Several studies have recently been published evaluating the administration of intravenous ascorbate (vitamin C) for the treatment of sepsis.\textsuperscript{1-3} While these studies have methodological limitations, given the interest surrounding their publication, and that a large number of trials evaluating ascorbate have commenced (NCT02106975, NCT03509662, NCT03380507, NCT02734147, NCT03258684, NCT03389555, NCT03422159, NCT03335124, NCT03338569, NCT03333278, NCT03380507, ACTRN12617000793314, ACTRN12617001553369p, ACTRN12617001392358p), it is worthwhile to review the biological roles of ascorbate. These underlying mechanisms provide a rationale for the potential use of intravenous ascorbate as an adjunctive treatment for a variety of acute inflammatory conditions and may inform dosing regimens.

Biological roles of ascorbate (vitamin C)

With a low ionisation energy allowing for undemanding transfer of electrons, ascorbate is well suited for its primary biochemical role as an electron-shuttling cofactor for a wide variety of enzymes and other proteins.\textsuperscript{4-6} There are many important in vivo biological reactions and processes known to involve ascorbate; these reactions include the synthesis of monoamine neurotransmitters (adrenaline, dopamine, noradrenaline and serotonin),\textsuperscript{7-9} collagen\textsuperscript{10,11} and nearly 50\% of all known biologically active peptides and peptide hormones (including vasopressin, endorphins and neurokinins, among others).\textsuperscript{12-14} As a cofactor, ascorbate is also involved in modulation of adrenergic, histaminergic and GABAergic receptor activity.\textsuperscript{15-19} In this role, ascorbate has been shown to increase the sensitivity of receptors to their respective ligands, particularly at lower concentrations. Ascorbate is known to play an important epigenetic role as a cofactor for methylcystosine diogenases, a family of enzymes responsible for DNA demethylation.\textsuperscript{20} Ascorbate is also required for the recycling of vitamin E and in multiple hepatic reactions.\textsuperscript{21-26} In addition, it is involved in modulation of (endothelial and neuronal) nitric oxide synthase (NOS) and in the continuous degradation of hypoxia-inducible transcription factor 1\(\alpha\) (HIF-1\(\alpha\)).\textsuperscript{4,26-33} Continuous HIF-1\(\alpha\) degradation is required to suppress activation of a diverse family of genes which are under the control of hypoxia response element containing promoters. These genes are associated with chronic inflammation, dyoxia — which is the phenomenon of altered mitochondrial oxygen handling in spite of normoxia\textsuperscript{34} — and other potentially deleterious responses.\textsuperscript{26,29,30,35,36} Ascorbate is also required for the generation of the neutrophil/macrophage oxidative burst.\textsuperscript{31,37,38} Finally, ascorbate has been described as the blood’s most effective exogenous antioxidant, and is the only antioxidant known to be capable of preventing lipoprotein peroxidation.\textsuperscript{25,26,39}

The association between inflammation and endogenous ascorbate concentrations

In response to acute inflammatory conditions, plasma ascorbate levels fall rapidly.\textsuperscript{40-43} The precise mechanisms responsible for this marked decrease are currently unclear but are likely to be multifactorial. Critically ill patients appear prone to low plasma ascorbate levels because of a combination of decreased intake and absorption, redistribution, and acutely increased degradation and metabolism.\textsuperscript{4,40-42} In the highly oxidative environment that arises during the inflammatory response,\textsuperscript{43-45} ascorbate is rapidly oxidised to dehydroxyascorbate. Dehydroxyascorbate is then either recycled back to ascorbate via one of two recycling systems or undergoes irreversible ring cleavage and is then further catabolised via one of several possible degradation pathways.\textsuperscript{46,47} For reasons that are unclear, the ascorbate recycling systems are seemingly unable to prevent the rapid fall in plasma ascorbate levels that occurs during such acute inflammation.\textsuperscript{5,48,49} This oxidative consumption of ascorbate is believed to be the primary reason for the increased metabolic requirements of ascorbate that develop in critically ill patients.\textsuperscript{4}

