Chapter

Vitamin C and Sepsis

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Abstract

Vitamin C is a supplement used orally by several people globally. It may help in many other conditions, like sepsis, which is caused by an infection that leads to an imbalanced immune response involving pro (e.g., TNF-α, IL-1, IL-2, IL-6) and anti-inflammatory (e.g., IL-10, IL-4, IL-7) cytokines. Ascorbic acid is an antioxidant and acts against reactive oxygen species. At the same time, this vitamin influences cellular immune signaling, avoiding exacerbated transcription of pro-inflammatory cytokines. Very high intravenous doses have already shown to be beneficial in septic patients. Some clinical trials are still running to evaluate the real impact of vitamin C in this condition. To the moment, the combination of low-dose corticosteroids, high-dose parenteral ascorbate, and thiamine seems to be the most effective supportive treatment that could help septic patients recover.

Keywords: vitamin C, sepsis, emergency, Intensive Care Unit

1. Introduction

Vitamin C is a well-known potent antioxidant essential to various biological processes such as carnitine synthesis, neurotransmitter synthesis, hormone synthesis, and tyrosine metabolism. Furthermore, it stabilizes collagen and acts in iron absorption on the intestinal tract. Nevertheless, much is still discussed on its role in common cold, pneumonia, stress-related disorders, metabolic syndrome, and sepsis. Sepsis is a dysregulated host response to an infection that triggers the release of both pro and anti-inflammatory cytokines throughout its course. This "cytokine storm" is responsible for systemic septic symptoms such as vasodilatation, which leads to hypotension and hypoxia. Also, there is the activation of the clotting cascade leading to disseminated intravascular coagulation (DIC). This hemodynamic instability associated with high immune response makes sepsis a deadly disease. Having such nonspecific symptoms, treating sepsis is also problematic. However, the great majority of protocols include antimicrobial and fluid therapy, vasopressors, and inotropic agents. Using anticoagulants and corticosteroids is debated and varies according to symptoms and local protocols. The use of vitamin C in sepsis treatment is also a highly discussed subject, and there are many clinical trials ongoing trying to associate a better outcome with the help of vitamin C in high doses. Considering that sepsis leads to a depletion in vitamin C because of the increased need for reactive oxygen species (ROS) and the elevated cytokine release, it is fair to assume that supplementing it in high doses might help improve septic symptoms since it scavenges those oxygen-free radicals.

All things considered, this chapter intends to shed light on the pathophysiology of sepsis, and its current treatments, vitamin C's biochemical and therapeutic properties, and the pieces of evidence from clinical trials that applied vitamin C to treat sepsis and its outcomes.

2. Sepsis

2.1 Pathophysiology, molecular pathways, and mediators of sepsis

Sepsis is an overreaction to infections, resulting in multiple organ failure and septic shock, frequently leading to death [1]. Sepsis is commonly associated with a super systemic inflammatory condition followed by an immunosuppression phase in which secondary infections typically occur [2]. First sepsis models were developed using animal experiments after confirmation in human volunteers. Bacterial debris can stimulate an acute rise of pro-inflammatory cytokines, implying that this was the cause of the sepsis-associated organ failure. Guided by these results, several different proposed therapies failed to achieve a substantial positive outcome [3, 4]. Following the overwhelming inflammatory process, an intense anti-inflammatory reply leads to a lack of immune response, lymphopenia, and a high propensity for developing infections [5]. This information had provided the basis of the suppositions that the initial hyperinflammatory condition advances to a following immunosuppression [2]. Pro-inflammatory (as IL-6 and TNF) and anti-inflammatory (as IL-10) cytokines are elevated and death-related in septic patients [6].

After innate recognition of conserved microbial patterns, a substantial inflammatory response begins. The recognition, usually through Toll-Like receptors, leads to the activation of cytokines, growth factors and chemokines [7]. After CD4 T cells activation (**Figure 1**), both pro and anti-inflammatory cytokines are released [4]. The reason why CD4 T cells response is pro (Th1) or anti (Th2) inflammatory is supposed to be related to the size of the bacterial inoculum, pathogen type, and

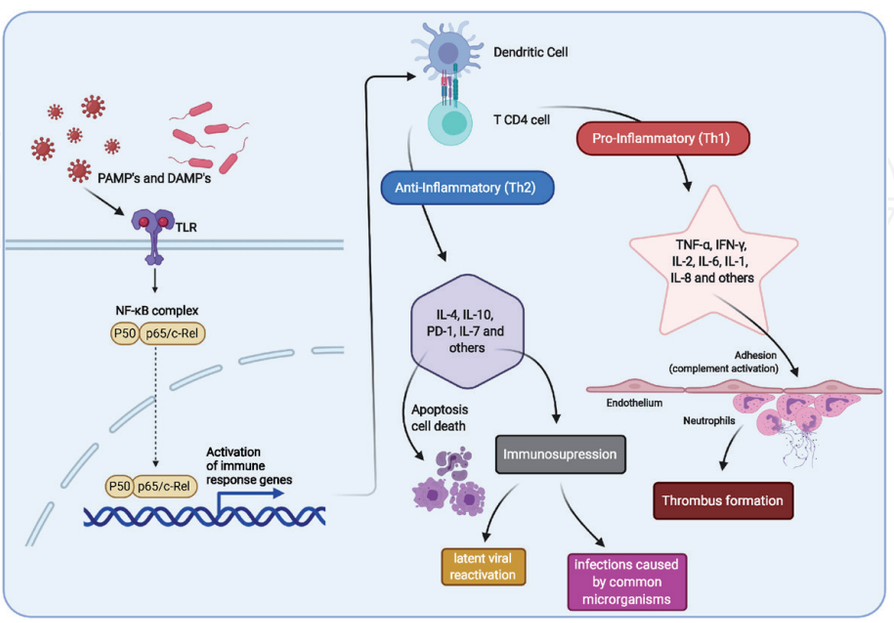

Figure 1.
Immune activation following microbial exposition or cellular damage. NF-κB: Nuclear Factor-κB; PAMP: Pathogen-associated molecular pattern; DAMP: Damage-associated molecular pattern; TLR: Toll-like Receptor; IL: Interleukin; TNF-α: Tumor Necrosis Factor-α; IFN-γ: Interferon-γ; PD-1: Programmed cell death protein 1.

the infected organ [8–11]. A more intense inflammatory response with higher cytokine levels is associated with severe sepsis situations. The spectrum of organic reactions is more intense, as well. General vasodilation, capillary leak, and lessened circulating fluid volume lead to blood clotting and multiple organ malfunction or failure [7].

2.2 Therapeutics of sepsis

The earlier the sepsis or septic shock diagnosis is achieved, the higher are the recovery chances. Broad-spectrum antibiotics (piperacillin/tazobactam, vancomycin, anidulafungin) are initiated while culture and antibiogram results are not available. Clindamycin associated with a β-lactam scheme can be recommended to avoid streptococcal toxic shock. Once the pathogen is identified and its susceptibility to antibiotics is defined, early interruption should be performed, depending on the patient's improvement [2, 12].

Supportive therapy is needed in virtually all cases. Fluid resuscitation, inotropic (e.g., dobutamine), and vasopressor agents (e.g., norepinephrine) are the most common, effective, and widespread therapies [13].

Other therapies have been studied over the years, but few or controversial results were obtained. Corticosteroids are frequently associated with septic shock therapy. Several randomized controlled trials focused on this issue, and some meta-analysis evaluated the outcomes. Considering all observed flaws of the trials (heterogeneity across studies, doses, the uncertainty of the statistical approach, time of observation, among others), the meta-analysis showed a small benefit using low doses of corticosteroids for a more extended period [14–20]. International Guidelines for Management of Sepsis and Septic Shock recommend corticosteroid therapy only if fluid resuscitation and vasopressor administration are not enough to restore patients' stability. Intravenous hydrocortisone (200 mg/day) and continuous evaluation of blood glucose and sodium (corticosteroids may induce hyperglycemia and hypernatremia) are the clinical guidance in those cases [12].

Anticoagulant therapy would be beneficial to oppose the disseminated intravascular coagulation that happens in sepsis conditions. However, antithrombin use did not show evidence to lower the mortality rate and was more prone to bleeding development [12, 21, 22]. On the other hand, thrombomodulin and heparin showed some positive effects on the mortality rate and reduced bleeding risk [23, 24].

Immunoglobulins are still controversial in sepsis. Studies using intravenous immunoglobulins could not show benefits on septic shock or sepsis conditions [25–29]. However, the majority of studies use a small sample size, so more extensive studies are still needed to evaluate its effectiveness [12, 29].

To the present, numerous researches are trying to achieve a satisfactory result for septic shock or sepsis. However, a long list of failures is along with all the tries. There is a rationale behind Vitamin C usage in these cases, and this chapter will then discuss what is already known and what still needs investigation.

