the combination of Hydrocortisone, Ascorbic Acid and Thiamine (HAT therapy) may reduce organ failure and mortality in patients with sepsis and septic shock. HAT therapy is based on the concept that a combination of readily available, safe and cheap agents, which target multiple components of the host's response to an infectious agent, will synergistically restore the dysregulated immune response and thereby prevent organ failure and death. This paper reviews the rationale for HAT therapy with a focus on vitamin C.

Keywords: sepsis; septic shock; hydrocortisone; vitamin C; ascorbic acid; thiamine

1. Introduction

The global burden of sepsis is substantial with an estimated 32 million cases and 5.3 million deaths per year per year with most of these cases occurring in low-income countries [1]. Data from the U.S. and Australia demonstrates that over the last two decades the annual incidence of sepsis has increased by approximately 13% with a decrease in in-hospital mortality from about 35% to 20% [2–4]. In 2013, over 1.3 million patients were hospitalized in the U.S. with a diagnosis of sepsis, of which over 300,000 died [5]. In addition to short-term mortality, septic patients suffer from numerous long-term complications with a reduced quality of life and an increased risk of death up to five years following the acute event [6–10]. Approximately 50% of sepsis survivors develop post-sepsis syndrome characterized by the development of new psychiatric and cognitive deficits [11]. Post sepsis-syndrome is similar in many respects to post-traumatic stress disorder (PTSD); patients suffer memory impairment, abnormalities of higher executive function, nightmares, anxiety disorders and depression [12]. Apart from the enormous financial costs of sepsis, the human toll of this disease is staggering and new interventions that limit the ravages of this disease are urgently required.

In patients with sepsis organ failure and death, it is usually a result of the host's response to the infecting pathogen rather than from the infecting pathogen itself. This was first recognized by Sir William Osler, who commented that “*except on few occasions, the patient appears to die from the body's response to infection rather than from the infection*” [13]. Sepsis is fundamentally an inflammatory disease mediated by the activation of the innate immune system by both pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs). Calvano et al. demonstrated that exposure of blood leukocytes to bacterial endotoxin (LPS) altered the expression of 3714 genes [14]. These include genes for pro- and anti-inflammatory cytokines, chemokines, adhesion molecules,

transcription factors, enzymes, clotting factors, stress proteins and anti-apoptotic molecules [15]. These inflammatory mediators have widespread pathophysiological consequences, including vasoplegic shock, myocardial dysfunction, altered microvascular flow and diffuse endothelial injury [16,17]. However, fundamentally, sepsis is characterized by the excessive production of reactive oxygen species (ROS) by the induction of enzymes such as nicotinamide adenine dinucleotide phosphate-oxidase (NOX) and the uncoupling of mitochondrial oxidative phosphorylation (see Figure 1) [18]. In addition, ROS are produced by xanthine oxidase, lipoxygenase and cyclooxygenase. Important ROS in sepsis pathogenesis includes hydrogen peroxide (H_2O_2), superoxide (O_2^-), hydroxyl radicals (HO^\cdot), peroxynitrite ($ONOO^-$) and hypochlorous acid ($HOCl$).

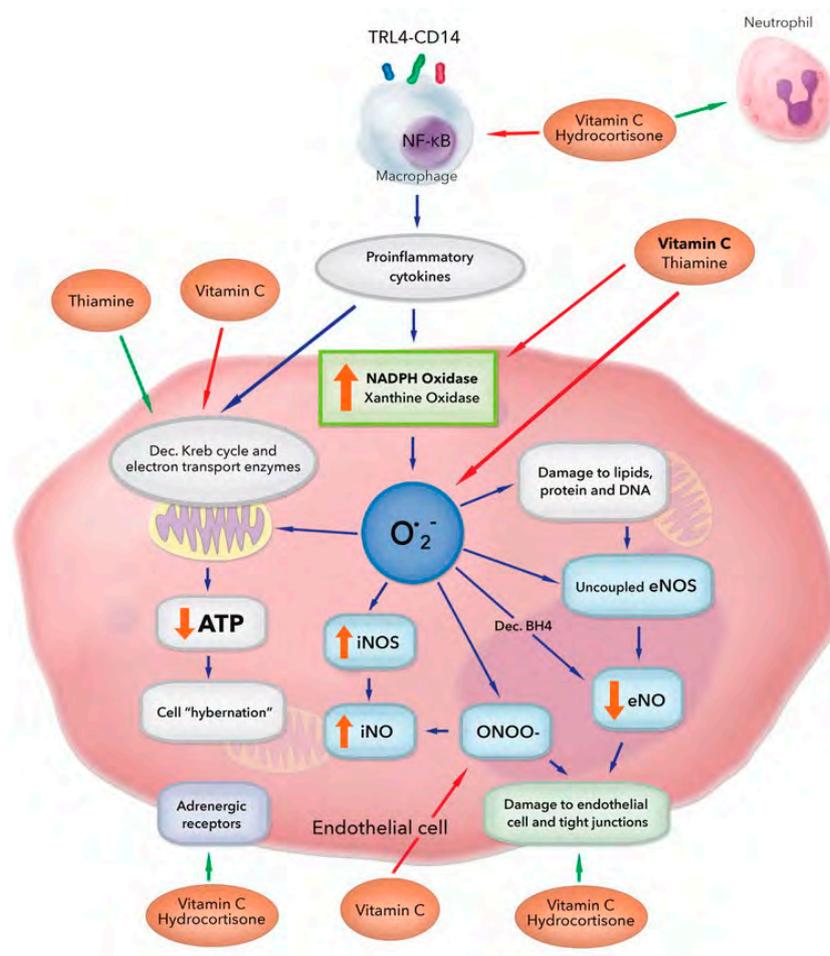


Figure 1. Multiple and overlapping effects of hydrocortisone, vitamin C, and thiamine in the setting of bacterial sepsis. Vitamin C and thiamine scavenge free radicals from superoxide (O_2^-) and inhibit activation of xanthine oxidase and NADPH oxidase. Vitamin C protects the mitochondria from oxidative stress caused by increased leakage of electrons from the dysfunctional electron transport chain and recovers tetrahydrobiopterin (BH4) from dihydrobiopterin (BH2), restoring endothelial nitric oxide synthase (eNOS) activity and increasing eNO bioavailability. Vitamin C inhibits inducible NOS (iNOS) activation, preventing profuse iNO production and peroxynitrite ($ONOO^-$) generation. Vitamin C scavenges $ONOO^-$, preventing loosening of the tight junctions of the endothelium. Vitamin C and hydrocortisone decrease the activation of nuclear factor κB (NF- κB), thereby decreasing the release of proinflammatory mediators. They restore endothelial tight junctions and increase adrenergic receptor function. Thiamine increases the activity of pyruvate dehydrogenase and alpha ketoglutarate dehydrogenase. These actions act in concert to restore cellular adenosine tri-phosphate (ATP) levels. \uparrow —increased levels/activity; \downarrow —decreased levels/activity. Figure adapted from Spoelstra-de Man et al. [19].

The excess production of ROS underlie many of the pathological processes characteristic of sepsis [20]. ROS has been shown to modulate the lipopolysaccharide-Toll like receptor 4 (LPS-TLR4) signaling pathway. ROS activate nuclear factor kappa-B (NF- κ B) by activation of inhibitory kappa-B kinase (I κ B kinase). NF- κ B increase the transcription of multiple pro-inflammatory mediators (see Figure 1). When the hosts antioxidant defenses are overwhelmed, ROS can induce injury to lipids, proteins and nucleic acids, thereby resulting in widespread endothelial dysfunction, mitochondrial dysfunction, cellular injury, and multiple organ dysfunction [20]. Isoprostanes are products of lipid peroxidation that are formed non-enzymatically and can be measured in blood, urine, or tissues. Because of their stability and high specificity, the F2-isoprostanes are currently considered to be the most reliable biomarkers of in vivo oxidative stress and lipid peroxidation. In a cohort of patients with sepsis, Ware et al. demonstrated that F2-isoprostane levels were higher in patients who developed organ failure and in those who died [21]. Similarly, Kumar et al. reported augmented levels of oxidants in patients with sepsis, as demonstrated by DMPO nitron adduct formation and plasma myeloperoxidase (MPO) level activity [22]. In this study, the SOFA and APACHE II scores correlated linearly with the MPO levels and inversely with the levels of antioxidants. This study supports the concept that the imbalance between oxidant and antioxidants plays a key role in the pathophysiology of organ failure in sepsis.

