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Vitamin C for sepsis intervention: from redox biochemistry to clinical medicine

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Abstract

Vitamin C, also known as ascorbic acid or ascorbate, is a water-soluble vitamin synthesized in plants as well as in animals except humans and several other animal species. Humans obtain vitamin C from dietary sources and via vitamin supplementation. Vitamin C possesses important biological functions, including serving as a cofactor for many enzymes, acting as an antioxidant and anti-inflammatory compound, and participating in regulating stem cell biology and epigenetics. The multifunctional nature of vitamin C contributes to its essentialness in maintaining and safeguarding physiological homeostasis, especially regulation of immunity and inflammatory responses. In this context, vitamin C has been investigated for its efficacy in treating diverse inflammatory disorders, including sepsis, one of the major causes of death globally and for which currently there is no cure. Accordingly, this Mini-Review surveys recent major research findings on the effectiveness of vitamin C and the underling molecular mechanisms in sepsis intervention in both experimental animal models and randomized controlled trials. To set a stage for discussing the effects and mechanisms of vitamin C in sepsis intervention, this Mini-Review begins with an overview of vitamin C redox biochemistry and its multifunctional properties.

Keywords Ascorbic acid · Inflammation · Mitochondria · Oxidative stress · Randomized controlled trial · Sepsis · Septic shock · Redox homeostasis · Vitamin C

Abbreviations

Asc	Ascorbate
DHA	Dehydroascorbate
DHAR	Dehydroascorbate reductase
GLUT	Glucose transporter
GSH	Reduced form of glutathione
HAT	Hydrocortisone, ascorbic acid, and thiamine
HO-1	Heme oxygenase-1
iNOS	Inducible nitric oxide synthase
LPS	Lipopolysaccharide;
NOX	NADPH oxidase
NQO1	NADPH:quinone oxidoreductase 1
SVCT	Sodium-dependent vitamin C transporter
Tet	Ten eleven translocase

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Introduction

Vitamin C, also known as ascorbic acid or ascorbate, was discovered in 1928 by Szent-Györgyi [1], who was subsequently awarded the Nobel Prize in Physiology or Medicine in 1937 for his discoveries in connection with the biological combustion processes, with special reference to vitamin C and the catalysis of fumaric acid. In addition to being produced by plant cells, this water-soluble vitamin is also synthesized endogenously in animal species except humans, monkeys, bats, guinea pigs, and some reptiles [2]. Humans lost this capability because of a series of inactivating mutations of the gene encoding gulonolactone oxidase (GULO), a key enzyme for the biosynthesis of vitamin C [3]. Humans normally acquire vitamin C from dietary sources through a substrate-saturable transport mechanism and oral vitamin C intake produces plasma concentrations that are tightly regulated. Once the oral intake of vitamin C exceeds 200 mg daily, it is difficult to further raise the plasma vitamin C concentration through increasing the oral intake [4]. The maximal plasma concentration attainable by oral intake of vitamin C has been estimated to be about 200 μM though the physiological plasma concentrations of vitamin C in

healthy humans range from 40 to 100 µM [4, 5]. On the other hand, parenteral administration (e.g., via intravenous injection) of a large dose of vitamin C (e.g., 10 g) can yield millimolar concentrations of plasma vitamin C [6, 7], and this megadose-based strategy has been employed for cancer treatment (reviewed in [8]).

Despite the micromolar concentration range of vitamin C in the plasma under physiological conditions and with typical dietary intake of vitamin C, intracellular levels of vitamin C are in the millimolar range. This high concentration is due to selective intracellular accumulation via a vitamin C transport system present in the plasma membrane [9] (see section 'Intracellular dynamics of vitamin C' for more details). The high intracellular concentration of vitamin C in mammalian tissues is in line with its essential roles in maintaining physiological homeostasis (reviewed in [4, 5, 10]). In this Mini-Review, we first describe the novel redox properties and functions of vitamin C, and then discuss major recent research evidence supporting its multitasking functions in controlling oxidative stress and inflammatory responses and in treating sepsis, a major contributor to the global burden of disease.