3. Vitamin C

3.1 Redox potential

Vitamin C (VitC, ascorbate) is an antioxidant vitamin. This classification is based on the emission of solvated electrons in aqueous media. In organisms, this process can be enzymatically induced. VitC quickly loses electrons in aqueous media, forming ascorbate free radicals. This is why ascorbate is classified as a very

potent electron donor. Peroxyl radicals may be formed under oxygenated conditions, by the reaction of solvated electrons with oxygen in aerated solutions [30].

Ascorbate can directly scavenge free radicals or restore other redox systems like α-tocopherol or glutathione (**Figure 2**). Simultaneously, it is vital to the activity of several iron and copper-dependent enzymes [31]. VitC and monodehydroascorbate radicals have low electron reduction potentials [32] to reduce more common radicals present in metabolic conditions.

It is well established that a severe dietary undersupply of vitamin C will result in scurvy. But vitamin C has also a role as a cofactor in several enzymes. It takes part in carnitine synthesis, which is essential for the transport of fatty acids into mitochondria for ATP generation [33, 34]; in the biosynthesis of norepinephrine from dopamine [35, 36], peptide hormones [37, 38], and tyrosine metabolism [39, 40]; in collagen synthesis, increasing its stability [41–43]; finally, it acts in nonheme iron absorption on the intestinal tract [44].

However, ascorbic acid's role in preventing or treating common and complex diseases is still uncertain. Even the widely held assumption that ascorbic acid is a significant biological antioxidant and has a prominent role in disease prevention has not been definitively validated [45, 46].

3.2 Evaluation of vitamin C therapeutic efficacy

Hundreds of studies have been published over the years on vitamin C's effects and its roles in preventing or treating several diseases. There have been many controversial outcomes from this association, whether they are positive or negative. **Table 1** presents the reviews that summarize those outcomes.

Nevertheless, when they are critically analyzed, one can realize they show many inconsistencies regarding the methodology. Recently, Lykkesfeldt interestingly analyzed some more expressive clinical trials and unraveled many of the study's limitations and flaws, as described below, which should be avoided in future researches in this field [59] (**Table 2**).

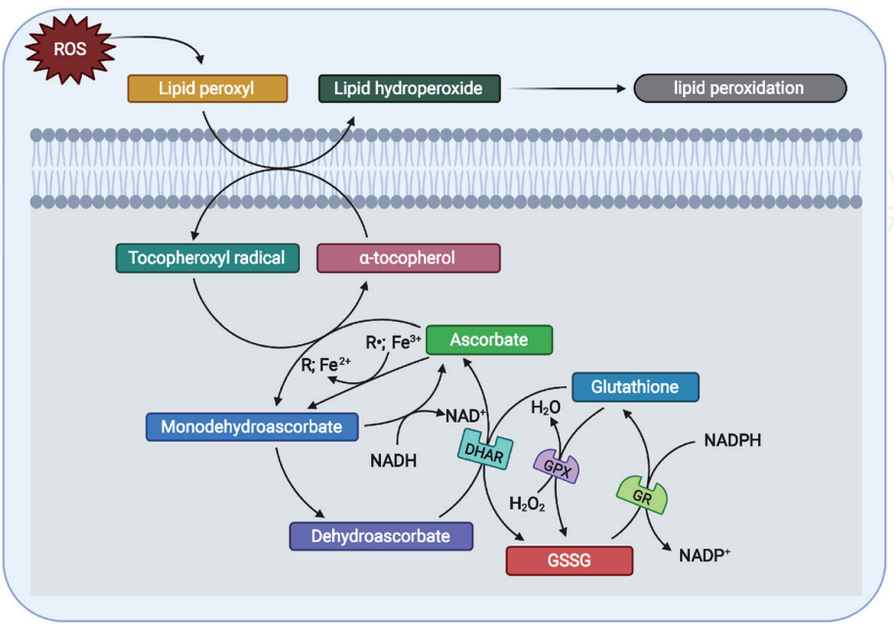

Figure 2.
Antioxidant network. Ascorbate plays a central role in the human antioxidant system. ROS: Reactive Oxygen Species; R·: Free Radical; DHAR: Dehydroascorbate Reductase; GPX: Glutathione Peroxidase; GSSG: Glutathione Disulfide; GR: Glutathione Reductase.

Effect	Conclusion of the study	Reference
Cardiovascular protection	Vitamin C deficiency is associated with a higher risk of cardiovascular disease (CVD) mortality.	[47]
	High vitamin C intake from supplements is associated with an increased risk of CVD mortality in postmenopausal women with diabetes.	[48]
	Population with optimal plasma levels of VitC has no benefit from supplementation. People with VitC deficiency have a higher risk of developing CVD.	[49]
Neurologic protection	Antiexcitotoxic, neuromodulator, and neurotrophic effects of ascorbic acid over the CNS are critical for neuroprotective strategies. Clinical trials have demonstrated that ascorbate supplementation produces beneficial results for depression and anxiety. More controlled clinical trials are still necessary to better understand the action mechanisms in stress-related disorders.	[50]
Metabolic syndrome	A direct positive effect of vitamin C alone on Metabolic syndrome needs to be confirmed in animals and human populations. Combination of vitamin C with other antioxidants may be worthwhile in managing Metabolic syndrome.	[51]
Common cold (CC) treatment and prevention	In adults, the duration of colds was reduced by 8% and in children by 14%. The severity of colds was also reduced by vitamin C administration during the cold process. No reliable effect of vitC was seen on the duration or severity of colds in the therapeutic trials.	[52]
	Regular supplementation has shown that ascorbate reduces the duration and severity of CC.	[53]
	Supplementation with vitamin C appears to be able to both prevent and treat respiratory and systemic infections.	[54]
Pneumonia treatment and prevention	Due to the small number of included studies and the low quality of the existing evidence, data is uncertain about the effect of vitamin C supplementation on preventing and treating pneumonia.	[55]
Exercise recovery	Vitamin C supplementation attenuates the oxidative stress (lipid peroxidation) and inflammatory response (IL-6) to a single exercise bout. No effects of vitamin C supplementation were found on creatine kinase (CK), C-reactive protein (CRP), cortisol levels, muscle soreness, and muscle strength.	[56]
Cancer treatment	Ascorbate can be positive as a pro-oxidative factor as well. VitC would promote the removal of 8-Oxo-2′-deoxyguanosine from DNA by upregulation of repair enzymes due to pro-oxidative properties. Vitamin C showed protection against radiation-induced cell damage.	[57]
	No clinically relevant positive effect of vitamin C in cancer patients on the overall survival, clinical status, quality of life, and performance status. The quality of the evaluated studies, however, is low. Small advantages were more associated to intravenous than oral administration.	[58]

Table 1.
Summary of reviews about therapeutic evidence associated with VitC.

Considering the lack of high-quality data to evaluate the efficacy of ascorbate in less severe or more chronic conditions, it is fair to assume that an acute and severe disease such as sepsis is a hard-to-evaluate condition. Vitamin C in sepsis has some particularities such as a diverse route of administration and a peculiar dose–response relationship. The scientific rationale behind this therapeutic proposal to sepsis is discussed in the rest of this chapter.

Concern	Trouble	Resolution
Measurement of Vit C intake vs. status	Focus on VitC intake rather than its status. Even large cohort studies used estimates of micronutrient intakes from self-reported questionnaires or diaries. Lack of precision due to recall error, loss of vitamin from storage and preparation, diet change over time, and possible different polymorphisms.	Retrieving blood samples from fasted individuals.
Lack of stability	Fasted blood samples can be obtained, but there are significant challenges in correlating vitamin C status to disease risk. This is due to the lability of ascorbate. Ascorbate is quickly oxidized ex vivo, and the resulting oxidation products are quickly degraded or metabolized	Process samples in a cold (4 °C) environment. Avoid hemolysis. Choose HPLC with electrochemical detection.
Study Design	Random Controlled Trials may require very long intervention periods to accumulate sufficient disease endpoints. This perspective is needed to observe an accumulated preventive potential of a lifelong VitC intake of both placebo and intervention groups up to the trial. This issue has been completely neglected in the available literature.	Perform multicentric randomized follow-up clinical trials.
Healthy Enrolee Effect	A tendency towards recruiting health-conscious, self-motivated subjects eating a healthy diet already rich in micronutrients, with higher exercise frequency and lower disease rate than the background population.	Work with more significant samples, baseline adjustment among groups, previous genetic evaluation

Table 2.
Concerns about clinical trials performed to evaluate ascorbate efficacy on diseases, according to Lykkesfeldt [59] (modified by the authors).