The unbalanced production of mitochondrial ROS impairs mitochondrial structure, enzymatic function, and biogenesis. Mitochondria are both the target and source of ROS in sepsis. In an in vitro model of sepsis-induced kidney injury, Quoilin et al. demonstrated that LPS induces NADPH oxidase and inducible nitric oxide synthetase (iNOS) expression. Following this, uncoupling of the mitochondrial respiratory chain due to the inhibition of complex IV and reduction in ATP levels were observed [23]. Mitochondrial dysfunction was associated with cytochrome c release and the loss of mitochondrial membrane potential. Alterations in fatty acid metabolism with decreased beta-oxidation and abnormalities of the citric acid cycle appear to be a characteristic feature of the mitochondrial dysfunction in sepsis [24,25]. These changes lead to decreased ATP production. In the heart, myocyte oxidative injury is accompanied by increased proteolysis, mitochondrial damage, dysregulated nitric oxide metabolism, β -adrenoceptor down-regulation and calcium mishandling [26–28]. The unbalanced production of mitochondrial ROS impairs mitochondrial structure, enzymatic function and biogenesis, and plays a role in the metabolic failure of sepsis. These data suggest that excessive production of ROS plays a pivotal role in the pathophysiology of sepsis and that interventions that neutralize oxidants would have a protective role in sepsis. Furthermore, proinflammatory mediators increase the expression of pyruvate dehydrogenase kinase isoenzymes (PDK4), which inactivate the pyruvate dehydrogenase complex (PDC) [29,30]. Inactivation of the PDC prevents pyruvate entering the Krebs cycle potentiating the metabolic failure caused by mitochondrial dysfunction. This phenomenon is compounded by thiamine deficiency and cytokine-mediated down regulation of expression of the PDC [29].

Over the last three decades, over 100 phase II and phase III clinical trials have been performed testing various novel pharmacologic agents and therapeutic interventions in an attempt to improve the outcome of patients with severe sepsis and septic shock; all of these efforts ultimately failed to produce a novel pharmacologic agent that reduced organ failure and improved the survival of patients with sepsis [31]. All these studies used a single agent that targeted a specific molecule or pathway; due to the thousands of mediators involved and the redundancy of the multiple pathways, such an approach was doomed to fail. Furthermore, the central role of ROS was ignored. Dr Aird commented that *“the best hope for therapeutic advances [in sepsis] will depend on broad-base targeting, in which multiple components are targeted at the same time”* [32]. We believe that the combination of Hydrocortisone, Ascorbic Acid and Thiamine (HAT therapy) achieves these goals. The premise behind HAT therapy is the use of a combination of readily available, safe and cheap agents that target multiple components of the host’s response to an infectious agent such that they synergistically restore the dysregulated host immune response, neutralize damaging oxidants, and restore mitochondrial function. We believe

this unique and novel approach has the potential to reduce the global burden of sepsis, reduce the post-sepsis syndrome without side-effects, and be highly cost-effective. A cost analysis indicates that HAT therapy has the potential to save billions of dollars and millions of life-years in the United States [33]. This paper reviews the rationale for HAT therapy with a focus on vitamin C.

2. Vitamin C

It has been known for over 20 years that in acutely-ill patients [34–36] as well as in experimental models of sepsis that an acute deficiency of vitamin C develops, characterized by low serum and intracellular levels of the vitamin [37–41]. Critically ill septic patients typically have very low or undetectable serum levels of vitamin C, resulting in an acute scorbutic condition [35,42–44]. Recently Carr and co-authors demonstrated that 100% of septic patients had low vitamin C levels, 88% had hypovitaminosis C ($<23 \mu\text{mol/L}$), while 38% had a severe deficiency ($<11 \mu\text{mol/L}$) [45]. Low vitamin C levels in critically ill patients are associated with increased vasopressor requirements, kidney injury, multiple organ dysfunction (higher SOFA scores) and increased mortality [35,44]. The underlying cause of vitamin C deficiency is likely due to increased oxidation (metabolic consumption), decreased absorption, and increased urinary losses of the vitamin. In a murine caecal-ligation and perforation model (CLP), Armour et al. reported that the plasma ascorbate level fell rapidly by 50% and this was associated with a 1000% increase in the urine ascorbate concentration [38]. Sepsis induced glomerular hyperfiltration and/or tubular dysfunction results in decreased tubular reabsorption of filtered vitamin C with increased urinary losses [46].

Vitamin C is a potent antioxidant, which directly scavenges oxygen free radicals, restores other cellular antioxidants including tetrahydrobiopterin and α -tocopherol and is an essential co-factor for iron and copper containing enzymes [18,47]. Vitamin C is a key cellular antioxidant, detoxifying exogenous oxidants radical species that have entered cells or which have arisen within cells due to excess superoxide generation by mitochondrial metabolism, by NADPH oxidase, xanthine oxidase or by uncoupled nitric oxide synthase (NOS) [18,48]. The low electron reduction potential of both vitamin C and its one-electron oxidation product, the ascorbyl radical, enable them to reduce most clinically important radicals and oxidants.

Dehydroascorbic acid, the two-electron oxidation product of ascorbic acid, is transported via the GLUT1 transporter into mitochondria, where it converted to ascorbic acid and acts as a potent antioxidant limiting mitochondrial oxidant injury [49,50]. Considering that the mitochondrial respiratory chain is a main source of ROS in live cells and mitochondrial dysfunction plays a prominent role in sepsis pathogenesis, antioxidants targeting the intra-mitochondrial environment could be pivotal role in the treatment of sepsis. Furthermore, ascorbic acid is required for the synthesis of carnitine, which is required for the transport of fatty acids into the mitochondrial matrix and for beta-oxidation [24,49]. Carnitine deficiency may occur in the context of sepsis and preliminary data suggests that an infusion of L-carnitine may be beneficial in patients with septic shock [51]. In an experimental model, Dhar-Mascareno and colleagues demonstrated that oxidant induced mitochondrial damage and apoptosis in human endothelial cells were inhibited by vitamin C [52]. In a CLP sepsis model, Kim et al. administered 100 mg/kg ascorbic acid immediately after sepsis induction [53]. In this study, vitamin C attenuated the elevation in serum aminotransferase and hepatic lipid peroxide levels. Studies using *N*-acetylcysteine (a synthetic anti-oxidant) have proven to be ineffective and potentially harmful in patients with sepsis, possibly due to the limited ability of this drug to enter into the mitochondria and its inability to regenerate BH4 [18,54,55].

Vitamin C suppresses activation of NF- κ B by inhibiting tumor necrosis factor- α (TNF α) induced phosphorylation of inhibitory kappa-B kinase (I κ B kinase) [56]. Ascorbic acid decreases high mobility group box 1 (HMGB1) secretion [57]; HMGB1 is an important late pro-inflammatory cytokine. Vitamin C may decrease the synthesis and inactivate histamine [58]; histamine has been shown to play an important role in sepsis [59]. Vitamin C is an essential co-factor for the synthesis of norepinephrine, epinephrine and vasopressin; in addition vitamin C increases adrenergic transmission [60]. Vitamin C

may decrease the immunosuppression associated with sepsis. It has been known for over 60 years that vitamin C has immune-enhancing properties. In 1949, Dr Fred Klenner from Reidsville, North Carolina, reported on the use of intravenous vitamin C in the treatment of polio and other viral illnesses [61]. It was initially assumed that vitamin C was directly viricidal (in vivo) and this mistaken belief underlies the recommendations of Linus Pauling who promoted the use of large doses of oral vitamin C (up to 18 g/day) for the prevention and treatment of the common cold [62]. A number of RCTs have reported that vitamin C supplementation had no effect on the incidence of the common cold [63]. However, vitamin C has been shown to decrease the incidence of the common cold if the person is under enhanced stress, e.g., cold temperatures and/or physical stress [64]. While high dose vitamin C has in-vitro viricidal properties [65,66], there is no data or physiologic rationale to suggest that this occurs in vivo. Rather, the “anti-viral” effect of vitamin C are likely due to that fact that vitamin C has specific immune-enhancing effects. Vitamin C is concentrated in leucocytes, lymphocytes and macrophages, reaching high concentrations in these cells [67]. Vitamin C improves chemotaxis, enhances neutrophil phagocytic capacity and oxidative killing, stimulates interferon production, and supports lymphocyte proliferation [68–70]. The major presumed beneficial effects of vitamin C in patients with sepsis are outlined in Table 1.

Table 1. Summary of key roles of Vitamin C in sepsis.