Redox chemistry of vitamin C

On the one hand, the redox chemistry of vitamin C determines its biological functions. On the other hand, the biological milieu also influences the redox chemistry of vitamin C. In this context, vitamin C has been shown to exist in different redox forms in biological systems [11]. As illustrated in Fig. 1, vitamin C ($AscH_2$) has two ionizable hydroxyl groups. At a physiological pH, vitamin C exists predominantly as a monoanion, i.e., ascorbate monoanion ($AscH^-$). $AscH^-$ acts as a reducing agent and is converted to ascorbate radical ($Asc^{\cdot-}$, also known as semidehydroascorbate) after donating one-electron. After losing another electron, $Asc^{\cdot-}$ is converted to dehydroascorbate (DHA) [4]. DHA is commonly known as the oxidized form of vitamin C. Likewise, ascorbic acid ($AscH_2$ or $AscH^-$) is commonly known as the reduced form of vitamin C. Hence, vitamin C can be considered as a generic name referring to both ascorbic acid and DHA. For this reason, ascorbic acid or ascorbate is more commonly used in research literature as it specifies the redox form of the vitamin [4].

It should be noted that the above redox reactions are reversible. For example, $Asc^{\cdot-}$ can be reduced by one-electron to $AscH^-$. DHA can also be reduced by either one-electron to $Asc^{\cdot-}$ or by two electrons to $AscH^-$. The two-electron reduction of DHA to $AscH^-$ is catalyzed by DHA reductase (DHAR) using the reduced form of glutathione (GSH) as an electron donor [12]. The reduction of DHA to $AscH^-$ can be catalyzed also by the selenoenzyme, thioredoxin reductase [13], highlighting the intimate interaction between vitamin C and other cellular redox factors.

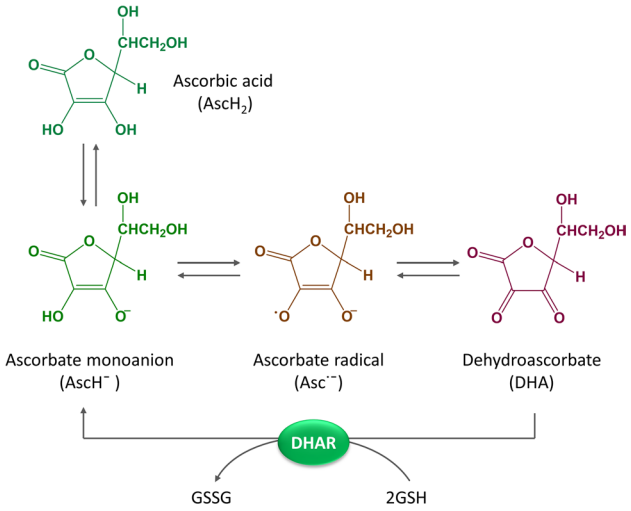

Fig. 1 Redox chemistry of vitamin C. As depicted, ascorbate can undergo two sequential one-electron oxidation reactions to form ascorbate radical and DHA, respectively. Conversely, DHA can undergo two sequential one-electron reductions to yield ascorbate radical and ascorbate, respectively. Moreover, DHA can be reduced by two electrons to form ascorbate, and this two-electron reduction reaction is catalyzed by DHA reductase (DHAR) using the reduced form of glutathione (GSH) as an electron donor. During the reaction, GSH is oxidized to glutathione disulfide (GSSG)

Intracellular dynamics of vitamin C

Specific transporting systems are involved in the absorption of vitamin C as well as its intracellular distribution (reviewed in [8, 10]). Historically, vitamin C transport across cell membranes had been an area of extensive research in neurobiology. In this context, the highest tissue concentrations of vitamin C are found in the brain and in neuroendocrine tissues, especially adrenal glands, which may range from 1 to 3 mM. These concentrations are 15–50 times higher than those in the plasma [14, 15], pointing to the existence of active transporting mechanisms. Early seminal work by Diliberto et al. showed that adrenomedullary cells accumulate vitamin C through a saturable and energy-dependent process and that the newly taken-up vitamin C is also secreted from the cells through specific transporter mechanisms [16–19]. It is now well-established that vitamin C enters and accumulates in neurons as well as other types of cells via two different transporting systems—(i) sodium-dependent vitamin C transporters and (ii) glucose transporters. As described below, utilization of the specific transporting systems also depends on the redox forms of vitamin C.

Transport of the reduced form of vitamin C

As reviewed previously [8], the reduced form of vitamin C is transported into cells via sodium-dependent vitamin C transporters (SVCT1 and SVCT2). SVCT1 (the product of the SLC23A1 gene in humans) is primarily expressed in intestinal and renal epithelial cells, where it helps mediate absorption and re-absorption of vitamin C, respectively. SVCT2 (the product of the SLC23A2 gene in humans) is found in cells of most other tissues. Both SVCT1 and SVCT2 mediate high affinity, sodium- and energy-dependent transport of vitamin C into cells and are essential to establish steep concentration gradients of vitamin C across the plasma membrane [14]. Notably, SVCTs, particularly SVCT2, also transport the reduced form of vitamin C from the cytosol into the mitochondrial matrix [20] (also reviewed in [21]).