Ascorbate (vitamin C) uptake and transport mechanisms

In health, ascorbate uptake from the gastrointestinal tract and into cells throughout the body is mediated by a family of constitutively and differentially expressed transport proteins. Ascorbate transport is mediated by sodium vitamin C transport (SVCT) proteins 1 and 2, while dehydroxyascorbate...
transport is mediated by glucose transport (GLUT) proteins 1 and 3.\textsuperscript{50,51} Intestinal, renal, integumentary, hepatic and pulmonary uptake of ascorbate is primarily mediated by SVCT1.\textsuperscript{50,51} SVCT2 mediate ascorbate transport in immune cells, adrenal glands, central nervous tissues and striated and cardiac muscle tissues.\textsuperscript{51} In the gut, SVCT1 are localised to the luminal side and thus determine ascorbate uptake rates, while SVCT2 are localised to the basal side and are responsible for transporting vitamin C from the enteroocyte into the interstitium.\textsuperscript{52} GLUT1 are widely present throughout the endothelium, while GLUT3 are primarily responsible for central nervous system glucose handling.\textsuperscript{53,54} SVCT1 and SVCT2 differ in terms of affinity and inducible expression in response to stress.\textsuperscript{51} SVCT2 have a comparatively higher ascorbate affinity and are upregulated in response to inflammation.\textsuperscript{50}

In health, the recommended levels of dietary intake are usually sufficient.\textsuperscript{51} Excess ascorbate conditions are rare because ascorbate is readily recycled and any excess ingested ascorbate is either not absorbed due to SVCT1 saturation or excreted in urine when plasma levels rise above renal ascorbate reuptake thresholds (about 60 \textmu mol).\textsuperscript{55,56}

**Consequences of low ascorbate concentrations in acute inflammation**

Once concentrations of ascorbate decrease beyond a certain threshold, marked alteration across multiple biological systems is observed.\textsuperscript{4,32,57} It is interesting to note that many of the pathological features of septic shock — microvascular dysfunction,\textsuperscript{33,58} dysregulated inflammation,\textsuperscript{31,33} microvascular thrombosis,\textsuperscript{59} increased endothelial permeability and oedema,\textsuperscript{60,61} dysoxia,\textsuperscript{26,39} and white cell anergy\textsuperscript{37,38} — are also present in experimental models of severe ascorbate deficiency.

HIF-1\textalpha continuous degradation is known to be impaired in sepsis leading to activation of pro-inflammatory hypoxia response element-controlled genes.\textsuperscript{36,62} Hypoxia response element is also known to be activated by acute ascorbate deficiency due to impairment of HIF-1\textalpha degradation.\textsuperscript{29,36} Thus, acute ascorbate deficiency provides a plausible mechanistic link between sepsis and dysoxia, but this needs to be confirmed experimentally. Endothelial (eNOS) and neuronal nitric oxide synthase (nNOS) enzymes, which are responsible for homeostatic microvascular production of nitric oxide, uncouple in response to low ascorbate concentrations.\textsuperscript{4,32,33,58,63} Uncoupling is a biochemical term used to describe the phenomenon of an enzyme either altering or losing its catalytic activity in response to a stimulus due to conformational changes and/or dissociation of subunits. NOS uncoupling results in not only the loss of anti-inflammatory nitric oxide production but also leads to the generation of pro-inflammatory superoxide free radicals as the NOS complex oxygenase domain is activated.\textsuperscript{28,32,33,57,58} eNOS uncoupling also leads to loss of endothelial integrity and enhanced stress fibre formation through a cAMP-mediated mechanism that is attenuated by ascorbate.\textsuperscript{33,60} Thus, eNOS uncoupling, as a consequence of ascorbate deficiency, triggers reinforcement and amplification of the inflammatory process, and enhances microvascular dysfunction and endothelial permeability.\textsuperscript{28,32,33,57,60} However, whether ascorbate administration is able to recouple eNOS in clinical practice remains to be proven and will be informed by results from upcoming randomised controlled trials.