Key Role	Mechanism
Antioxidant	Scavenges extracellular, intracellular and mitochondrial ROS; limits oxidation of mitochondrial proteins, enzymes, lipoproteins, cell membrane, etc.
Anti-inflammatory	Inhibits activation of NFκB, decreases HMGB1, inhibits histamine, prevents NETosis, inactivates HIF-1α
Microcirculation	Increases eNOS, decreases iNOS, preserves tight junctions
Immune function	Supports lymphocyte proliferation, increases neutrophil bacteriocidal action, improves chemotaxis, stimulates interferon production, decreases T regulatory cells (Tregs)
Anti-thrombotic	Decreases platelet activation and tissue factor expression, increases thrombomodulin
Synthesis of catecholamines	Acts as a cofactor in synthesis of epinephrine, dopamine and vasopressin. Increases adrenergic sensitivity
Wound Healing	Hydroxylation of procollagen, increased expression of collagen mRNA

ROS = reactive oxygen species; NFκB = nuclear factor κB; HIF-1α = hypoxia-inducible transcription factor-1α; HMGB1 = high mobility group box 1; eNOS endothelial nitric oxide synthetase; iNOS = inducible nitric oxide synthetase; HO-1 = heme oxygenase-1; HIF-1α = hypoxia-inducible transcription factor-1α2. Vitamin C: Dose response and pro-oxidant effect.

While vitamin C is the most potent and important anti-oxidant in mammals, in the presence of transition metals (iron and copper), vitamin C may paradoxically be associated with a pro-oxidant effect [71]. In the presence of free iron, vitamin C may reduce free ferric iron to the ferrous form. The ferrous form then undergoes a Fenton-type reaction with hydrogen peroxide yielding hydroxyl or hydroxyl-like reactions. Vitamin C may generate ROS in in-vitro, cell culture or tissue incubation experiments, where free metal ions might exist [71–73]. Normally, iron is tightly bound to protein and does not exist in the free form. However, in conditions such as hypoxia (ischemia/reperfusion) and sepsis, free iron may be released from ferritin. Furthermore, sepsis is associated with hemolysis and the release of free heme. Free heme can be highly cytotoxic in the presence of proinflammatory mediators [74,75]. The divalent iron atom contained within its protoporphyrin IX ring can promote the production of free radicals. In the presence of free iron or free heme and depending on the dose of vitamin C and the timing of the administration of the dose in relationship to inciting event, vitamin C may either act as an anti-oxidant or pro-oxidant. This concept was elegantly demonstrated in a hepatic ischemia/reperfusion model where Seo and Lee demonstrated that an infusion of 30 mg/kg and

100 mg/kg of vitamin C decreased markers of oxidative injury, whereas these markers were increased with a dose of 300 mg/kg and 1000 mg/kg [76]. Similarly, in a hepatic ischemia/reperfusion model, Park and colleagues demonstrated that oxidative injury was attenuated at ascorbic acid concentrations of 0.25 mM and 0.5 mM, however they were augmented at a concentration of 2 mM [77]. This concept is further supported by a number of studies using a cardiac arrest ischemia/reperfusion model. In two murine studies, vitamin C in a dose of 50 mg/kg and 100 mg/kg IV decreased myocardial damage, improved neurological outcome and the survival rate [68,69]. However, using a similar model, Motl and colleagues reported that a 250 mg/kg dose was harmful [78]. In a murine CLP model, Tysl et al. demonstrated that the delayed (24 h) administration of vitamin C (in a dose of 76 mg/kg) restored blood pressure and microvascular perfusion [41]. Using human umbilical vein endothelial cells Kuck and colleagues demonstrated that cell free hemoglobin increased endothelial permeability in part through depletion of intracellular ascorbate [79]. In this study the addition of ascorbate up to a concentration of 60 μ mol/L (physiologic concentrations) attenuated the increase in permeability mediated by cell free hemoglobin [79]. This study reaffirms that low dose vitamin C may reduce the toxicity of cell free hemoglobin.

In the absence of free iron or heme, vitamin C acts as an oxidant only in extremely high pharmacologic doses (>100 g) when used as adjunctive treatment in patients with cancer [80–83]. Repeated intravenous injection of 750–7500 mg/day of vitamin C for six days in healthy volunteers did not induce a pro-oxidant change in plasma markers [84]. Furthermore, intravenous infusion of very high-dose ascorbate (1584 mg/kg over 24 h) lowered serum malondialdehyde concentration (a marker of oxidative stress) in severely burned patients [85]. When used prophylactically (before the inciting event) prior to the oxidant injury with release of free iron, it is likely that a lower dose of vitamin C may act as a powerful antioxidant. Basili et al. demonstrated that a single 1 g infusion of vitamin C prior to the performance of percutaneous coronary reperfusion significantly improved microcirculatory perfusion with a marked reduction in the markers of oxidant injury [86]. Similarly, Wang and colleagues demonstrated that 3 g vitamin C IV prior to elective percutaneous coronary reperfusion was associated with less myocardial injury [87]. A metaanalysis, which included 8 randomized controlled trials, demonstrated that vitamin C up to a dose of 2 g IV given pre-operatively significantly reduced the risk of atrial fibrillation in patients undergoing cardiac surgery [88]. Oxidant injury is postulated to play a role in contrast-induced renal dysfunction. A systematic review by Xu and colleagues demonstrated that prophylactic vitamin C (oral and IV up to a total dose of 7 g) significantly reduced the risk of renal impairment [89].

These data suggest that in the setting of sepsis and ischemia/reperfusion, a narrow dose response curve exists. In preliminary observational data in patients with severe sepsis and septic shock and using procalcitonin as a biomarker, we have similarly observed a narrow dose response curve; the optimal daily dose appears to be approximately 6 g/day with an attenuated effect at a dose of 2 g and 10 g, respectively (personal observations). In treating over 800 patients with a 6 g/day dose, we are unaware of any patient who had a pro-oxidant effect (as reflected by the procalcitonin trajectory), even when the treatment was delayed. It is possible that delayed treatment with a higher dose may have a pro-oxidant deleterious effect. It should be recognized that there is a delicate balance between protective oxidant signaling and the detrimental effects of ROS. Additional studies are required to further elucidate the dose response of vitamin C in various clinical situations. Furthermore, we endorse the recommendation of Spoelstra-de Man and colleagues who suggest administering “*high-dose vitamin C for a short course of four days only, e.g., during the overwhelming oxidative stress when organ damage occurs. After four days, plasma concentrations will be supranormal and vitamin C can be continued in a low (nutritional) dose to allow generation of low concentrations of ROS, which are essential for physiological signaling and repair.*” [19].

3. Hydrocortisone

Glucocorticoids have diverse anti-inflammatory properties. These are briefly reviewed here; the reader is referred to recent publications for a more comprehensive review of this topic [90–93]. Classically, glucocorticoid binding to the glucocorticoid receptor (GR) activates or represses gene transcription, with glucocorticoids regulating up to 20% of the genome [94]. Glucocorticoids affect nearly every cell of the immune system. Glucocorticoids suppress inflammation by multiple mechanisms that impact both the innate and adaptive immune responses. The primary anti-inflammatory action of glucocorticoids is to repress a large number of pro-inflammatory genes, which encode cytokines, chemokines, inflammatory enzymes, cell adhesion molecules coagulation factors and receptors. GR-mediated repression of the transcriptional activity of NF- κ B and AP-1 play a major role in mediating the anti-inflammatory actions of glucocorticoids. In addition to attenuating the pro-inflammatory response, low-dose glucocorticoids have immune-stimulating effects, which may limit the anti-inflammatory immunosuppressive state [95]. The immune-enhancing effects of glucocorticoids and the balance between the immune suppressing and enhancing effects of the drug are critically dependent on the dose and duration of treatment as well as the state of immune activation of the host.

Over the last 40 years, 22 randomized controlled trials have been conducted investigating the benefits of glucocorticoids in patients with septic shock [96]. Many of these studies are limited by their small sample size, high degree of bias, and the fact that they were conducted over a 40-year period during which the treatment for sepsis has improved and the mortality from sepsis and septic shock has decreased significantly [2,4]. The earlier studies used a short course of high-dose corticosteroid (30 mg/kg methylprednisone for up to 4 doses); this approach increased mortality and complications and was abandoned [97]. This was followed by numerous studies using a prolonged course (5–7 days) of physiologic “stress-doses” of glucocorticoids (typically 200–300 mg hydrocortisone/day). The results of these studies were mixed with some demonstrating a survival benefit, while other did not [98,99]. In 2018, two large randomized controlled trials (RCTs) were published evaluating the role of hydrocortisone in patients with septic shock [100,101]. The Activated Protein C and Corticosteroids for Human Septic Shock (APROCCHSS) study demonstrated a reduction in 90-day mortality whereas the Adjunctive Corticosteroid Treatment in Critically Ill Patients with Septic Shock (ADRENAL) study demonstrated no mortality benefit. Both studies, however, demonstrated a reduction in vasopressor dependency, duration of mechanical ventilation and ICU stay with no increased risk of complications. These studies indicate that while glucocorticoids (alone) have a biological effect in patients with septic shock, their effect on patient centered outcomes is limited. However, as indicated below, we believe that glucocorticoids act synergistically with both vitamin C and thiamine to reduce the complications and mortality associated with sepsis.