Transport of the oxidized form of vitamin C

Fourteen glucose transporters (GLUTs) are expressed in the human, and they include transporters for substrates other than glucose, such as fructose, myoinositol, and urate [22, 23]. The primary physiological substrates for at least half of the 14 GLUT proteins are either uncertain or completely unknown. The four well-established GLUT isoforms, namely, GLUTs 1–4, have been demonstrated to have distinct regulatory and/or kinetic properties that reflect their specific roles in cellular and whole-body glucose homeostasis [22, 23]. Besides transporting glucose, GLUTs 1, 3, and 4, have been shown to also transport the oxidized form of vitamin C (i.e., DHA) from extracellular milieu into cells. It is noteworthy that, of all cell types, human erythrocytes express the highest level of GLUT1. However, glucose transport decreases during human erythropoiesis despite a more than 3-log increase in GLUT1 transcripts. In contrast, GLUT1-mediated transport of DHA is dramatically enhanced. Mechanistically, stomatin, an integral erythrocyte membrane protein, is responsible for regulating the switch from glucose to DHA transport [24].

Some GLUT isoforms are also expressed in the mitochondrial inner membrane. GLUT1 is the most extensively studied GLUT isoform in terms of mediating the transport of DHA into the mitochondrial matrix [25, 26]. In addition to GLUT1, a recent study demonstrated that GLUT10 is also expressed in mitochondria and may participate in transporting DHA from the cytosol into the mitochondrial matrix [27]. Once transported into the matrix, DHA is reduced to ascorbic acid (the reduced form of vitamin C) primarily by the mitochondrial electron transport chain [28]. The high concentrations of vitamin C in the mitochondrial matrix suggest a role for this molecule in maintaining mitochondrial redox homeostasis and function. Indeed, depletion of mitochondrial vitamin C causes mitochondrial oxidative stress and dysfunction, leading to neurodegeneration in an animal model of Alzheimer's disease [29]. On the other hand, selective targeting of vitamin C to mitochondrial compartment protects against oxidative stress in this organelle [30].

Vitamin C as a multifunctional molecule

As a cofactor for conventional enzymes

Vitamin C serves as a cofactor for multiple well-known enzymes in humans [4]. The most notable ones are proline hydroxylase and lysine hydroxylase, which are involved in collagen synthesis (reviewed in [31]). The other enzymes for which vitamin C acts as a cofactor are involved in carnitine synthesis, catecholamine synthesis, peptide amidation, and tyrosine metabolism [28]. Due to its essential role in collagen biosynthesis, deficiency of vitamin C compromises the integrity of blood vessels, leading to scorbutic gums and pinpoint hemorrhage, characteristic manifestations of vitamin C deficiency [32].

As an antioxidant

Vitamin C is notable for its antioxidative activity and, as such, is widely recognized as a natural antioxidant [33]. Mechanistically, as described below, vitamin C may fulfill its antioxidative function via four means: (i) directly scavenging free radicals and reactive oxygen/nitrogen species (ROS/RNS); (ii) downregulating ROS/RNS-generating enzymes; (iii) facilitating the action of other cellular antioxidants; and (iv) activating Nrf2 signaling.

Scavenging ROS/RNS

The unique redox chemistry of vitamin C renders it the readiness to directly react with free radicals and ROS/RNS [33]. In many in vitro systems, vitamin C has been found to quench free radicals and ROS/RNS and protect cells from oxidative damage. Accumulation of vitamin C in mitochondria, a major source of cellular ROS [34], is particularly important for counteracting cellular oxidative stress and inflammation. In this regard, mitochondrial ROS play a critical role in both oxidative stress injury [35, 36] and pro-inflammatory responses [37, 38].

Downregulating ROS/RNS-generating enzymes

Vitamin C inhibits the expression of NADPH oxidase (NOX) subunit $p47^{phox}$ induced by inflammatory insults, thereby decreasing the formation of ROS from this important cellular source [39]. Mechanistically, vitamin C reduces the oxidative activation of Jak2/Stat1/IRF1 pathway that is

involved in the inducible expression of $p47^{phox}$ [39]. In addition to NOX, the inducible expression of inducible nitric oxide synthase (iNOS) in septic mice is also inhibited by vitamin C [40].