3.3 Dose-response in supplementation versus high dose

Sepsis is a condition associated with VitC deficiency because of its high consumption due to enhanced reactive oxygen species (ROS) production. Ascorbate supplementation is thus necessary, and the best results are thought to be achieved through high intravenous doses [60, 61]. The absorption, distribution, metabolism, and excretion (ADME) of vitC in humans are distinct from other small molecules.

3.3.1 Pharmacokinetics of vitamin C

3.3.1.1 Oral and intravenous administration

In biological systems, VitC exists in two main chemical forms (**Figure 3**), the reduced and predominant ascorbate anion and the oxidized dehydroascorbate (DHA). Due to the DHA reductase activity (**Figure 2**), virtually every cell can recycle DHA. Therefore, total ascorbate is considered the sum of VitC and DHA. The membrane transport can be performed by three possible mechanisms: passive or facilitated diffusion and active transport, the most relevant of the three [62].

Orally, VitC is absorbed by the saturable mucosal sodium-dependent Vitamin C transporter 1 (SVCT1) [60]. Ascorbate oral absorption is limited, achieving a plateau after 200-300 mg (**Figure 4**). SVCT transporters are widely distributed throughout organs and are responsible for most VitC passage across membranes, even against a concentration gradient [63–65].

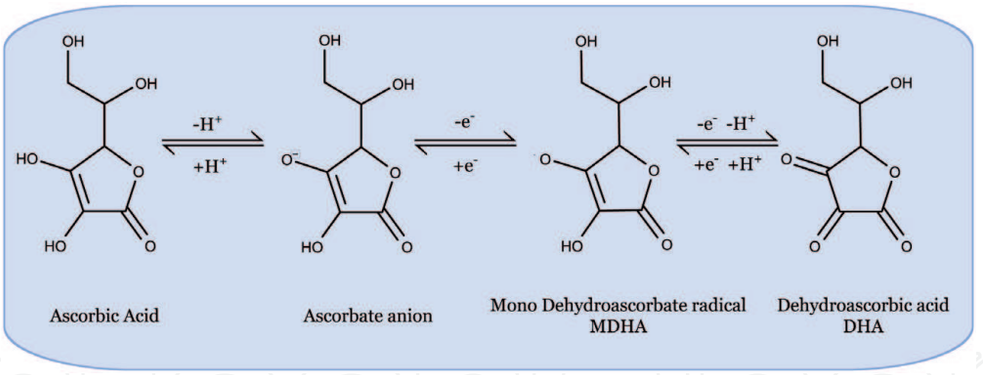

Figure 3.
VitC chemical forms in biological systems – Redox cycle. MDHA: Monodehydroascorbate radical. DHA: Dehydroascorbate.

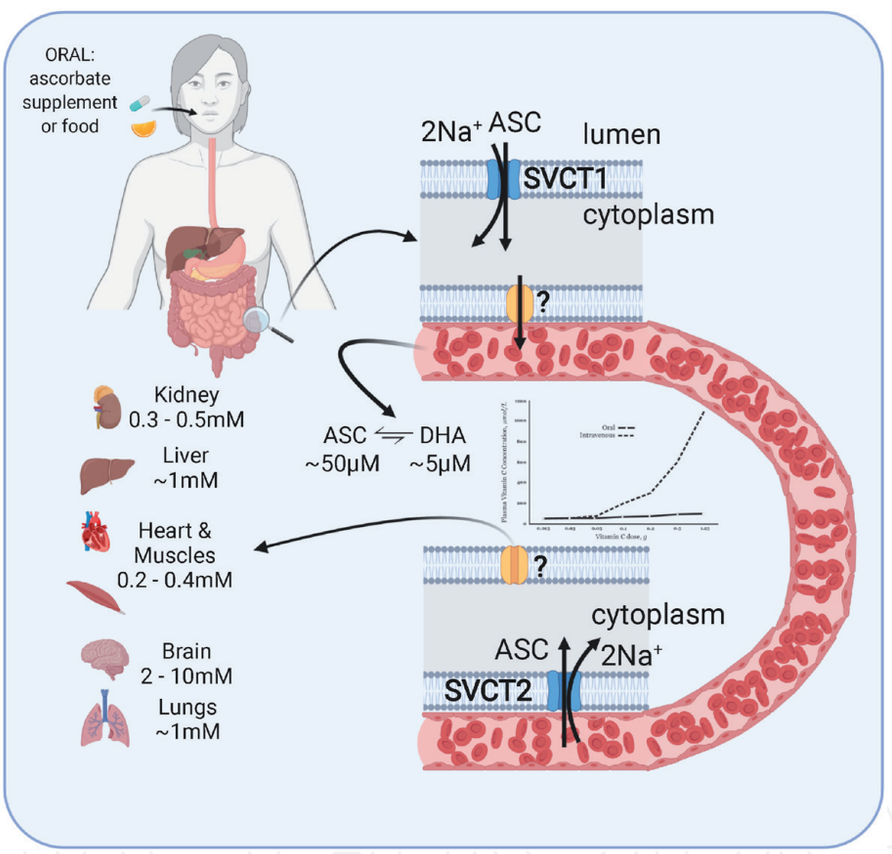

Figure 4.
VitC oral absorption and distribution. ASC: Ascorbate, SVCT1 and 2: sodium-dependent Vitamin C transporter 1 and 2, DHA: Dehydroascorbate.

Some SVCT polymorphisms have already been identified, which may be associated with a critical pharmacokinetic variation. Some of those SVCT alleles are supposed to lead to permanent ascorbate deficiency (plasma concentrations <23 µM) [62].

Humans do not synthesize vitC, so the oral ingestion of food is the primary source of vitC. There is enough ascorbate for healthy individuals in the average diets that contain food rich in it. However, pathologic conditions associated with low ascorbate levels may need supplementation to achieve the minimum plasma concentrations [62].

Intravenously, plasma levels of ascorbic acid continuously increase, producing plasma levels up to 70-fold higher than the maximum oral doses, achieving the millimolar concentration [66]. A linear relationship between dose and Cmax

(maximum concentration plasma level) was observed in doses up to about 70 g/m^2, leading to nearly 50 mM plasma levels. Apparently, the pharmacokinetic of vitC changes from zero to first-order after high-dose intravenous administration [62].

3.3.1.2 Distribution

Intracellular levels of ascorbate vary between 0.5 to 10 mM, which is much higher than the 50–80 μM usually found in healthy individuals' plasma. Simultaneously, human erythrocytes can turn DHA to VitC and keep an intracellular ascorbate level similar to that of plasma. This recycling ability of the red blood cells is essential as an antioxidant reserve [62].

As it happens at the absorption phase, distribution depends on active transport as well. Ascorbate exits the bloodstream and crosses the organ's cell membranes through SVCT2 carriers (**Figure 4**). Yet, even in the steady-state achieved concentration after regular ascorbate dosage, different tissues present highly diverse concentrations. This may happen because of distinct levels of SVCT2 expression [62].

3.3.1.3 Metabolism

Metabolism of VitC is essentially associated with the redox cycle involved with the antioxidant function (**Figure 3**). As previously cited, ascorbate is an electron donor, and it can reduce free radicals (**Figure 2**) by oxidizing itself to the stable radical monodehydroascorbate (MDHA). This radical can react to another equal, providing an ascorbate molecule and the DHA metabolite that can be reduced, as mentioned before, to ascorbate through DHA reductase activity [62].

3.3.1.4 Excretion

VitC is a highly water-soluble (about 330 g/L) small molecule (about 8 Å large, 176.1 g/mol), it has a pKa of 4.2, and is almost insoluble in hydrophobic organic solvents [67]. Like other molecules with similar solubility, ascorbate is filtered through the glomerulus and is concentrated after water resorption. At this time, local pH drops to five, leading to an increase of the non-ionized ascorbic acid fraction. However, passive reabsorption does not occur because of the highly hydrophilic characteristic of the molecule. In the proximal tubules, the reuptake of ascorbate is controlled by the saturable active transporter SVCT1. In individuals with saturated plasma levels, supplemental vitC is excreted quantitatively [68].

After high-dose intravenous administration, vitC is rapidly eliminated through glomerular filtration. Reuptake is non-significant under this condition, and the half-life is constant, about two hours (after discontinuation of infusion), and first-order kinetic applies to this case. In about 16 h, physiological levels are back to normal [62, 69–71].

3.3.1.5 Pharmacokinetics in critically ill patients

Critically ill patients, such as those in septic shock conditions, have an increased ascorbate turnover, needing a dose many folds higher (oral or intravenous) than would be expected to saturate a healthy person. Systemic inflammation and severe pressure due to oxidative stress increase VitC consumption [61, 72, 73]. Mathematically predicted plasmatic ascorbate values are much higher than what is achieved in critically ill patients, suggesting that pharmacokinetics in this group of patients is changed.