4. Thiamine

Thiamine is the precursor of thiamine pyrophosphate (TPP), the essential coenzyme of several decarboxylases required for glucose metabolism, the Krebs cycle, the generation of ATP, the pentose phosphate pathway, and the production of NADPH. TPP is a critical co-enzyme for the pyruvate dehydrogenase complex, the rate-limiting step in the citric acid cycle [102]. Thiamine plays an important role in many enzymatic processes involved in brain function, maintenance, and interneuronal communication [103]. Thiamine is involved in nerve tissue repair, myelin synthesis and nerve signal modulation. It plays a role in the uptake of serotonin, which in turn affects the activity of the cerebellum, the hypothalamus, and hippocampus [103]. In addition, thiamine has anti-inflammatory effects, suppressing the oxidative stress-induced activation of NF- κ B [103].

Thiamine deficiency is common among septic patients, with a range in prevalence between 20% and 70%, depending on measurement techniques and inclusion criteria [104–106]. A deficiency in thiamine leads to decreased activity of thiamine-dependent enzymes, which triggers a sequence of metabolic events leading to energy compromise and decreased ATP production. Thiamine deficiency

is associated with excitotoxic-mediated neuronal cell death [107]. Furthermore, thiamine deficiency is associated with an increased production of ROS, as well as increased expression of heme oxygenase (HO-1) and eNOS [107–109]. Thiamine can reverse oxidative stress that is not related to thiamine deficiency, suggesting that thiamine may act as a site-directed antioxidant [108]. It is therefore likely that thiamine deficiency compounds the oxidative mitochondrial injury and bioenergetic failure caused by vitamin C depletion.

In a pilot randomized controlled trial, Donnino et al. randomized 88 patients with septic shock to receive 200 mg thiamine twice daily for seven days [106]. In the predefined subgroup of patients with thiamine deficiency, those in the thiamine treatment group had statistically significantly lower lactate levels at 24 h and a lower mortality at 30 days. Furthermore, in a secondary analysis of this study, the need for renal replacement therapy and serum creatinine were greater in the placebo group [110]. Similarly, in a propensity matched observational study in patients with septic shock, Woolum et al. demonstrated that thiamine supplementation increased lactate clearance and decreased 28-day mortality [111].

5. Hydrocortisone, Ascorbic Acid, and Thiamine (HAT) in Combination

The overlapping anti-inflammatory properties of glucocorticoids and vitamin C reduce the production of pro-inflammatory mediators and ROS, which are associated with endothelial injury, mitochondrial damage, and organ failure characteristic of sepsis (see Figure 1). Furthermore, both agents have immuno-enhancing effects, which limit the immunosuppression that occurs in patients with prolonged sepsis. These agents may synergistically restore the dysregulated immune system which characterizes sepsis (see Figure 2) [112]. Thiamine may act synergistically with glucocorticoids and vitamin C to limit mitochondrial oxidative injury and restore mitochondrial function and energy production. The anti-inflammatory properties of these agents likely restore the activity of the PDC, thereby improving ATP production. However, the interaction between thiamine and ascorbic acid is complex, and likely dependent on the clinical context and ascorbic acid dosing.

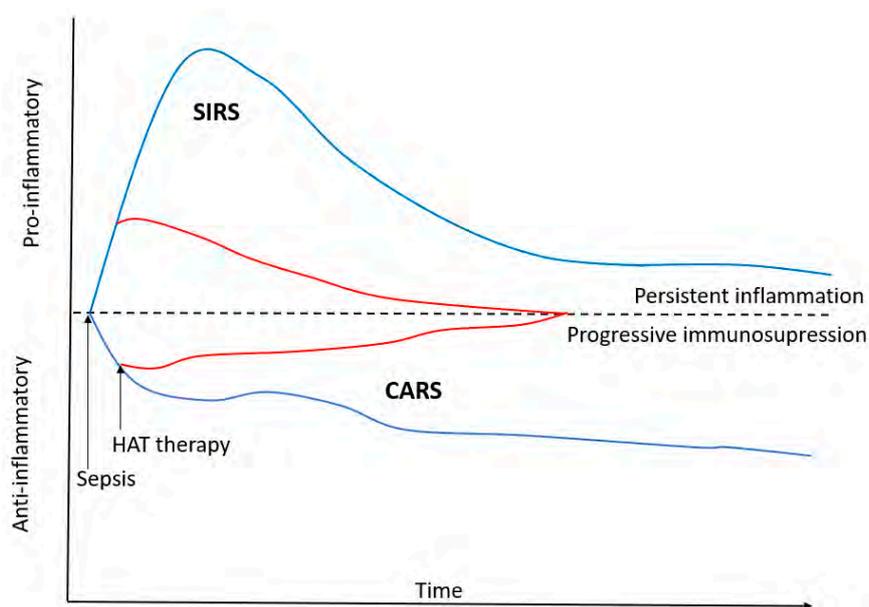


Figure 2. Treatment with Hydrocortisone, vitamin C and Thiamine attenuates both the pro- and anti-inflammatory response in patients with sepsis. HAT—hydrocortisone, ascorbic acid and thiamine; SIRS—Systemic Inflammatory Response Syndrome; CARS—Compensatory Anti-inflammatory Response Syndrome.

The synergy between glucocorticoids and vitamin C has been established in experimental studies. Barabutis et al. have demonstrated that hydrocortisone together with vitamin C protects the vascular endothelium from damage by endotoxin, while neither agent alone had this effect [113]. Azari et al. compared the protective effects of Vitamin C (in a dose of 50 mg/kg), vitamin E and hydrocortisone alone and in combination, in a murine renal and intestinal ischemia-reperfusion model [114,115]. In these studies, both vitamin C and hydrocortisone reduced the ischemia-reperfusion injury with the combination having synergistic protective effects. In a small, retrospective, before-after-study, we demonstrated that the combination of hydrocortisone, ascorbic acid (6 g/day) and thiamine (HAT Rx) improved organ function (as reflected by the SOFA score) with a significant reduction in mortality [43]. In a similar before-after, propensity adjusted observational study, Kim et al. demonstrated a significant reduction of mortality in patients with severe pneumonia using the same treatment strategy (HAT Rx) [116]. According to the U.S. National Library of Medicine's ClinicalTrials.gov website (<https://clinicaltrials.gov/>), in excess of 12 randomized controlled trials are currently underway testing vitamin C alone and in combination with hydrocortisone and thiamine in patients with severe sepsis and or septic shock. The results of these studies should provide definite information on the role of this treatment strategy in the management of patients with severe sepsis and septic shock.

6. Conclusions

Glucocorticoids, vitamin C and thiamine have important biological effects in patients with sepsis and septic shock. Due to the overlapping and synergistic effects of these remarkably safe and inexpensive drugs, the combination of these agents (HAT therapy) likely restores the dysregulated immune system and bioenergetic failure that characterizes sepsis. We, therefore, propose that HAT therapy will improve both the short-term (mortality) and long-term (post-sepsis syndrome) outcome of patients with sepsis and septic shock. Multiple randomized controlled trials are currently underway to test this hypothesis.

Funding: This research received no external funding.

Conflicts of Interest: The author declares no conflict of interest.