Facilitating the action of other cellular antioxidants

Vitamin C acts to regenerate α-tocopherol and coenzyme Q from α-tocopherol radical and coenzyme Q radical, respectively, and thereby plays a critical role in maintaining the antioxidant activities of α-tocopherol and coenzyme Q, two important lipophilic antioxidants in cells [41]. Vitamin C is able to also reduce 1-Cys peroxiredoxin [42]. Peroxiredoxin is crucial for the detoxification of hydrogen peroxide and peroxynitrite [43]. As noted above (section 'Redox chemistry of vitamin C'), vitamin C and GSH cooperate to act as an efficient dual-antioxidant system in mammalian cells [44].

Activating Nrf2 signaling

Nrf2 is a major regulator of cellular antioxidative and other cytoprotective genes [45]. In a rat model of severe acute pancreatitis and an in vitro model of taurocholate-induced injury in AR42J rat pancreatic acinar cells, vitamin C has been shown to attenuate pancreatic cell injury likely via activating the Nrf2/NQO1/HO-1 pathway [46]. Notably, the protective effects of vitamin C on taurocholate-induced AR42J cell injury was attenuated by Nrf2 knockdown [46], suggesting a causal role for Nrf2 signaling in mediating vitamin C cytoprotection. Presently, how vitamin C activates Nrf2 signaling remains unclear though an early study suggested a possible involvement of phosphoinositide 3-kinase [47].

As an anti-inflammatory compound

Vitamin C shows an efficacy in protecting against inflammatory disorders, including sepsis (see section 'Vitamin C in sepsis intervention'). As outlined below, several mechanisms may contribute to the anti-inflammatory activities of vitamin C.

Reducing ROS/RNS flux

As ROS/RNS are intimately involved in inflammation [48], quenching these reactive species or inhibiting their generation (via decreasing NOX and iNOS expression [39, 40]) by vitamin C would blunt inflammatory tissue injury.

Nrf2 activation

On the one hand, Nrf2 positively regulates antioxidant gene expression, boosting cellular antioxidant defense capacity [49]. On the other hand, Nrf2 is also a negative regulator of proinflammatory genes, and activation of Nrf2 signaling leads to suppression of inflammatory responses [50, 51]. Furthermore, vitamin C induces heme oxygenase-1 (HO-1) [52], an enzyme that produces the anti-inflammatory molecule—carbon monoxide [53]. Hence, activation of Nrf2 signaling by vitamin C may also contribute to its anti-inflammatory activities. Indeed, an early study showed that vitamin C protected against lipopolysaccharide (LPS)-induced endotoxemia in mice in an Nrf2-dependent manner [47].

NF-κB suppression

NF-κB is a crucial transcription factor involved in the positive regulation of proinflammatory gene expression [54]. Vitamin C reduces NF-κB activation via two mechanisms. One is through inhibiting ROS-mediated activation of NF-κB [55]. The other is via inhibition of a kinase by DHA (the oxidized form of vitamin C). DHA, but not ascorbic acid, directly inhibits IκBα kinase β (IKKβ) and IKKα enzymatic activity, thereby leading to inhibition of NF-κB activation [56]. Hence, vitamin C possesses a dual molecular action on NF-κB signaling—Ascorbic acid quenches ROS involved in the activation of NF-κB and is oxidized to DHA, which directly inhibits IKKβ and IKKα enzymatic activity [56].

As an important player in stem cell biology and epigenetics

Multiple studies suggest an important role for vitamin C in stem cell biology and epigenetics. Somatic cells can be reprogrammed into induced pluripotent stem cells (iPSCs) by defined factors, such as Oct4/Klf4/Sox2 or Oct4/Klf4/Sox2/cMyc [57]. However, the low efficiency and slow kinetics of the reprogramming process have hampered progress with this technology. Two studies found that vitamin C enhances the reprogramming efficiency of mouse and human fibroblasts transduced with either three (Oct4/Klf4/Sox2) or four (Oct4/Klf4/Sox2/cMyc) factors [58, 59]. Mechanistically, vitamin C may alleviate cell senescence (a roadblock for reprogramming) by p53 repression and accelerate reprogramming by synergizing with epigenetic regulators [58, 59]. More recently, several studies have also identified a novel function of vitamin C in promoting Tet enzyme-mediated generation of 5-hydroxymethylcytosine (5-hmC), suggesting that the availability of vitamin C may have a profound effect on many cellular functions dictated by DNA demethylation (reviewed in [60]). Here Tet enzymes denote the DNA demethylases ten eleven translocases. Indeed, vitamin C acts as a critical mediator of the interface between the genome and environment [61–64], especially in mediating intergenerational epigenetic effects [63] and in suppressing tumorigenesis [64]. In this regard, vitamin