3.4 Vitamin C in septic conditions

In sepsis conditions, the mitochondrial impairment may be a relevant route to cell death and organ collapse. Anomalies in the citric acid cycle and reduction of the fatty acid's beta-oxidation seem to be a characteristic aspect of this mitochondrial disorder [74].

While ascorbate is transported across membranes through SVCT's proteins, DHA can be transported by glucose transporters GLUT1, 3, and 4 [75]. DHA is transported into the mitochondria by GLUT1 and converted to ascorbate (**Figure 5**), where it works as an antioxidant, avoiding damage to the organelle [76]. Ascorbate can also act as a cofactor to the mitochondrial Trimethyllysine dioxygenase (TMLD) enzyme, responsible for the first L-carnitine synthesis, needed for the β-oxidation of fatty acids [77].

The heart is a vital organ that may be affected by sepsis. Proteolysis, mitochondrial injury, and calcium homeostasis dysfunction are expected consequences of the oxidative myocyte damage. Experimental models show that supplementation of the redox scavengers can diminish cardiac disorder [78]. Ascorbate can decrease apoptosis and improve mitochondrial integrity in myocytes through the blockage of the mitochondrial permeability transition pore opening, limiting calcium profusion [79].

VitC can achieve high concentrations in leukocytes, especially lymphocytes and macrophages. In other defense cells, VitC acts to improve chemotaxis, stimulating interferon expression, and promoting lymphocyte proliferation. In neutrophils, ascorbate increases phagocytic capacity and oxidative burst, and decreases NET (neutrophil-extracellular-trap) formation [61, 80].

VitC can mediate immune modulation. VitC inhibits nuclear Factor Kappa-B (NF-κB) activation. The mechanism that underlies this suppression involves the blockade of the TNFα-induced activation of NIK (NFκB-inducing kinase) and

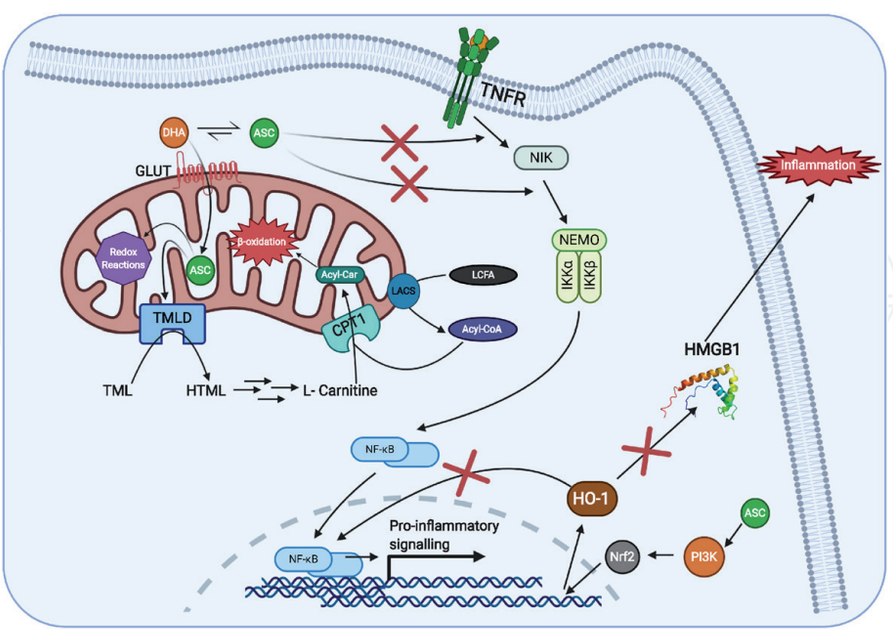

Figure 5.
Vitamin C multiple anti-inflammatory mechanisms. DHA: Dehydroascorbate, ASC: Ascorbate, GLUT: Glucose Transporter, (H)TML(D): (Hydroxy) Trimethyllysine (Dioxygenase), CPT1: Carnitine Palmitoyltransferase 1, LCFA: Long-Chain Fatty Acids; LACS: Long-Chain Acyl-CoA Synthetase, TNFR: Tumor Necrosis Factor Receptor, NF-κB: Nuclear Factor Kappa-light-chain-enhancer of activated B cells, NIK: NF-κB-Inducing Kinase, NEMO: NF-κB Essential Modulator, IKKα and β: IκBα and β kinases, PI3K: Phosphoinositide 3-Kinase, Nrf2: Nuclear Factor Erythroid 2-Related Factor 2, HO-1: Heme Oxygenase 1, HMGB1 - High Mobility Group Box 1.

IKKβ kinases (**Figure 5**) [81]. Further modulation is provided by the VitC induced decrease in the late pro-inflammatory cytokine HMGB1 (high mobility group box 1) secretion and through the lowering of histamine levels [82, 83].

3.4.1 Clinical trials: vitamin C and sepsis or other critically ill conditions

Table 1 shows studies that were performed to evaluate VitC efficacy in many pathological conditions. In critically ill patients, several clinical trials have already been completed or are still ongoing. Until December 2020, 39 studies involving ascorbate and some critically ill conditions were registered at the United States National Library of Medicine (NLM) databank clinicaltrials.gov. The list with all referred studies and links to the clinicaltrials.gov forms are available at the end of this chapter.

To the present date, 25 of the cited trials are already finished, 12 are ongoing, and two will begin in 2021. Twelve of these studies tested VitC alone, with no other experimental therapeutics except the usually applied in sepsis cases (i.e., antimicrobial and fluid therapy, vasopressors, and inotropic agents). Seventeen trials used a combination of hydrocortisone, ascorbate, and thiamine (HAT).

Ten studies experimented a combination of VitC with a corticosteroid only (2 trials) or VitC with VitB1 (5 trials) or VitC in combination with some other therapeutic agent (3 trials). Even if there is no consensus about intravenous doses to be used in critically ill patients, 23 of the 39 trials employed 6 g/day doses, mostly in a 6 h-interval regimen (1.5 g each). Five studies used doses below 6 g/day, and nine studies used doses above 6 g/day, mostly in a protocol of 200 mg/kg/day in a 6 h interval regimen (about 14 g/day to a 70 kg patient).

Sadly, from the 25 already finished trials, only 5 reported their results to clinicaltrials.gov or published them in a peer-reviewed journal. One of those was a pharmacokinetic study [84], so no outcomes were evaluated. The other four studies that reported results were called REDOXS [85], ORANGES [86], VITAMINS [87], and CITRIS-ALI [88].

REDOXS used ascorbate in 1.5 g/day dose administered enterally associated with glutamine and other antioxidants. The study was planned to evaluate glutamine associated with a pool of antioxidants effect on critically ill patients. Results reported no difference when compared to placebo for the primary endpoint (28-day mortality rate).

ORANGES was a study intended to evaluate the HAT protocol in septic patients. They evaluated almost 70 patients (in each group) in a protocol that involved 6 g/day ascorbate (1.5 g per dose) for a maximum of 4 days after ICU admission. The study concluded that HAT could decrease the duration of shock, but not the 28-day mortality rate in patients with sepsis, probably due to ascorbate administration (they had an arm of the study that received only corticosteroids).

VITAMINS used the same HAT and ascorbate dosage as described above. They evaluated about 100 patients (in each group). The difference between the ORANGES trial is the control group. While ORANGES intervention in control was essentially placebo, the VITAMINS used a corticoid and thiamine (when clinicians evaluated its need). VITAMINS results indicate that treatment with intravenous ascorbate, hydrocortisone, and thiamine, did not significantly improve the duration of mortality rate and discontinuation of vasopressor administration over seven days.

The CITRIS-ALI trial evaluated the administration of VitC alone in sepsis, associated with acute respiratory distress syndrome (ARDS) patients, in a dose of 200 mg/kg/day (about 14 g/day to a 70 kg patient). The primary outcome evaluated

the change in the Sequential Sepsis Related Organ Failure Score (SOFA) and two plasma biomarkers (C-reactive protein and thrombomodulin). The study assessed groups of about 80 patients. Circa 65% of patients (from both control and treatment groups) received corticosteroids during the study, and the mortality rate was significantly lower in the VitC group. However, since this outcome was not a primary outcome, the authors did not consider it in this study. Authors concluded that patients with sepsis and ARDS did not have an improvement in organ dysfunction scores, nor did they have altered markers of inflammation and vascular injury after a 96-hour infusion of vitamin C compared with placebo.

Outside the clinicaltrial.gov, several studies have investigated the use of IV ascorbate in critically ill patients. Cases of trauma, severe burn, and septic shock were evaluated, in various dosage schemes, from 7 g until 110 g/day. No severe adverse effects related to the vitamin C infusion were reported in any of the studies. A decrease in the incidence of multiple system organ failure, trends to reduced mortality, and ICU stay length was the usual results achieved [89–91].