References

1. Fleischmann, C.; Scherag, A.; Adhikari, N.K.; Hartog, C.S.; Tsaganos, T.; Angus, D.C.; Reinhart, K. Assessment of global incidence and mortality of hospital-treated sepsis. Current estimates and limitations. *Am. J. Respir. Crit. Care Med.* **2016**, *193*, 259–272. [[CrossRef](#)] [[PubMed](#)]
2. Gaieski, D.F.; Edwards, J.M.; Kallan, M.J.; Carr, B.G. Benchmarking the incidence and mortality of severe sepsis in the United States. *Crit. Care Med.* **2013**, *41*, 1167–1174. [[CrossRef](#)] [[PubMed](#)]
3. Stevenson, E.K.; Rubenstein, A.R.; Radin, G.T.; Wiener, R.S.; Walkey, A.J. Two decades of mortality trends among patients with severe sepsis: A comparative meta-analysis. *Crit. Care Med.* **2014**, *42*, 625–631. [[CrossRef](#)] [[PubMed](#)]
4. Kaukonen, K.M.; Bailey, M.; Suzuki, S.; Pilcher, D.; Bellomo, R. Mortality related to severe sepsis and septic shock among critically ill patients in Australia and New Zealand, 2000–2012. *JAMA* **2014**, *311*, 1308–1316. [[CrossRef](#)] [[PubMed](#)]
5. Torio, C.M.; Moore, B.J. *National Inpatient Hospital Costs: The Most Expensive Conditions by Payer, 2013: Statistical Brief #240*; Healthcare Costs and Utilization Project (HCUP) Statistical Briefs; Agency for Healthcare Research and Quality: Rockville, MD, USA, 2016.
6. Karlsson, S.; Ruokonen, E.; Varpula, T.; Ala-Kokko, T.I.; Pettila, V. Long-term outcome and quality-adjusted life years after severe sepsis. *Crit. Care Med.* **2009**, *37*, 1268–1274. [[CrossRef](#)] [[PubMed](#)]
7. Yende, S.; Austin, S.; Rhodes, A.; Finfer, S.; Opal, S.; Thompson, T.; Bozza, F.; Angus, D.C. Long-term quality of life among survivors of severe sepsis: Analyses of two international trials. *Crit. Care Med.* **2016**, *44*, 1461–1467. [[CrossRef](#)] [[PubMed](#)]

8. Ou, S.M.; Chu, H.; Chao, P.W.; Lee, Y.J.; Kuo, S.C.; Chen, T.J.; Tseng, C.M. Long-term mortality and major adverse cardiovascular events in sepsis survivors. A Nationwide population-based study. *Am. J. Respir. Crit. Care Med.* **2016**, *194*, 209–217. [[CrossRef](#)] [[PubMed](#)]
9. Prescott, H.C.; Langa, K.M.; Liu, V.; Escobar, G.J. Increased 1-year healthcare use in survivors of severe sepsis. *Am. J. Respir. Crit. Care Med.* **2014**, *190*, 62–69. [[CrossRef](#)] [[PubMed](#)]
10. Liu, V.; Lei, X.; Prescott, H.C.; Kipnis, P.; Iwashyna, T.J. Hospital readmission and healthcare utilization following sepsis in community settings. *J. Hosp. Med.* **2014**, *9*, 502–507. [[CrossRef](#)] [[PubMed](#)]
11. Shah, F.A.; Pike, F.; Alvarez, K.; Angus, D.C.; Newman, A.B. Bidirectional relationship between cognitive function and pneumonia. *Am. J. Respir. Crit. Care Med.* **2013**, *188*, 586–592. [[CrossRef](#)] [[PubMed](#)]
12. Prescott, J.C.; Angus, D.C. Enhancing recovery from sepsis. *JAMA* **2018**, *319*, 62–75. [[CrossRef](#)] [[PubMed](#)]
13. Osler, W. *The Evolution of Modern Medicine*; Yale University Press: New Haven, CT, USA, 1921.
14. Calvano, S.E.; Xiao, W.; Richards, D.R.; Felciano, R.M.; Baker, H.V.; Cho, R.J.; Moldawer, L.L.; Lowry, S.F. A network-based analysis of systemic inflammation in humans. *Nature* **2005**, *437*, 1032–1037. [[CrossRef](#)] [[PubMed](#)]
15. Tang, B.M.; Huang, S.J.; McLean, A.S. Genome-wide transcription profiling of human sepsis: A systematic review. *Crit Care* **2010**, *14*, R237. [[CrossRef](#)] [[PubMed](#)]
16. Landry, D.W.; Oliver, J.A. Pathogenesis of vasodilatory shock. *N. Engl. J. Med.* **2001**, *345*, 588–595. [[CrossRef](#)] [[PubMed](#)]
17. Lee, W.L.; Slutsky, A.S. Sepsis and endothelial permeability. *N. Engl. J. Med.* **2010**, *363*, 689–691. [[CrossRef](#)] [[PubMed](#)]
18. May, J.M.; Harrison, F.E. Role of vitamin C in the function of the vascular endothelium. *Antioxid. Redox Signal.* **2013**, *19*, 2068–2083. [[CrossRef](#)] [[PubMed](#)]
19. Spoelstra-de Man, A.M.; Elbers, P.W.; Oudemans-van Straaten, H.M. Making sense of early high-dose intravenous vitamin C in ischemia/reperfusion injury. *Crit Care* **2018**, *22*, 70. [[CrossRef](#)] [[PubMed](#)]
20. Prauchner, C.A. Oxidative stress in sepsis: Pathophysiological implications justifying antioxidant co-therapy. *Burns* **2017**, *43*, 471–485. [[CrossRef](#)] [[PubMed](#)]
21. Ware, L.B.; Fessel, J.P.; May, A.K.; Roberts, L.J. Plasma biomarkers of oxidant stress and development of organ failure in severe sepsis. *Shock* **2011**, *36*, 12–17. [[CrossRef](#)] [[PubMed](#)]
22. Kumar, S.; Gupta, E.; Kaushik, S.; Kumar Srivastava, V.; Mehta, S.K. Evaluation of oxidative stress and antioxidant status: Correlation with the severity of sepsis. *Scand. J. Immunol.* **2018**, *87*, e12653. [[CrossRef](#)] [[PubMed](#)]
23. Quoilin, C.; Mouithys-Mickalad, A.; Lecart, S.; Fontaine-Aupart, M.P.; Hoebeke, M. Evidence of oxidative stress and mitochondrial respiratory chain dysfunction in an in vitro model of sepsis-induced kidney injury. *Biochim. Biophys. Acta* **2014**, *1837*, 1790–1800. [[CrossRef](#)] [[PubMed](#)]
24. Langley, R.J.; Tsalik, E.L.; van Velkinburgh, J.C.; Glickman, S.W. An integrated clinico-metabolomic model improves prediction of death in sepsis. *Sci. Transl. Med.* **2013**, *5*, 195ra195. [[CrossRef](#)] [[PubMed](#)]
25. Langley, R.J.; Tipper, J.L.; Bruse, S.; Baron, R.M.; Tsalik, E.L.; Huntley, J.; Choi, A.M. Integrative “omic” analysis of experimental bacteremia identifies a metabolic signature that distinguishes human sepsis from systemic inflammatory response syndromes. *Am. J. Respir. Crit. Care Med.* **2014**, *190*, 445–455. [[CrossRef](#)] [[PubMed](#)]
26. Beesley, S.J.; Weber, G.; Sarge, T.; Nikravan, S. Septic cardiomyopathy. *Crit. Care Med.* **2018**, *46*, 625–634. [[CrossRef](#)] [[PubMed](#)]
27. Haileselassie, B.; Su, E.; Pozios, I.; Nino, D.F.; Liu, H.; Lu, D.Y.; Ventoulis, I. Myocardial oxidative stress correlates with left ventricular dysfunction on strain echocardiography in a rodent model of sepsis. *Intensive Care Med. Exp.* **2017**, *5*, 21. [[CrossRef](#)] [[PubMed](#)]
28. Galley, H.F. Oxidative stress and mitochondrial dysfunction in sepsis. *Br. J. Anaesth.* **2011**, *107*, 57–64. [[CrossRef](#)] [[PubMed](#)]
29. Alamdari, N.; Constantin-Teodosiu, D.; Murton, A.J.; Gardiner, S.M.; Bennett, T.; Layfield, R.; Greenhaff, P.L. Temporal changes in the involvement of pyruvate dehydrogenase complex in muscle lactate accumulation during lipopolysaccharide infusion in rats. *J. Physiol.* **2008**, *586*, 1767–1775. [[CrossRef](#)] [[PubMed](#)]
30. Thomas, G.W. Potential dysregulation of the pyruvate dehydrogenase complex by bacterial toxins and insulin. *J. Trauma* **2009**, *67*, 628–633. [[CrossRef](#)] [[PubMed](#)]