C accumulates within hematopoietic stem cells (HSC) to promote Tet activity, thereby suppressing leukemogenesis [64]. As Tet enzymes play an important role in maintaining the homeostasis of immunity [65–67], activation of Tet by vitamin C might counteract dysregulated immune responses seen in inflammatory disorders, such as sepsis. The interaction between vitamin C and Tet enzymes as well as stem cell homeostasis in the context of inflammatory syndrome and sepsis warrants investigations. Such studies would help delineate the detailed molecular mechanisms underlying vitamin C redox biology and its role in disease intervention.

Vitamin C in sepsis intervention

Overview

According to the Third International Consensus Definition for Sepsis and Septic Shock (Sepsis-3) [68], sepsis is defined as life-threatening organ dysfunction resulting from dysregulated host responses to infection, and septic shock is defined as a subset of sepsis in which underlying circulatory, cellular, and metabolic abnormalities are profound enough to substantially increase the risk of mortality. The extent of organ dysfunction is assessed by Sequential Organ Failure Assessment score, commonly known as the SOFA score [69]. Based on the Sepsis-3 definition, sepsis is now classified into two categories: (i) sepsis, and (ii) septic shock. The traditional classification of sepsis into (i) sepsis, (ii) severe sepsis, and (iii) septic shock is obsolete and has now changed to (i) infection, (ii) sepsis, and (iii) septic shock. A key criterion for the current Sepsis-3 definition is the occurrence of organ dysfunction due to infection. Without organ dysfunction, the condition is categorized as infection only [70].

Pathophysiologically, sepsis is the culmination of complex interactions between the infecting microorganisms and the host immune, inflammatory, and coagulation responses, leading to death due to multiorgan failure. Although the overall outcomes of sepsis management have improved over the past decade, mortality remains exceptionally high which accounts for over 30% of all hospital deaths in the United States and nearly 20% of all global deaths [71]. Currently, there is no cure for sepsis, and developing therapies that improve clinical outcomes of sepsis remains a global priority [72].

Recent advances in delineating the molecular pathophysiology of sepsis facilitate the development of novel and effective mechanistically based therapeutic modalities for this dread disorder [72–74]. In this context, substantial evidence suggests an important role for oxidative stress and dysregulated inflammation in the initiation and progression of multiorgan dysfunction and injury in sepsis in both experimental animals and human subjects [72–74]. Regarding the molecular basis of oxidative stress in sepsis, multiple studies show that, in inflammatory cells, mitochondrial ROS play an important role in activating NF-κB-dependent pro-inflammatory responses to cause multiorgan dysfunction in sepsis [38]. On the other hand, activation of Nrf2 signaling suppresses sepsis in animal models [75]. These mechanistic findings have prompted the search for effective therapies targeting the multiple molecular and signaling pathways underlying sepsis. Due to its multifunctional properties (see section 'Vitamin C as a multifunctional molecule'), vitamin C has been investigated extensively over the past two decades as a potential treatment of sepsis. The sections below survey latest major research findings on vitamin C in sepsis intervention in both animal models and human subjects with a focus on key findings from major randomized controlled trials.

Studies on experimental sepsis

The effectiveness of vitamin C in treating sepsis has been demonstrated extensively in animal models of experimental sepsis, including LPS-induced endotoxemia and polymicrobial sepsis. In these experimental models, treatment with parenteral vitamin C (given either intravenously or intraperitoneally) results in: (i) suppression of oxidative stress and NF-κB-driven pro-inflammatory responses; (ii) amelioration of microcirculation abnormalities and multiorgan dysfunction, and (iii) improvement of survival [76–80]). In addition, combination of vitamin C with glucocorticoid drugs shows synergistic effects in treating experimental sepsis [81]. This synergism observed in experimental sepsis has also prompted clinical trials to evaluate the efficacy of the vitamin C-glucocorticoid combination therapy in sepsis and septic shock (see section 'Randomized controlled trials'). Moreover, three drug-combination treatment (Hydrocortisone, Ascorbic acid, and Thiamine)—the so called HAT therapy has been shown to dramatically decrease oxidative stress, ameliorate cardiovascular dysfunction, and improve survival in experimental sepsis in mice [82]. The efficacy of HAT therapy in treating severe sepsis or septic shock has also been evaluated in multiple randomized controlled trials (see section 'Randomized controlled trials').