One of the most commented studies about the effects of the HAT approach, and maybe the reference for several of the clinical trials, was published by Dr. Marik from Eastern Virginia Medical School in 2017 [92]. This study proposed the early HAT protocol using ascorbate IV (1.5 g every 6 h for 4 days or until ICU discharge), hydrocortisone (50 mg every 6 h for 7 days or until ICU discharge), as well as IV thiamine (200 mg every 12 h for 4 days or until ICU discharge). VitC is administered as an infusion over 30 to 60 min and mixed in a 100 mL solution of either dextrose 5% in water or normal saline. Dr. Marik's results showed that early use of intravenous VitC, with hydrocortisone and thiamine, would be used effectively to prevent progressive organ impairment, including acute kidney damage, and reduce patients' mortality with severe sepsis and septic shock. However, the published work evaluated a small sample, and as the authors say at the end of the manuscript, additional studies are required to confirm their preliminary findings [92].

High doses of IV ascorbate, thiamine, and glucocorticoids can reduce pro-inflammatory mediators, ROS, and decrease immunosuppression. Thiamine is useful to energy production as a precursor of thiamine pyrophosphate and acts as an antioxidant. Thiamine is essential because ascorbate may cause oxalate accumulation in the kidneys, and the concomitant use can prevent it since thiamine pyrophosphate is a cofactor required for the oxidation of glyoxylate to carbon dioxide by the enzyme glyoxylate aminotransferase. Thiamine deficiency increases the conversion of glyoxylate to oxalate. At the same time, thiamine deficiency is common in septic patients and is associated with an increased risk of death [61, 93].

The VitC in critically ill patients is still a dilemma to be solved. There is a rationale behind its use that seems to be optimal. HAT therapy's premise is the use of a combination of drugs that aim at multiple sectors of the patient's response to an infectious agent, synergistically restoring the impaired immune system, avoid damage due to oxidants, and restore mitochondrial activity. However, to evaluate the clinical features and impact of this scheme, most of the studies performed were small, doses used between trials were highly different, and the risk of bias was usually uncertain or high. Secondary outcomes need bigger sample sizes, and so were yet harder to evaluate. The studies' duration was not uniform, so the follow-up and comparison analysis were possible only to the longest available time in each trial. Finally, the heterogeneity between treatment schemes made comparisons hard. Isolated analysis of VitC ignores any synergistic effects that could be seen with HAT therapy [94].

4. Conclusions

Vitamin C is a powerful antioxidant that takes part in many vital biological processes. Due to its properties, it has been proposed that VitC could improve sepsis and septic shock symptoms. Because of its pharmacokinetics, it is imperative that ascorbic acid is administered IV in high dosage to explore its full potential in sepsis. Furthermore, the inclusion of hydrocortisone and thiamine to compose the HAT protocol has shown to improve patients outcomes in some clinical trials. Nevertheless, there is still much debate on whether the HAT protocol can actually exert this improvement. To further investigate this proposal, trials should increase sample sizes and come to an agreement on treatment schemes so they can be accurately compared, in addition to sharing the results of the research on Clinical Trials.

Acknowledgements

JChem for Word was used for **Figure 3**, Product version 20.21.0.768, ChemAxon (https://www.chemaxon.com). All other images were created with BioRender.com

Conflict of interest

The authors declare no conflict of interest.

Appendix

Trial name	Internet link to the trial
High-dose Intravenous Vitamin C as an Adjunctive Treatment for Sepsis in Rwanda	https://clinicaltrials.gov/ct2/show/NCT04088591
Outcome Following Vitamin C Administration in Sepsis	https://clinicaltrials.gov/ct2/show/NCT01590303
VICTAS Vitamin C, Thiamine, and Steroids in Sepsis	https://clinicaltrials.gov/ct2/show/study/NCT03509350
Hydrocortisone, Vitamin C, and Thiamine for the Treatment of Sepsis and Septic Shock	https://clinicaltrials.gov/ct2/show/NCT03258684
Therapy With Hydrocortisone, Ascorbic Acid, Thamine in Patients With Sepsis	https://clinicaltrials.gov/ct2/show/NCT04160676
Vitamin C & Thiamine in Sepsis	https://clinicaltrials.gov/ct2/show/NCT03592277
Vitamin C Infusion for Treatment in Sepsis and Alcoholic Hepatitis	https://clinicaltrials.gov/ct2/show/NCT03829683
Vitamin C, Vitamin B1 and Steroid in Sepsis	https://clinicaltrials.gov/ct2/show/NCT04039815
Effect of Intravenous Vitamin Con SOFA Score Among Septic Patients	https://clinicaltrials.gov/ct2/show/NCT04137276
Ascorbic Acid, Corticosteroids, and Thiamine in Sepsis (ACTS) Trial	https://clinicaltrials.gov/ct2/show/NCT03389555
Pilot Study on the Use of Hydrocortisone, Vitamin c and Thiamine in Patient With Sepsis and Septic Shock	https://clinicaltrials.gov/ct2/show/NCT04111822
Vitamin C, Thiamine, Cyanocobalamine, Pyridoxine and Hydrocortisone in Sepsis	https://clinicaltrials.gov/ct2/show/NCT04197115

Trial name	Internet link to the trial
High Dose of Vitamin C on Mechanically Ventilated Septic Patients in Intensive Care Unit	https://clinicaltrials.gov/ct2/show/NCT04029675
Ascorbic Acid (Vitamin C) Infusion in Human Sepsis	https://clinicaltrials.gov/ct2/show/NCT01434121
The Effect of Vitamin C, Thiamine and Hydrocortisone on Clinical Course and Outcome in Patients With Severe Sepsis and Septic Shock	https://clinicaltrials.gov/ct2/show/NCT03335124
Effect of Anti-inflammatory and Anti-microbial Cosupplementations in Traumatic ICU Patients at High Risk of Sepsis	https://clinicaltrials.gov/ct2/show/NCT04216459
Ascorbic Acid and Thiamine Effect in Septic Shock	https://clinicaltrials.gov/ct2/show/NCT03756220
ASTER (Acetaminophen and Ascorbate in Sepsis: Targeted Therapy to Enhance Recovery)	https://clinicaltrials.gov/ct2/show/study/NCT04291508
ViCiS (Vitamin C to Reduce Vasopressor Dose in Septic Shock)	https://clinicaltrials.gov/ct2/show/NCT03835286
Vitamin C and Septic Shock	https://clinicaltrials.gov/ct2/show/NCT03338569
Comparative, Between Triple Therapy Regimen to Hydrocortisone Monotherapy in Reducing the MR in Septic Shock Patients	https://clinicaltrials.gov/ct2/show/study/NCT04508946
Outcomes of Septic Shock Patients Treated With a Metabolic Resuscitation Bundle Consisting of Intravenous Hydrocortisone, Ascorbic Acid and Thiamine	https://clinicaltrials.gov/ct2/show/NCT03913468
LOVIT (Lessening Organ Dysfunction With Vitamin C)	https://clinicaltrials.gov/ct2/show/NCT03680274
Vitamin C, Thiamine and Hydrocortisone for the Treatment of Septic Shock	https://clinicaltrials.gov/ct2/show/NCT03872011
CORVICTES (Vitamin C, Hydrocortisone and Thiamine for Septic Shock)	https://clinicaltrials.gov/ct2/show/NCT03592693
CORVICTES-ΥΜ (Vitamin C, Steroids, and Thiamine, and Cerebral Autoregulation and Functional Outcome in Septic Shock)	https://clinicaltrials.gov/ct2/show/NCT03649633
Effect of IV Vitamin C, Thiamine, and Steroids on Mortality of Septic Shock	https://clinicaltrials.gov/ct2/show/NCT03828929
Thiamine, Vitamin C and Hydrocortisone in the Treatment of Septic Shock	https://clinicaltrials.gov/ct2/show/NCT03540628
Effects of Glucocorticoid Combined With Vitamin C and Vitamin B1 on Microcirculation in Severe Septic Shock	https://clinicaltrials.gov/ct2/show/NCT03821714
Clinical Trial of Antioxidant Therapy in Patients With Septic Shock	https://clinicaltrials.gov/ct2/show/NCT03557229
STASIS (Steroids, Thiamine and Ascorbic Acid in Septic Shock)	https://clinicaltrials.gov/ct2/show/NCT04134403
HYVITS (Evaluation of Hydrocortisone, Vitamin C and Thiamine for the Treatment of Septic Shock)	https://clinicaltrials.gov/ct2/show/NCT0338050
AVoCaDO (Administration of Intravenous Vitamin C in Novel Coronavirus Infection (COVID-19) and Decreased Oxygenation)	https://clinicaltrials.gov/ct2/show/NCT04357782

Trial name	Internet link to the trial
REDOXS (Trial of Glutamine and Antioxidant Supplementation in Critically Ill Patients)	https://clinicaltrials.gov/ct2/show/study/NCT00133978
Pharmacokinetics of Two Different High-dose Regimens of Intravenous Vitamin C in Critically Ill Patients	https://clinicaltrials.gov/ct2/show/study/NCT02455180
High Dose Intravenous Ascorbic Acid in Severe Sepsis	https://clinicaltrials.gov/ct2/show/results/NCT02734147
ORANGES - Metabolic Resuscitation Using Ascorbic Acid, Thiamine, and Glucocorticoids in Sepsis.	https://clinicaltrials.gov/ct2/show/NCT03422159
VITAMINS The Vitamin C, Hydrocortisone and Thiamine in Patients With Septic Shock Trial	https://clinicaltrials.gov/ct2/show/NCT03333278
CITRIS-ALI Vitamin C Infusion for Treatment in Sepsis Induced Acute Lung Injury	https://clinicaltrials.gov/ct2/show/study/NCT02106975

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References

[1] WHO. Global report on the epidemiology and burden of sepsis: current evidence, identifying gaps and future directions. WHO. 2020. 56 p.