31. Artenstein, A.W.; Higgins, T.L.; Opal, S.M. Sepsis and scientific revolutions. *Crit. Care Med.* **2013**, *41*, 2770–2772. [[CrossRef](#)] [[PubMed](#)]
32. Aird, W.C. The role of the endothelium in severe sepsis and multiple organ dysfunction syndrome. *Blood* **2003**, *101*, 3765–3777. [[CrossRef](#)] [[PubMed](#)]
33. Blythe, R.; Cook, D.; Graves, N. Septicemia: The impact on the health system and patients of dealying new treatments with uncertain evidence: A case study of the sepsis bundle. *F1000Research* **2018**, *7*, 500. [[CrossRef](#)] [[PubMed](#)]
34. Galley, H.F.; Davies, M.J.; Webster, N.R. Ascorbyl radical formation in patients with sepsis: Effect of ascorbate loading. *Free Radic. Biol Med.* **1996**, *20*, 139–143. [[CrossRef](#)]
35. Borrelli, E.; Roux-Lombard, P.; Grau, G.E.; Girardin, E.; Ricou, B.; Dayer, J.; Suter, P.M. Plasma concentrations of cytokines, their soluble receptors, and antioxidant vitamins can predict the development of multiple organ failure in patients at risk. *Crit. Care Med.* **1996**, *24*, 392–397. [[CrossRef](#)] [[PubMed](#)]
36. Evans-Olders, R.; Eintracht, S.; Hoffer, L.J. Metabolic origin of hypovitaminosis C in acutely hospitalized patients. *Nutrition* **2010**, *26*, 1070–1074. [[CrossRef](#)] [[PubMed](#)]
37. Rojas, C.; Cadenas, S.; Herrero, A.; Mendez, J.; Batja, G. Endotoxin depletes ascorbate in the guinea pig heart. Protective effects of vitamins C and E against oxidative stress. *Life Sci.* **1996**, *59*, 649–657. [[CrossRef](#)]
38. Armour, J.; Tyml, K.; Lidington, D.; Wilson, J.X. Ascorbate prevents microvascular dysfunction in the skeletal muscle of the septic rat. *J. Appl. Physiol.* **2001**, *90*, 795–803. [[CrossRef](#)] [[PubMed](#)]
39. Victor, V.M.; Guayerbas, N.; Puerto, M.; De la Fuente, M. Changes in the ascorbic acid levels of peritoneal lymphocytes and macrophages of mice with endotoxin-induced oxidative stress. *Free Radic. Res.* **2001**, *35*, 907–916. [[CrossRef](#)] [[PubMed](#)]
40. Wu, F.; Wilson, J.X.; Tyml, K. Ascorbate inhibits iNOS expression and preserves vasoconstrictor responsiveness in skeletal muscle of septic mice. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **2003**, *285*, R50–R56. [[CrossRef](#)] [[PubMed](#)]
41. Tyml, K.; Li, F.; Wilson, J.X. Delayed ascorbate bolus protects against maldistribution of microvascular blood flow in septic rat skeletal muscle. *Crit. Care Med.* **2005**, *33*, 1823–1828. [[CrossRef](#)] [[PubMed](#)]
42. Fowler, A.A.; Syed, A.A.; Knowlson, S.; Sculthorpe, R.; Farthing, D.; DeWilde, C.; Farthing, C.A.; Laruss, T.L.; Martin, E.; Brophy, D.F. Phase 1 safety trial of intravenous ascorbic acid in patients with severe sepsis. *J. Transl. Med.* **2014**, *12*, 32. [[CrossRef](#)] [[PubMed](#)]
43. Marik, P.E.; Khangoora, V.; Rivera, R.; Hooper, M.H.; Catravas, J. Hydrocortisone, Vitamin C and Thiamine for the treatment of severe sepsis and septic shock: A retrospective before-after study. *Chest* **2017**, *151*, 1229–1238. [[CrossRef](#)] [[PubMed](#)]
44. De Grooth, H.M.; Spoeistra-de Man, A.M.; Oudermans-van Straaten, H.M. Early plasma vitamin C concentration, organ dysfunction and ICU mortality [Abstract]. *Intensive Care Med.* **2014**, *40*, S199.
45. Carr, A.C.; Rosengrave, P.C.; Bayer, S.; Chambers, S.; Mehrtens, J.; Shaw, G.M. Hypovitaminosis C and vitamin C deficiency in critically ill patients despite recommended enteral and parenteral intakes. *Crit Care* **2017**, *21*, 300. [[CrossRef](#)] [[PubMed](#)]
46. De Grooth, H.J.; Manubulu-Choo, W.P.; Zandvliet, A.; Spoelstra-de Man, A.M.; Swart, E. Oudermans-van Straaten HM. Vitamin C pharmacokinetics in critically ill patients: A randomized trial of four intravenous regimens. *Chest* **2018**, *153*, 1368–1377. [[CrossRef](#)] [[PubMed](#)]
47. Wilson, J.X. Evaluation of vitamin C for adjuvant sepsis therapy. *Antioxid. Redox Signal.* **2013**, *19*, 2129–2140. [[CrossRef](#)] [[PubMed](#)]
48. Oudemans-van Straaten, H.M.; Spoelstra-de Man, A.M.; de Waard, M.C. Vitamin C revisited. *Crit Care* **2014**, *18*, 460. [[CrossRef](#)] [[PubMed](#)]
49. Sagun, K.C.; Carcamo, J.M.; Golde, D.N. Vitamin C enters mitochondria via facilitative glucose transporter 1 (Glut1) and confers mitochondrial protection against oxidative injury. *FASEB J.* **2005**, *19*, 1657–1667.
50. Lowes, D.A.; Webster, N.R.; Galley, H.F. Dehydroascorbic acid as pre-conditioner: Protection from lipopolysaccharide induced mitochondrial damage. *Free Radic. Res.* **2010**, *44*, 283–292. [[CrossRef](#)] [[PubMed](#)]
51. Puskarich, M.A.; Kline, J.A.; Krabill, V.; Claremont, H.; Jones, A.E. Preliminary safety and efficacy of L-carnitine infusion for the treatment of vasopressor-dependent septic shock: A randomized control trial. *JPEN* **2014**, *38*, 736–743. [[CrossRef](#)] [[PubMed](#)]

52. Dhar-Mascreno, M.; Carcamo, J.M.; Golde, D.W. Hypoxia-reoxygenation-induced mitochondrial damage and apoptosis in human endothelial cells inhibited by vitamin C. *Free Radic. Biol. Med.* **2005**, *38*, 1311–1322. [[CrossRef](#)] [[PubMed](#)]
53. Kim, J.Y.; Lee, S.M. Effect of ascorbic acid on hepatic vasoregulatory gene expression during polymicrobial sepsis. *Life Sci.* **2004**, *75*, 2015–2026. [[CrossRef](#)] [[PubMed](#)]
54. Szakmany, T. N-acetylcysteine for sepsis and systemic inflammatory response in adults. *Cochrane Database Syst. Rev.* **2012**, *9*, CD006616. [[CrossRef](#)] [[PubMed](#)]
55. Molnar, Z. N-acetylcysteine as the magic bullet: Too good to be true. *Crit. Care Med.* **2008**, *36*, 645–646. [[CrossRef](#)] [[PubMed](#)]
56. Carcamo, J.M.; Pedraza, A.; Borquez-Ojeda, O.; Golde, D.W. Vitamin C suppresses TNF-alpha induced NFkB activation by inhibiting Ikb-alpha phosphorylation. *Biochemistry* **2002**, *41*, 12995–13002. [[CrossRef](#)] [[PubMed](#)]
57. Kim, S.R.; Kim, Y.M.; Park, E.J.; Kim, J.W.; Park, S.W.; Kim, H.J. Ascorbic acid reduces HMGB1 secretion in lipopolysaccharide-activated RAW 264.7 cells and improves survival rate in septic mice by activation of Nrf2/HO-1 signals. *Biochem. Pharmacol.* **2015**, *95*, 279–289. [[CrossRef](#)] [[PubMed](#)]
58. Hagel, A.F.; Layritz, C.M.; Hagel, W.H.; Hagel, H.J.; Hagel, E.; Dauth, W.; Kressel, J.; Regnet, T. Intravenous infusion of ascorbic acid decreases serum histamine concentrations in patients with allergic and non-allergic diseases. *Naunyn-Schmiedebergs Arch. Pharmacol.* **2013**, *386*, 789–793. [[CrossRef](#)] [[PubMed](#)]
59. Hattori, M.; Yamazaki, M.; Ohashi, W.; Tanaka, S.; Hattori, K.; Todoroki, K. Critical role of endogenous histamine in promoting end-organ tissue injury in sepsis. *Intensive Care Med. Exp.* **2016**, *4*, 36. [[CrossRef](#)] [[PubMed](#)]
60. Carr, A.C.; Shaw, G.; Fowler, A.A.; Natarajan, R. Ascorbate-dependent vasopressor synthesis- a rationale for vitamin C administration in severe sepsis and septic shock? *Crit Care* **2015**, *19*, 418. [[CrossRef](#)] [[PubMed](#)]
61. Klenner, F.R. The treatment of poliomyelitis and other virus diseases with vitamin C. *South. Med. Surg.* **1949**, *111*, 209–214. [[PubMed](#)]
62. Pauling, L. Ascorbic acid and the common cold. *Am. J. Clin. Nutr.* **1971**, *24*, 1294. [[CrossRef](#)] [[PubMed](#)]
63. Hemila, H.; Chalker, E. Vitamin C for preventing and treating the common cold. *Cochrane Database Syst. Rev.* **2013**, *1*, CD000980. [[CrossRef](#)] [[PubMed](#)]
64. Hemila, H. Does vitamin C alleviate the symptoms of the common cold?—A review of current evidence. *Scand. J. Infect. Dis.* **1994**, *26*, 1–6. [[CrossRef](#)] [[PubMed](#)]
65. Furuya, A.; Uozaki, M.; Yamasaki, H.; Arakawa, T.; Arita, M.; Koyama, A.H. Antiviral effects of ascorbic and dehydroascorbic acids in vitro. *Int. J. Mol. Med.* **2008**, *22*, 541–545. [[PubMed](#)]
66. Madhusudana, S.N. In vitro inactivation of the rabies virus by ascorbic acid. *Int. J. Infect. Dis.* **2004**, *8*, 21–25. [[CrossRef](#)] [[PubMed](#)]
67. Anderson, R. Vitamin C and cellular immune functions. Protection against hypochlorous acid-mediated inactivation of glyceraldehyde-3-phosphate dehydrogenase and ATP generation in human leukocytes as a possible mechanism of ascorbate-mediated immunostimulation. *Ann. N. Y. Acad. Sci.* **1990**, *587*, 34–48. [[CrossRef](#)] [[PubMed](#)]
68. Manzella, J.P.; Roberts, N.J. Human macrophage and lymphocyte responses to mitogen stimulation after exposure to influenza virus, ascorbic acid, and hyperthermia. *J. Immunol.* **1979**, *123*, 1940–1944. [[PubMed](#)]
69. Siegel, B.V. Enhancement of interferon production by poly(rI)-poly(rC) in mouse cell cultures by ascorbic acid. *Nature* **1975**, *254*, 531–532. [[CrossRef](#)] [[PubMed](#)]
70. Jeong, Y.J.; Kim, J.H.; Hong, J.M.; Kang, J.S.; Kim, H.R. Vitamin C treatment of mouse bone marrow-derived dendritic cells enhanced CD8(+) memory T cell production capacity of these cells in vivo. *Immunobiology* **2014**, *219*, 554–564. [[CrossRef](#)] [[PubMed](#)]
71. Carr, A.; Frei, B. Does vitamin C act as a pro-oxidant under physiological conditions? *FASEB J.* **1999**, *13*, 1007–1024. [[CrossRef](#)] [[PubMed](#)]
72. Paolini, M.; Pozzetti, L.; Pedulli, G.F.; Marchesi, E.; Canteli-Forti, G. The nature of prooxidant activity of vitamin C. *Life Sci.* **1999**, *64*, L-8. [[CrossRef](#)]
73. Prat, A.G.; Turrens, J.F. Ascorbate- and hemoglobin-dependent brain chemiluminescence. *Free Radic. Biol. Med.* **1990**, *8*, 319–325. [[CrossRef](#)]
74. Larsen, R.; Gozzelino, R.; Jeney, V.; Tokaji, L.; Bozza, F.A.; Japiassu, A.M.; Bonaparte, D.; Cavalcante, M.M.; Chora, A.; Ferreira, A.; et al. A central role for free heme in the pathogenesis of severe sepsis. *Sci. Transl. Med.* **2010**, *2*, 51ra71. [[CrossRef](#)] [[PubMed](#)]