Randomized controlled trials

The clinical trials on vitamin C in sepsis intervention can be categorized into the following three groups: (i) vitamin C compared with placebo; (ii) HAT therapy compared with hydrocortisone alone; and (iii) HAT therapy compared with placebo.

Vitamin C versus placebo

Multiple earlier preliminary clinical studies suggested a potential efficacy for intravenous megadose vitamin C in treating patients with sepsis or endotoxemia [83–86]. Recently, the potential clinical efficacy of high doses of vitamin C therapy in patients with sepsis has been investigated in the CITRIS-ALI randomized, placebo-controlled trial. Up to date, this is the only major randomized trial comparing vitamin C versus placebo in sepsis intervention. The CITRIS-ALI trial, involving 167 patients (mean age 54.8), aimed primarily to determine the effect of intravenous vitamin C infusion (50 mg/kg in dextrose 5% in water every 6 h for 96 h) on organ dysfunction and biological markers of inflammation and vascular injury in patients with sepsis and acute respiratory distress syndrome (ARDS) [87]. Upon perusing only the abstract of CITRIS-ALI trial, one would easily notice that the trial produced null results—"a 96-h infusion of vitamin C compared with placebo did not significantly improve organ dysfunction scores or alter markers of inflammation and vascular injury."

Scrutinization of the data reported in the CITRIS-ALI trial, however, may lead to an intriguing preliminary conclusion. In this regard, the trial did show a potential benefit of vitamin C therapy in reducing the mortality: at day 28, mortality was 46.3% (38/82) in the placebo group versus 29.8% (25/84) in the vitamin C group ($p=0.03$) [87]. This significant reduction (36% reduction compared to placebo) in mortality with the vitamin C therapy, though not mentioned in the abstract of the paper, is perhaps one of the most notable findings of the trial. This finding, though being considered preliminary, points to an exciting opportunity of using high doses of vitamin C to save lives in patients with sepsis and ARDS. This preliminary finding in sepsis patients is also consistent with the well-demonstrated effectiveness of vitamin C in treating experimental sepsis in animal models (see section 'Studies on experimental sepsis'). Apparently, the efficacy of high-dose vitamin C therapy in patients with sepsis and ARDS warrants further randomized controlled trials with a focus on mortality reduction and long-term survival. The potential survival benefit of high-dose vitamin C infusion suggested by the CITRIS-ALI trial may also necessitate clinical studies of this readily available, low-cost therapy in other life-threatening respiratory disorders, such as severe Covid-19 currently in pandemic. Indeed, a trial is currently underway to assess the efficacy of high-dose vitamin C infusion in treating Covid-19 [88].

HAT therapy versus hydrocortisone alone

The efficacy of the HAT therapy versus hydrocortisone alone in treating septic shock has recently evaluated in the VITAMIN randomized clinical trial [89]. This trial, involving 216 patients (mean age 61.7 years), was designed to determine the effects of the combination of hydrocortisone, ascorbate, and thiamine (i.e., HAT therapy; hydrocortisone, 50 mg every 6 h; ascorbate, 1.5 g every 6 h; thiamine, 200 mg every 12 h), compared with hydrocortisone alone (50 mg every 6 h), on the duration of time alive and free of vasopressor administration in patients with septic shock [89]. The trial concluded that "In patients with septic shock, treatment with intravenous vitamin C, hydrocortisone, and thiamine, compared with intravenous hydrocortisone alone, did not significantly improve the duration of time alive and free of vasopressor administration over 7 days." It is worth noting that the VITAMIN trial involved only critically ill patients with septic shock. In addition, the vitamin C dose (1.5 g every 6 h for 4 days is also lower than the one used in the CITRIS-ALI trial (50 mg/kg every 6 h for 4 days; this would translate to 3.5 g every 6 h for 4 days for an average adult body weight of 70 kg).

The potential effectiveness of HAT therapy versus hydrocortisone alone in pediatric septic shock was also investigated in a single-centered, propensity score-matched cohort study involving 557 children with septic shock [90]. The study reported that pediatric patients who received HAT therapy had lower mortality than notched hydrocortisone alone at 30 days (9% versus 28%, $p=0.03$) and 90 days (14% versus 33%, $p<0.04$) [90]. The study concluded that HAT therapy, when administered early in the clinical course could reduce mortality in children with septic shock. This encouraging finding should prompt randomized controlled trials to further evaluate the efficacy of the vitamin C-based therapy in pediatric sepsis.