[2] Cecconi M, Evans L, Levy M, Rhodes A. Sepsis and septic shock. Lancet (London, England). 2018;392(10141):75-87. DOI: 10.1016/S0140-6736(18)30696-2

[3] Bone RC. Why sepsis trials fail. JAMA. 1996;276(7):565-566.

[4] Hotchkiss RS, Karl IE. The pathophysiology and treatment of sepsis. N Engl J Med. 2003;348(2):138-150. DOI: 10.1056/NEJMra021333

[5] Venet F, Chung C-S, Kherouf H, Geeraert A, Malcus C, Poitevin F, et al. Increased circulating regulatory T cells (CD4(+)CD25 (+)CD127 (−)) contribute to lymphocyte anergy in septic shock patients. Intensive Care Med. 2009;35(4):678-686. DOI: 10.1007/s00134-008-1337-8

[6] Kellum JA, Kong L, Fink MP, Weissfeld LA, Yealy DM, Pinsky MR, et al. Understanding the inflammatory cytokine response in pneumonia and sepsis: results of the Genetic and Inflammatory Markers of Sepsis (GenIMS) Study. Arch Intern Med. 2007;167(15):1655-1663. DOI: 10.1001/archinte.167.15.1655

[7] Boyd JH, Russell JA, Fjell CD. The meta-genome of sepsis: host genetics, pathogens and the acute immune response. J Innate Immun. 2014;6(3):272-283. DOI: 10.1159/000358835

[8] Abbas AK, Murphy KM, Sher A. Functional diversity of helper T lymphocytes. Nature. 1996;383(6603):787-793. DOI: 10.1038/383787a0

[9] Kim D-M, Jung S-I, Jang H-C, Lee CS, Lee SH, Yun NR, et al. Vibrio vulnificus DNA load and mortality. J Clin Microbiol. 2011;49(1):413-415. DOI: 10.1128/JCM.01913-09

[10] Darton T, Guiver M, Naylor S, Jack DL, Kaczmarski EB, Borrow R, et al. Severity of meningococcal disease associated with genomic bacterial load. Clin Infect Dis an Off Publ Infect Dis Soc Am. 2009;48(5):587-594. DOI: 10.1086/596707

[11] Hackett SJ, Guiver M, Marsh J, Sills JA, Thomson APJ, Kaczmarski EB, et al. Meningococcal bacterial DNA load at presentation correlates with disease severity. Arch Dis Child. 2002;86(1):44-46. DOI: 10.1136/adc.86.1.44

[12] Rhodes A, Evans LE, Alhazzani W, Levy MM, Antonelli M, Ferrer R, et al. Surviving Sepsis Campaign: International Guidelines for Management of Sepsis and Septic Shock: 2016. Intensive Care Med. 2017;43(3):304-377. DOI: 10.1007/s00134-017-4683-6

[13] Dugar S, Choudhary C, Duggal A. Sepsis and septic shock: Guideline-based management. Cleve Clin J Med. 2020;87(1):53-64. DOI: 10.3949/ccjm.87a.18143

[14] Annane D, Bellissant E, Bollaert PE, Briegel J, Keh D, Kupfer Y, et al. Corticosteroids for treating sepsis in children and adults. Cochrane database Syst Rev. 2019;12(12):CD002243. DOI: 10.1002/14651858.CD002243.pub4

[15] Rochwerg B, Oczkowski SJ, Siemieniuk RAC, Agoritsas T, Belley-Cote E, D'Aragon F, et al. Corticosteroids in Sepsis: An Updated Systematic Review and Meta-Analysis. Crit Care Med. 2018;46(9):1411-1420. DOI: 10.1097/CCM.0000000000003262

[16] Bollaert PE, Charpentier C, Levy B, Debouverie M, Audibert G, Larcan A. Reversal of late septic shock with supraphysiologic doses of hydrocortisone. Crit Care Med. 1998;26(4):645-650. DOI: 10.1097/00003246-199804000-00010

[17] Briegel J, Forst H, Haller M, Schelling G, Kilger E, Kuprat G, et al. Stress doses of hydrocortisone reverse hyperdynamic septic shock: a prospective, randomized, double-blind, single-center study. Crit Care Med. 1999;27(4):723-732. DOI: 10.1097/00003246-199904000-00025

[18] Sprung CL, Annane D, Keh D, Moreno R, Singer M, Freivogel K, et al. Hydrocortisone therapy for patients with septic shock. N Engl J Med. 2008;358(2):111-124. DOI: 10.1056/NEJMoa071366

[19] Sligl WI, Milner DAJ, Sundar S, Mphatswe W, Majumdar SR. Safety and efficacy of corticosteroids for the treatment of septic shock: A systematic review and meta-analysis. Clin Infect Dis an Off Publ Infect Dis Soc Am. 2009;49(1):93-101. DOI: 10.1086/599343

[20] Annane D, Bellissant E, Bollaert PE, Briegel J, Keh D, Kupfer Y. Corticosteroids for treating sepsis. Cochrane database Syst Rev. 2015;2015(12):CD002243. DOI: 10.1002/14651858.CD002243.pub3

[21] Warren BL, Eid A, Singer P, Pillay SS, Carl P, Novak I, et al. Caring for the critically ill patient. High-dose antithrombin III in severe sepsis: a randomized controlled trial. JAMA. 2001;286(15):1869-1878. DOI: 10.1001/jama.286.15.1869

[22] Allingstrup M, Wetterslev J, Ravn FB, Møller AM, Afshari A. Antithrombin III for critically ill patients: a systematic review with meta-analysis and trial sequential analysis. Intensive Care Med. 2016;42(4):505-520. DOI: 10.1007/s00134-016-4225-7

[23] Vincent J-L, Ramesh MK, Ernest D, LaRosa SP, Pachl J, Aikawa N, et al. A randomized, double-blind, placebo-controlled, Phase 2b study to evaluate the safety and efficacy of recombinant human soluble thrombomodulin, ART-123, in patients with sepsis and suspected disseminated intravascular coagulation. Crit Care Med. 2013;41(9):2069-2079. DOI: 10.1097/CCM.0b013e31828e9b03

[24] Zarychanski R, Abou-Setta AM, Kanji S, Turgeon AF, Kumar A, Houston DS, et al. The efficacy and safety of heparin in patients with sepsis: a systematic review and metaanalysis. Crit Care Med. 2015;43(3):511-518. DOI: 10.1097/CCM.0000000000000763

[25] Alejandria MM, Lansang MAD, Dans LF, Mantaring JB 3rd. Intravenous immunoglobulin for treating sepsis, severe sepsis and septic shock. Cochrane database Syst Rev. 2013;2013(9):CD001090. DOI: 10.1002/14651858.CD001090.pub2

[26] Laupland KB, Kirkpatrick AW, Delaney A. Polyclonal intravenous immunoglobulin for the treatment of severe sepsis and septic shock in critically ill adults: a systematic review and meta-analysis. Crit Care Med. 2007;35(12):2686-2692.

[27] Kreymann KG, de Heer G, Nierhaus A, Kluge S. Use of polyclonal immunoglobulins as adjunctive therapy for sepsis or septic shock. Crit Care Med. 2007;35(12):2677-2685.

[28] Soares MO, Welton NJ, Harrison DA, Peura P, Shankar- Hari M, Harvey SE, et al. An evaluation of the feasibility, cost and value of information of a multicentre randomised controlled trial of intravenous immunoglobulin for sepsis (severe sepsis and septic shock): incorporating a systematic review,

meta-analysis and value of informat. Health Technol Assess. 2012;16(7):1-186. DOI: 10.3310/hta16070

[29] Werdan K, Pilz G, Bujdoso O, Fraunberger P, Neeser G, Schmieder RE, et al. Score-based immunoglobulin G therapy of patients with sepsis: the SBITS study. Crit Care Med. 2007;35(12):2693-2701.