75. Jeney, V.; Balla, J.; Yachie, A.; Varga, Z.; Vercellotti, G.M.; Eaton, J.W.; Balla, G. Pro-oxidant and cytotoxic effects of circulating heme. *Blood* **2002**, *100*, 879–887. [[CrossRef](#)] [[PubMed](#)]
76. Seo, M.Y.; Lee, S.M. Protective effect of low dose of ascorbic acid on hepatobiliary function in hepatic ischemia/reperfusion in rats. *J. Hepatol.* **2002**, *36*, 72–77. [[CrossRef](#)]
77. Park, S.W.; Lee, S.M. Antioxidant and prooxidant properties of ascorbic acid on hepatic dysfunction induced by cold ischemia/reperfusion. *Eur. J. Pharmacol.* **2008**, *580*, 401–406. [[CrossRef](#)] [[PubMed](#)]
78. Motl, J.; Radhakrishnan, J.; Ayoub, I.M.; Grmec, S.; Gazmuri, R.J. Vitamin C compromises cardiac resuscitability in a rat model of ventricular fibrillation. *Am. J. Ther.* **2014**, *21*, 352–357. [[CrossRef](#)] [[PubMed](#)]
79. Kuck, J.L.; Bastarache, J.A.; Shaver, C.M.; Fessel, J.P.; Dikalov, S.I.; May, J.M.; Ware, L.B. Ascorbic acid attenuates endothelial permeability triggered by cell-free hemoglobin. *Biochem. Biophys. Res. Commun.* **2018**, *495*, 433–437. [[CrossRef](#)] [[PubMed](#)]
80. Chen, Q.; Espey, M.G.; Sun, A.; Lee, J.H.; Krishna, M.C.; Shacter, E.; Levine, M. Ascorbate in pharmacologic concentrations selectively generates ascorbate radical and hydrogen peroxide in extracellular fluid in vivo. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 8749–8754. [[CrossRef](#)] [[PubMed](#)]
81. Chen, Q.; Espey, M.G.; Krishna, M.C.; Mitchell, J.B.; Corpe, C.P. Pharmacologic ascorbic acid concentrations selectively kill cancer cells: Action as a pro-drug to deliver hydrogen peroxide to tissues. *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 13604–13609. [[CrossRef](#)] [[PubMed](#)]
82. Chen, Q.; Espey, M.G.; Sun, A.; Pooput, C.; Kirk, K.L.; Krishna, M.C.; Khosh, D.B.; Levine, M. Pharmacologic doses of ascorbate act as a prooxidant and decrease growth of aggressive tumor xenografts in mice. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 11105–11109. [[CrossRef](#)] [[PubMed](#)]
83. Levine, M.; Padayatty, S.J.; Espey, M.G. Vitamin C: A concentration-function approach yields pharmacology and therapeutic discoveries. *Adv. Nutr.* **2011**, *2*, 78–88. [[CrossRef](#)] [[PubMed](#)]
84. Muhlhofer, A.; Mrosek, S.; Schlegel, B.; Trommer, W.; Rozario, F.; Bohles, H.; Schremmer, D.; Zoller, W.G.; Biesalski, H.K. High-dose intravenous vitamin C is not associated with an increase of pro-oxidative biomarkers. *Eur. J. Clin. Nutr.* **2004**, *58*, 1151–1158. [[CrossRef](#)] [[PubMed](#)]
85. Tanaka, H.; Matsuda, T.; Miyagantani, Y.; Yukioka, T.; Matsuda, H.; Shimazaki, S. Reduction of resuscitation fluid volumes in severely burned patients using ascorbic acid administration: A randomized, prospective study. *Arch. Surg.* **2000**, *135*, 326–331. [[CrossRef](#)] [[PubMed](#)]
86. Basili, S.; Tanzilli, G.; Mangieri, E.; Raparelli, V.; Di Santo, S. Intravenous ascorbic acid infusion improves myocardial perfusion grade during elective percutaneous coronary intervention: Relationship with oxidative stress markers. *JACC Cardiovasc. Interv.* **2010**, *3*, 221–229. [[CrossRef](#)] [[PubMed](#)]
87. Wang, Z.J. The effect of intravenous vitamin C infusion on periprocedural myocardial injury for patients undergoing elective percutaneous coronary intervention. *Can. J. Cardiol.* **2014**, *30*, 96–101. [[CrossRef](#)] [[PubMed](#)]
88. Hu, X. Efficacy and safety of vitamin C for atrial fibrillation after cardiac surgery: A meta-analysis with trial sequential analysis of randomized controlled trials. *Int. J. Surg.* **2017**, *37*, 58–64. [[CrossRef](#)] [[PubMed](#)]
89. Xu, Y.; Zheng, X.; Liang, B.; Gao, J.; Gu, Z. Vitamins for prevention of contrast-induced acute kidney injury: A systematic review and trial sequential analysis. *Am. J. Cardiovasc. Drugs* **2018**, *18*, 373–386. [[CrossRef](#)] [[PubMed](#)]
90. Cain, D.W.; Cidlowski, J.A. Immune regulation by glucocorticoids. *Nat. Rev. Immunol.* **2017**, *17*, 233–247. [[CrossRef](#)] [[PubMed](#)]
91. Cruz-Topete, D.; Cidlowski, J.A. One hormone, two actions: Anti- and pro-inflammatory effects of glucocorticoids. *Neuroimmunomodulation* **2015**, *22*, 20–32. [[CrossRef](#)] [[PubMed](#)]
92. Busillo, J.M.; Cidlowski, J.A. The five Rs of glucocorticoid action during inflammation: Ready, reinforce, repress, resolve and restore. *Trends Endocrinol. Metab.* **2013**, *24*, 109–119. [[CrossRef](#)] [[PubMed](#)]
93. Barnes, P.J. Glucocorticosteroids: Current and future directions. *Br. J. Pharmacol.* **2011**, *163*, 29–43. [[CrossRef](#)] [[PubMed](#)]
94. Galon, J.; Franchimont, D.; Hiroi, N.; Frey, G.; Boettner, A.; Ehrhart-Bornstein, M.; O’Shea, J.J.; Chrousos, G.P.; Bornstein, S.R. Gene profiling reveals unknown enhancing and suppressive actions of glucocorticoids on immune cells. *FASEB J.* **2002**, *16*, 61–71. [[CrossRef](#)] [[PubMed](#)]