HAT therapy versus placebo

In patients with sepsis or septic shock In a retrospective before-after clinical study involving 94 patients with severe sepsis and septic shock, in 2017, Marik et al. reported that the early use of intravenous vitamin C, together with corticosteroids and thiamine, was associated with significantly attenuated progressive organ dysfunction, including acute kidney injury, and reduced mortality (8.5% in treated group versus 40.4% in control group) of the patients [91]. Subsequently, in 2020, in a randomized, placebo-controlled trial (the ORANGES trial) involving 137 patients with sepsis or septic shock (mean age 68.5 years), Iglesias et al. [92] showed that compared with placebo (normal saline), the combination of intravenous hydrocortisone, ascorbic acid, and thiamine (hydrocortisone, 50 mg every 6 h; ascorbic acid, 1.5 g every 6 h; thiamine, 200 mg every 12 h; all for a maximum of 4 days) significantly reduced the time to resolution of shock (27 versus 53 h). The study, however, did not show a difference in SOFA score, ICU stay, or hospital mortality.

On the other hand, in a randomized controlled trial (the HYVCTTSSS trial) involving 80 patients with sepsis and septic shock (mean age 61.6 years), Chang et al. [93] demonstrated a significant improvement in SOFA score (3.5 versus 1.8, $p = 0.02$) following HAT therapy (hydrocortisone, 50 mg every 6 h for 7 days; vitamin C, 1.5 g every 6 h for 4 days; thiamine, 200 mg every 12 h for 4 days) as compared with placebo (normal saline). Although the study did not reveal a statistically significant reduction in 28-day all-cause mortality (27.5% versus 35%, $p = 0.47$), in prespecified subgroup analysis, patients of the HAT treatment subgroup diagnosed with sepsis within 48 h showed lower mortality than those in the control subgroup (13.6% versus 47.6%, $p = 0.02$) [93]. This suggests that the efficacy of vitamin C-based therapy may vary among different subgroups of septic patients.

In critically ill patients with septic shock The ACTS randomized trial [94] was designed to evaluate the efficacy of HAT therapy in septic shock rather than in a mixed population of patients with sepsis or septic shock. This multicenter trial involved 205 patients with septic shock (mean age 68 years), and the patients were randomly assigned to receive intravenous hydrocortisone (50 mg), ascorbic acid (1.5 g), and thiamine (100 mg) every 6 h for 4 days or placebo in matching volumes at the same time points. Overall, the study found no statistically significant difference between the HAT treatment and placebo regarding SOFA score and mortality, though subgroup analysis suggested an improvement in cardiovascular function in the HAT group [94].

More recently, the effectiveness of HAT therapy was further evaluated in the VICTAS randomized trial [95]. This multicenter, placebo-controlled trial involved 501 critically ill patients (mean age 62 years) with sepsis-induced respiratory and/or cardiovascular dysfunction. The trial concluded that among critically ill patients with sepsis, treatment with vitamin C, thiamine, and hydrocortisone (vitamin C, 1.5 g; thiamine, 100 mg; hydrocortisone, 50 mg; all given every 6 h for 4 days) compared with placebo, did not significantly increase ventilator-and vasopressor-free days within 30 days. On the other hand, the authors stated that the trial was terminated early for administrative reasons and may have been underpowered to detect a clinically important difference [95].

The overall null findings from the ACTS and the VICTAS trials indicate that the vitamin C-based HAT therapy (with a vitamin C dose regimen of 1.5 g every 6 h for 4 days) might not be useful for critically ill patients with septic shock or sepsis-induced respiratory and/or cardiovascular dysfunction. This notion, however, does not necessarily invalidate the vitamin C-based therapy in treating sepsis. As discussed below, many factors must be taken into consideration when interpreting a null finding from a clinical trial.