[30] Getoff N. Vitamin C: electron emission, free radicals and biological versatility. In Vivo. 2013;27(5):565-570.

[31] Marik PE. Hydrocortisone, Ascorbic Acid and Thiamine (HAT Therapy) for the Treatment of Sepsis. Focus on Ascorbic Acid. Nutrients. 2018;10(11). DOI: 10.3390/nu10111762

[32] Tur'yan YI, Kohen R. Formal redox potentials of the dehydro-l-ascorbic acid/l-ascorbic acid system. J Electroanal Chem. 1995;380(1):273-277. DOI: Https://doi.org/10.1016/0022-0728(94)03524-7

[33] Rebouche CJ. Ascorbic acid and carnitine biosynthesis. Am J Clin Nutr. 1991;54(6 Suppl):1147S–1152S. DOI: 10.1093/ajcn/54.6.1147s

[34] Dunn WA, Rettura G, Seifter E, Englard S. Carnitine biosynthesis from gamma-butyrobetaine and from exogenous protein-bound 6-N-trimethyl-L-lysine by the perfused guinea pig liver. Effect of ascorbate deficiency on the in situ activity of gamma-butyrobetaine hydroxylase. J Biol Chem. 1984;259(17):10764-10770.

[35] Levine M, Dhariwal KR, Washko P, Welch R, Wang YH, Cantilena CC, et al. Ascorbic acid and reaction kinetics in situ: a new approach to vitamin requirements. J Nutr Sci Vitaminol (Tokyo). 1992;Spec No:169-172. DOI: 10.3177/jnsv.38.special_169

[36] Kaufman S. Dopamine-beta-hydroxylase. J Psychiatr Res. 1974;11:303-316. DOI: 10.1016/0022-3956(74)90112-5

[37] Eipper BA, Milgram SL, Husten EJ, Yun HY, Mains RE. Peptidylglycine alpha-amidating monooxygenase: a multifunctional protein with catalytic, processing, and routing domains. Protein Sci. 1993;2(4):489-497. DOI: 10.1002/pro.5560020401

[38] Eipper BA, Stoffers DA, Mains RE. The biosynthesis of neuropeptides: peptide alpha-amidation. Annu Rev Neurosci. 1992;15:57-85. DOI: 10.1146/annurev.ne.15.030192.000421

[39] Englard S, Seifter S. The biochemical functions of ascorbic acid. Annu Rev Nutr. 1986;6:365-406. DOI: 10.1146/annurev.nu.06.070186.002053

[40] Lindblad B, Lindstedt G, Lindstedt S. The mechanism of enzymic formation of homogentisate from p-hydroxyphenylpyruvate. J Am Chem Soc. 1970;92(25):7446-7449. DOI: 10.1021/ja00728a032

[41] Prockop DJ, Kivirikko KI. Collagens: molecular biology, diseases, and potentials for therapy. Annu Rev Biochem. 1995;64:403-434. DOI: 10.1146/annurev.bi.64.070195.002155

[42] Peterkofsky B. Ascorbate requirement for hydroxylation and secretion of procollagen: relationship to inhibition of collagen synthesis in scurvy. Am J Clin Nutr. 1991;54(6 Suppl):1135S–1140S. DOI: 10.1093/ajcn/54.6.1135s

[43] Kivirikko KI, Myllylä R. Post-translational processing of procollagens. Ann N Y Acad Sci. 1985;460:187-201. DOI: 10.1111/j.1749-6632.1985.tb51167.x

[44] Hallberg L, Brune M, Rossander L. The role of vitamin C in iron absorption. Int J Vitam Nutr Res Suppl = Int Zeitschrift fur Vitamin- und

Ernahrungsforschung Suppl. 1989;30:103-108.

[45] Padayatty SJ, Katz A, Wang Y, Eck P, Kwon O, Lee J-H, et al. Vitamin C as an antioxidant: evaluation of its role in disease prevention. J Am Coll Nutr. 2003;22(1):18-35. DOI: 10.1080/07315724.2003.10719272

[46] Padayatty SJ, Levine M. Vitamin C: the known and the unknown and Goldilocks. Oral Dis. 2016;22(6):463-493. DOI: 10.1111/odi.12446

[47] Moser MA, Chun OK. Vitamin C and Heart Health: A Review Based on Findings from Epidemiologic Studies. Int J Mol Sci. 2016;17(8). DOI: 10.3390/ijms17081328

[48] Lee D-H, Folsom AR, Harnack L, Halliwell B, Jacobs DRJ. Does supplemental vitamin C increase cardiovascular disease risk in women with diabetes? Am J Clin Nutr. 2004;80(5):1194-1200. DOI: 10.1093/ajcn/80.5.1194

[49] Granger M, Eck P. Dietary Vitamin C in Human Health. Adv Food Nutr Res. 2018;83:281-310. DOI: 10.1016/bs.afnr.2017.11.006

[50] Moritz B, Schmitz AE, Rodrigues ALS, Dafre AL, Cunha MP. The role of vitamin C in stress-related disorders. J Nutr Biochem. 2020;85:108459. DOI: 10.1016/j.jnutbio.2020.108459

[51] Wong SK, Chin K-Y, Ima-Nirwana S. Vitamin C: A Review on its Role in the Management of Metabolic Syndrome. Int J Med Sci. 2020;17(11):1625-1638. DOI: 10.7150/ijms.47103

[52] Hemilä H, Chalker E. Vitamin C for preventing and treating the common cold. Cochrane database Syst Rev. 2013;(1):CD000980. DOI: 10.1002/14651858.CD000980.pub4

[53] Rondanelli M, Miccono A, Lamburghini S, Avanzato I, Riva A, Allegrini P, et al. Self-Care for Common Colds: The Pivotal Role of Vitamin D, Vitamin C, Zinc, and Echinacea in Three Main Immune Interactive Clusters (Physical Barriers, Innate and Adaptive Immunity) Involved during an Episode of Common Colds-Practical Advice on Dosages a. Evid Based Complement Alternat Med. 2018;2018:5813095. DOI: 10.1155/2018/5813095

[54] Carr AC, Maggini S. Vitamin C and Immune Function. Nutrients. 2017;9(11). DOI: 10.3390/nu9111211

[55] Padhani ZA, Moazzam Z, Ashraf A, Bilal H, Salam RA, Das JK, et al. Vitamin C supplementation for prevention and treatment of pneumonia. Cochrane database Syst Rev. 2020;4(4):CD013134. DOI: 10.1002/14651858.CD013134.pub2

[56] Righi NC, Schuch FB, De Nardi AT, Pippi CM, de Almeida Righi G, Puntel GO, et al. Effects of vitamin C on oxidative stress, inflammation, muscle soreness, and strength following acute exercise: meta-analyses of randomized clinical trials. Eur J Nutr. 2020;59(7):2827-2839. DOI: 10.1007/s00394-020-02215-2

[57] Kaźmierczak-Barańska J, Boguszewska K, Adamus-Grabicka A, Karwowski BT. Two Faces of Vitamin C-Antioxidative and Pro-Oxidative Agent. Nutrients. 2020;12(5). DOI: 10.3390/nu12051501

[58] van Gorkom GNY, Lookermans EL, Van Elssen CHMJ, Bos GMJ. The Effect of Vitamin C (Ascorbic Acid) in the Treatment of Patients with Cancer: A Systematic Review. Nutrients. 2019;11(5). DOI: 10.3390/nu11050977

[59] Lykkesfeldt J. On the effect of vitamin C intake on human health: How to (mis)interprete the clinical evidence. Redox Biol. 2020;34:101532. DOI: 10.1016/j.redox.2020.101532

[60] Spoelstra-de Man AME, Elbers PWG, Oudemans-Van Straaten HM. Vitamin C: should we supplement? Curr Opin Crit Care. 2018;24(4):248-255. DOI: 10.1097/MCC.0000000000000510

[61] Marik PE. Vitamin C for the treatment of sepsis: The scientific rationale. Pharmacol Ther. 2018;189:63-70. DOI: 10.1016/j.pharmthera.2018.04.007

[62] Lykkesfeldt J, Tveden-Nyborg P. The Pharmacokinetics of Vitamin C. Nutrients. 2019;11(10). DOI: 10.3390/nu11102412

[63] Tsukaguchi H, Tokui T, Mackenzie B, Berger U V, Chen XZ, Wang Y, et al. A family of mammalian Na+−dependent L-ascorbic acid transporters. Nature. 1999;399(6731):70-75. DOI: 10.1038/19986

[64] Lindblad M, Tveden-Nyborg P, Lykkesfeldt J. Regulation of vitamin C homeostasis during deficiency. Nutrients. 2013;5(8):2860-2879. DOI: 10.3390/nu5082860

[65] Wilson JX. Regulation of vitamin C transport. Annu Rev Nutr. 2005;25:105-125. DOI: 10.1146/annurev.nutr.25.050304.092647

[66] Padayatty SJ, Sun H, Wang Y, Riordan HD, Hewitt SM, Katz A, et al. Vitamin C pharmacokinetics: implications for oral and intravenous use. Ann Intern Med. 2004;140(7):533-537. DOI: 10.7326/0003-4819-140-7-200404060-00010

[67] Davies MB, Austin J, Partridge DA. Vitamin C: Its Chemistry and Biochemistry. Royal Society of Chemistry; 1991. 153 p.