95. Keh, D.; Boehnke, T.; Weber-Cartens, S.; Schulz, C.; Ahlers, O.; Bercker, S.; Volk, H.D.; Doecke, W.D.; Falke, K.J.; Gerlach, H. Immunologic and hemodynamic effects of “low-dose” hydrocortisone in septic shock: A double-blind, randomized, placebo-controlled, crossover study. *Am. J. Respir. Crit. Care Med.* **2003**, *167*, 512–520. [[CrossRef](#)] [[PubMed](#)]
96. Rygaard, S.L.; Butler, E.; Granholm, A.; Moller, M.H.; Cohen, J.; Finfer, S.; Perner, A.; Myburgh, J.; Venkatesh, B.; Delaney, A. Low-dose corticosteroids for adults with septic shock: A systematic review with meta-analysis and trial sequential analysis. *Intensive Care Med.* **2018**, *44*, 1003–1016. [[CrossRef](#)] [[PubMed](#)]
97. Minneci, P.C.; Deans, K.J.; Eichacker, P.Q.; Natanson, C. The effects of steroids during sepsis depend on dose and severity of illness: An updated meta-analysis. *Clin. Microbiol. Infect.* **2009**, *15*, 308–318. [[CrossRef](#)] [[PubMed](#)]
98. Volbeda, M.; Wetterslev, J.; Gluud, C.; Zijlstra, J.G.; van der Horst, I.C.; Keus, F. Glucocorticosteroids for sepsis: Systematic review with meta-analysis and trial sequential analysis. *Intensive Care Med.* **2015**, *41*, 1220–1234. [[CrossRef](#)] [[PubMed](#)]
99. Kalil, A.C.; Sun, J. Low-dose steroids for septic shock and severe sepsis; the use of Bayesian statistics to resolve clinical trial controversies. *Intensive Care Med.* **2011**, *37*, 420–429. [[CrossRef](#)] [[PubMed](#)]
100. Venkatesh, B.; Finfer, S.; Cohen, J.; Rajbhandari, D.; Arabi, Y.; Billot, L.; Glass, P.; Perner, A.; Bellomo, R.; Myburgh, J. Adjunctive glucocorticoid therapy in patients with septic shock. *N. Engl. J. Med.* **2018**, *378*, 797–808. [[CrossRef](#)] [[PubMed](#)]
101. Annane, D.; Renault, A.; Brub-Buisson, C.; Megarbane, Z.B.; Quenot, J.P.; Siami, S. Hydrocortisone plus fludrocortisone for adults with septic shock. *N. Engl. J. Med.* **2018**, *378*, 809–818. [[CrossRef](#)] [[PubMed](#)]
102. Collie, J.T.; Greaves, R.F.; Jones, O.A.; Lam, Q.; Eastwood, G.M.; Bellomo, R. Vitamin B1 in critically ill patients: Needs and challenges. *Clin. Chem. Lab. Med.* **2017**, *55*, 1652–1668. [[CrossRef](#)] [[PubMed](#)]
103. Manzetti, S.; Zhang, J.; van der Spoel, D. Thiamin function, metabolism, uptake, and transport. *Biochemistry* **2014**, *53*, 821–835. [[CrossRef](#)] [[PubMed](#)]
104. Cruickshank, A.M.; Telfer, A.B.; Shenkin, A. Thiamine deficiency in the critically ill. *Intensive Care Med.* **1988**, *14*, 384–387. [[CrossRef](#)] [[PubMed](#)]
105. Donnino, M.W.; Carney, E.; Cocchi, M.N.; Barbash, I.; Chase, M. Thiamine deficiency in critically ill patients with sepsis. *J. Crit. Care* **2010**, *25*, 567–581. [[CrossRef](#)] [[PubMed](#)]
106. Donnino, M.W.; Andersen, L.W.; Chase, M.; Berg, K.M.; Tidswell, M.; Giberson, T.; Wolfe, R.; Moskowitz, A. Randomized, double-blind, placebo-controlled trial of thiamine as a metabolic resuscitator in septic shock: A pilot study. *Crit. Care Med.* **2016**, *44*, 360–367. [[CrossRef](#)] [[PubMed](#)]
107. Hazell, A.S.; Faim, S.; Wertheimer, G.; Silva, V.R.; Marques, C.S. The impact of oxidative stress in thiamine deficiency: A multifactorial targeting issue. *Neurochem. Int.* **2013**, *62*, 796–802. [[CrossRef](#)] [[PubMed](#)]
108. Gibson, G.E.; Zhang, H. Interactions of oxidative stress with thiamine homeostasis promote neurodegeneration. *Neurochem. Int.* **2002**, *40*, 493–504. [[CrossRef](#)]
109. Gioda, C.R.; de Oliveira Barreto, T.; Primola-Gomes, T.N.; de Lima, D.C.; Campos, P.P.; Cruz, J.S. Cardiac oxidative stress is involved in heart failure induced by thiamine deprivation in rats. *Am. J. Physiol. Heart Circ. Physiol.* **2010**, *298*, H2039–H2045. [[CrossRef](#)] [[PubMed](#)]
110. Moskowitz, A.; Anderson, L.W.; Cocchi, M.N.; Karlsson, M.; Patel, P.V.; Donnino, M.W. Thiamine as a renal protective agent in septic shock: A secondary analysis of a randomized, double-blind, placebo-controlled trial. *Ann. Am. Thorac. Soc.* **2017**, *14*, 737–741. [[CrossRef](#)] [[PubMed](#)]
111. Woolum, J.A.; Abner, E.L.; Kelly, A.; Thompson Bastin, M.L.; Morris, P.E.; Flannery, A.H. Effect of thiamine administration on lactate clearance and mortality in patients with septic shock. *Crit. Care Med.* **2018**, *46*, 1747–1752. [[CrossRef](#)] [[PubMed](#)]
112. Singer, M.; Deutschman, C.S.; Seymour, C.W.; Shankar-Hari, M.; Annane, D.; Bauer, M.; Bellomo, R.; Bernard, G.R.; Chiche, J.D.; Cooper-Smith, C.M.; et al. The third international consensus definitions for sepsis and septic shock (Sepsis-3). *JAMA* **2016**, *315*, 801–810. [[CrossRef](#)] [[PubMed](#)]
113. Barabutis, N.; Khangoor, V.; Marik, P.E.; Catravas, J.D. Hydrocortisone and Ascorbic Acid synergistically protect and repair lipopolysaccharide-induced pulmonary endothelial barrier dysfunction. *Chest* **2017**, *152*, 954–962. [[CrossRef](#)] [[PubMed](#)]
114. Azari, O.; Kheirandish, R.; Azizi, S.; Farajli Abassi, M.; Bidi, M. Protective effects of hydrocortisone, Vitamin C and E alone or in combination against renal-ischemia-reperfusion injury rat. *Iran. J. Pathol.* **2015**, *10*, 272–280. [[PubMed](#)]

115. Tavasoli, M.; Azari, O.; Kheirandish, R. Evaluation of combination therapy with hydrocortisone, vitamin C and vitamin E in a rat model of intestine ischemia-reperfusion injury. *Comp. Clin. Pathol.* **2018**, *27*, 433–439. [[CrossRef](#)]
116. Kim, W.Y.; Jo, E.J.; Eom, J.S.; Mok, J.; Kim, M.H.; Kim, K.U.; Park, H.K.; Lee, M.K.; Lee, K. Combined vitamin, C.; hydrocortisone, and thiamine therapy for patients with severe pneumonia who were admitted to the intensive care unit: Propensity score-based analysis of a before-after cohort study. *J. Crit. Care* **2018**, *47*, 211–218. [[CrossRef](#)] [[PubMed](#)]



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