Clinical perspectives

Collectively, the findings from the randomized controlled trials on vitamin C either as "monotherapy" or in combination with hydrocortisone and thiamine are not clear-cut. This is in contrast to the effectiveness demonstrated consistently by numerous studies using experimental sepsis animal models. While the controversy on the clinical value of vitamin C in sepsis intervention continues, several points warrant consideration with regard to interpretation of current clinical research findings as well as guiding future clinical trials. Firstly, the inconsistency may arise due to the variations in vitamin C pharmacokinetics among individual septic patients and among the different vitamin C dosage regimens. In this context, a randomized trial of 4 intravenous vitamin C regimens in critically ill patients with multiple organ dysfunction found that the 2 g per day dose was associated with normal plasma concentrations, and the 10 g per day dose was associated with supranormal plasma concentrations, and more importantly, sustained therapy (infusion) was needed to prevent hypovitaminosis [7]. Secondly, the patients' age can be another major factor; studies in specific age groups would help delineate the age effect. Thirdly, the severity of sepsis (sepsis versus septic shock), the patients' nutritional status and comorbidities, and the timing and duration of vitamin C administration may also affect the overall clinical outcomes. Fourthly, the gender and ethnicity of the patients may influence plasma concentrations of vitamin C and subsequent responsiveness. In this context, studies showed that gender might affect vitamin C tissue distribution and pharmacokinetics [96, 97]. Lastly, but not the least, the optimal dosage regimen of vitamin C in treating sepsis remains poorly characterized.

The most-commonly used vitamin C dose regimen (i.e., 1.5 g every 6 h for 4 days) in clinical trials on sepsis may not be optimal for counteracting the pathophysiological processes (e.g., oxidative stress, dysregulated inflammation, and microcirculation dysfunction) underlying sepsis. The redox chemistry of vitamin C is significantly influenced by its concentrations as well as other substances in biological milieu (see section 'Redox chemistry of vitamin C'). For example, a supraphysiological concentration of vitamin C may cause oxidative stress, and this prooxidative potential of mega-dose vitamin C has been harnessed to treat various types of cancer [8]; cancer cells are more susceptible to oxidative stress-induced cytotoxicity due to their relative antioxidant deficiency. On the other hand, low doses of vitamin C may not be adequate for exerting sufficient antioxidative and anti-inflammatory action to counteract the pathophysiology underlying sepsis. Hence,

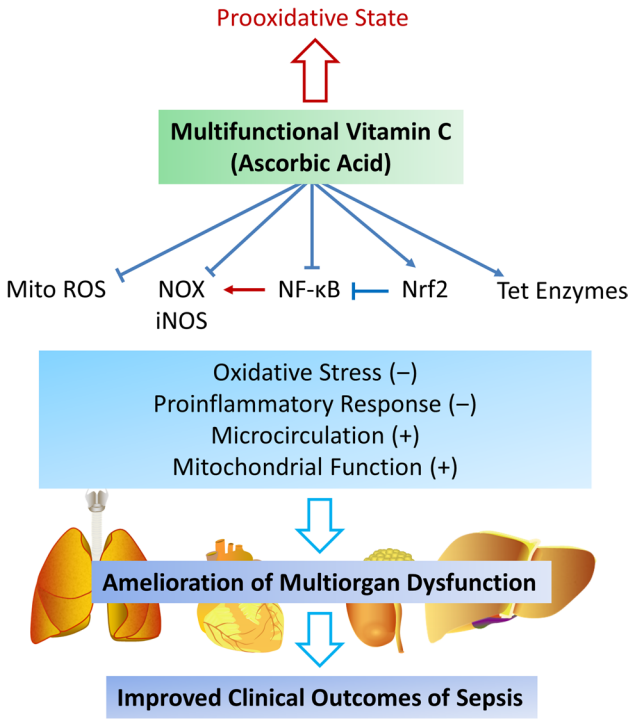

Fig. 2 Redox biology of multifunctional vitamin C in sepsis intervention. As illustrated, via targeting various cellular pathways, vitamin C treatment may lead to decreased oxidative stress and inflammatory response and improved microcirculation and mitochondrial function. These beneficial effects may ameliorate multiorgan dysfunction and thereby improve the clinical outcomes of septic patients. On the other hand, supraphysiological levels of vitamin C may result in a prooxidative state, causing harm to the body. As such, vitamin C dosage must be optimized to exert maximal beneficial effects and cause minimal oxidative stress so as to achieve a desired clinical efficacy in treating sepsis as well as other inflammatory disorders. Mito denotes mitochondrial

an optimal dose must be one that exerts maximal antioxidative/anti-inflammatory effects without causing a significant prooxidative state (Fig. 2).

While it is imperative to develop an optimal regimen for using vitamin C to treat septic patients, efforts should also be devoted to determining if vitamin C-based strategies could also be employed for preventive purpose in high-risk patients, such as those with advanced age, dietary insufficiency, comprised immunity, or other concomitant morbidities. In this regard, a recent systemic review and meta-analysis suggested that vitamin C supplement in normal individuals could reduce the risk of respiratory tract infections, including influenza [98].

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Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

Research involving human and/or animal participants This work does not involve animals or human subjects.

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