[68] Levine M, Conry-Cantilena C, Wang Y, Welch RW, Washko PW, Dhariwal KR, et al. Vitamin C pharmacokinetics in healthy volunteers: evidence for a recommended dietary allowance. Proc Natl Acad Sci U S A. 1996;93(8):3704-3709. DOI: 10.1073/pnas.93.8.3704

[69] Nielsen TK, Højgaard M, Andersen JT, Poulsen HE, Lykkesfeldt J, Mikines KJ. Elimination of ascorbic acid after high-dose infusion in prostate cancer patients: a pharmacokinetic evaluation. Basic & Clin Pharmacol & Toxicol. 2015;116(4):343—348. DOI: 10.1111/bcpt.12323

[70] Stephenson CM, Levin RD, Spector T, Lis CG. Phase I clinical trial to evaluate the safety, tolerability, and pharmacokinetics of high-dose intravenous ascorbic acid in patients with advanced cancer. Cancer Chemother Pharmacol. 2013;72(1):139-146. DOI: 10.1007/s00280-013-2179-9

[71] Ou J, Zhu X, Lu Y, Zhao C, Zhang H, Wang X, et al. The safety and pharmacokinetics of high dose intravenous ascorbic acid synergy with modulated electrohyperthermia in Chinese patients with stage III-IV non-small cell lung cancer. Eur J Pharm Sci Off J Eur Fed Pharm Sci. 2017;109:412-418. DOI: 10.1016/j.ejps.2017.08.011

[72] Carr AC, Rosengrave PC, Bayer S, Chambers S, Mehrtens J, Shaw GM. Hypovitaminosis C and vitamin C deficiency in critically ill patients despite recommended enteral and parenteral intakes. Crit Care. 2017;21(1):300. DOI: 10.1186/s13054-017-1891-y

[73] Carr AC, Shaw GM, Fowler AA, Natarajan R. Ascorbate-dependent vasopressor synthesis: a rationale for vitamin C administration in severe sepsis and septic shock? Crit Care. 2015;19:418. DOI: 10.1186/s13054-015-1131-2

[74] Langley RJ, Tipper JL, Bruse S, Baron RM, Tsalik EL, Huntley J, et al.

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(4):445-455. DOI: 10.1164/rccm.201404-0624OC

[75] Rumsey SC, Kwon O, Xu GW, Burant CF, Simpson I, Levine M. Glucose transporter isoforms GLUT1 and GLUT3 transport dehydroascorbic acid. J Biol Chem. 1997;272(30):18982-18989. DOI: 10.1074/jbc.272.30.18982

[76] Lowes DA, Webster NR, Galley HF. Dehydroascorbic acid as pre-conditioner: protection from lipopolysaccharide induced mitochondrial damage. Free Radic Res. 2010;44(3):283-292. DOI: 10.3109/10715760903468766

[77] Langley RJ, Tsalik EL, van Velkinburgh JC, Glickman SW, Rice BJ, Wang C, et al. An integrated clinico-metabolomic model improves prediction of death in sepsis. Sci Transl Med. 2013;5(195):195ra95. DOI: 10.1126/scitranslmed.3005893

[78] Haileselassie B, Su E, Pozios I, Niño DF, Liu H, Lu D-Y, et al. Myocardial oxidative stress correlates with left ventricular dysfunction on strain echocardiography in a rodent model of sepsis. Intensive care Med Exp. 2017;5(1):21. DOI: 10.1186/s40635-017-0134-5

[79] Hao J, Li W-W, Du H, Zhao Z-F, Liu F, Lu J-C, et al. Role of Vitamin C in Cardioprotection of Ischemia/Reperfusion Injury by Activation of Mitochondrial KATP Channel. Chem Pharm Bull (Tokyo). 2016;64(6):548-557. DOI: 10.1248/cpb.c15-00693

[80] Fisher BJ, Kraskauskas D, Martin EJ, Farkas D, Puri P, Massey HD, et al. Attenuation of sepsis-induced organ injury in mice by vitamin C. JPEN J Parenter Enteral Nutr. 2014;38(7):825-839. DOI: 10.1177/0148607113497760

[81] Cárcamo JM, Pedraza A, Bórquez-Ojeda O, Golde DW. Vitamin C suppresses TNF alpha-induced NF kappa B activation by inhibiting I kappa B alpha phosphorylation. Biochemistry. 2002;41(43):12995-13002. DOI: 10.1021/bi0263210

[82] Kim SR, Ha YM, Kim YM, Park EJ, Kim JW, Park SW, et al. 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(4):279-289. DOI: 10.1016/j.bcp.2015.04.007

[83] Hattori M, Yamazaki M, Ohashi W, Tanaka S, Hattori K, Todoroki K, et al. Critical role of endogenous histamine in promoting end-organ tissue injury in sepsis. Intensive care Med Exp. 2016;4(1):36. DOI: 10.1186/s40635-016-0109-y

[84] de Grooth H-J, Manubulu-Choo W-P, Zandvliet AS, Spoelstra-de Man AME, Girbes AR, Swart EL, et al. Vitamin C Pharmacokinetics in Critically Ill Patients: A Randomized Trial of Four IV Regimens. Chest. 2018;153(6):1368-1377. DOI: 10.1016/j.chest.2018.02.025

[85] Heyland D, Muscedere J, Wischmeyer PE, Cook D, Jones G, Albert M, et al. A randomized trial of glutamine and antioxidants in critically ill patients. N Engl J Med. 2013;368(16):1489-1497. DOI: 10.1056/NEJMoa1212722

[86] Iglesias J, Vassallo A V, Patel V V, Sullivan JB, Cavanaugh J, Elbaga Y. Outcomes of Metabolic Resuscitation Using Ascorbic Acid, Thiamine, and Glucocorticoids in the Early Treatment of Sepsis: The ORANGES Trial. Chest.

2020;158(1):164-173. DOI: 10.1016/j.chest.2020.02.049

[87] Fujii T, Luethi N, Young PJ, Frei DR, Eastwood GM, French CJ, et al. Effect of Vitamin C, Hydrocortisone, and Thiamine vs Hydrocortisone Alone on Time Alive and Free of Vasopressor Support Among Patients With Septic Shock: The VITAMINS Randomized Clinical Trial. JAMA. 2020;323(5):423-431. DOI: 10.1001/jama.2019.22176

[88] Fowler AA 3rd, Truwit JD, Hite RD, Morris PE, DeWilde C, Priday A, et al. Effect of Vitamin C Infusion on Organ Failure and Biomarkers of Inflammation and Vascular Injury in Patients With Sepsis and Severe Acute Respiratory Failure: The CITRIS-ALI Randomized Clinical Trial. JAMA. 2019;322(13):1261-1270. DOI: 10.1001/jama.2019.11825

[89] Nathens AB, Neff MJ, Jurkovich GJ, Klotz P, Farver K, Ruzinski JT, et al. Randomized, prospective trial of antioxidant supplementation in critically ill surgical patients. Ann Surg. 2002;236(6):814-822. DOI: 10.1097/00000658-200212000-00014

[90] 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(3):326-331. DOI: 10.1001/archsurg.135.3.326

[91] Zabet MH, Mohammadi M, Ramezani M, Khalili H. Effect of high-dose Ascorbic acid on vasopressor's requirement in septic shock. J Res Pharm Pract. 2016;5(2):94-100. DOI: 10.4103/2279-042X.179569

[92] Marik PE, Khangoora V, Rivera R, Hooper MH, Catravas J. Hydrocortisone, Vitamin C, and Thiamine for the Treatment of Severe Sepsis and Septic Shock: A Retrospective Before-After Study. Chest. 2017;151(6):1229-1238. DOI: 10.1016/j.chest.2016.11.036

[93] Mitchell AB, Ryan TE, Gillion AR, Wells LD, Muthiah MP. Vitamin C and Thiamine for Sepsis and Septic Shock. Am J Med. 2020;133(5):635-638. DOI: 10.1016/j.amjmed.2019.07.054

[94] Zhang M, Jativa DF. Vitamin C supplementation in the critically ill: A systematic review and meta-analysis. SAGE open Med. 2018;6:2050312118807615. DOI: 10.1177/2050312118807615