Using this model of pathogenesis, it follows that administration of ascorbate to restore adequate intracellular concentrations has the capacity to restore the system towards its previous non-inflammatory state. This is complicated by the oxidative environment created by the inflammatory process, which degrades ascorbate and impedes ascorbate accumulation in the area of inflammation. The oxidative environment provides a possible explanation as to why studies evaluating lesser doses of ascorbate have had negligible impact on surrogate (eg, haemodynamic) and patient-centred outcomes.\textsuperscript{41,64-66} It may also explain why trials using greater doses, which are able to overcome the oxidative environment, have yielded more promising results.\textsuperscript{1-3,61,67} Under this model, the apparent synergistic effect of steroids or vitamin E or other anti-oxidants and ascorbate may be explained, at least in part, by their action to reduce the oxidative environment and, thereby, attenuate inflammation-mediated ascorbate degradation.\textsuperscript{68} It should be noted that ascorbate is required for vitamin E recycling following reaction with inflammation-derived lipid hydroperoxyl radical species, and thus concomitant vitamin E administration may have an ascorbate sparing effect.\textsuperscript{69}

**Approach to dosing in the critically ill**

Normal plasma levels of ascorbate generally range between 30 and 80 \textmu mol/L.\textsuperscript{70} Levels below 21 \textmu mol/L are termed hypovitaminosis C, while levels below 11 \textmu mol/L are associated with severe deficiency.\textsuperscript{71} In the setting of acute inflammation, doses of ascorbate need to replace the existing plasma deficit and account for ongoing ascorbate metabolism. Recently, in a single-centre observational study of 44 critically ill patients, Carr and colleagues\textsuperscript{71} reported that 70\% of patients in intensive care had hypovitaminosis C despite receiving recommended doses of the vitamin. The proportion of patients with hypovitaminosis C increased to 90\% (40\% severely deficient) of the 24 patients who had septic shock.\textsuperscript{71} In a study involving 57 elective post-operative intensive care patients, Rümelin and colleagues\textsuperscript{72}
observed that up to 8 g intravenous ascorbate given in divided doses over 12 hours after admission to the intensive care unit was required to normalise plasma ascorbate levels in the most severely deficient patients. In an earlier study involving 14 critically ill patients, Long et al reported that 3 g intravenous daily ascorbate administered via total parenteral nutrition was able to restore ascorbate plasma levels within 5–6 days. In a blinded, randomised controlled feasibility study involving 24 patients, Fowler and colleagues reported that 50 mg/kg/24 h and 200 mg/kg/24 h doses of ascorbate restored plasma levels to normal levels within 6 hours of commencement. Ongoing infusions of 50 and 200 mg/kg/24 h ascorbate resulted in marked elevation in pharmacological plasma ascorbate levels above normal levels. Finally, in a recently published study examining ascorbate pharmacokinetics in the critically ill, De Grooth et al described that plasma ascorbate levels could be restored to above 21 μmol/L (ie, just below the lower threshold in health) with 2 g intravenous vitamin C every 12 hours (4 g per day) and that intravenous replacement needed to be sustained for at least 48 hours.

Bolus oral supplementation is considered inadequate to replace the existing ascorbate deficit and ongoing metabolic demand due to the inherent limitations of luminal SVCT1 channels in terms of its kinetic parameters (Km and Vmax values). This limited uptake ability is further compromised in the setting of critical illness, wherein disturbances in the interstitial milieu (of acid-base, redox potential, temperature, osmolality etc) and organ dysfunction (ileus, fluid overload) are common. Restoration of median plasma ascorbate levels to within normal range was possible in a cohort of 28 critically ill patients who received a continuous infusion of a proprietary enteral pharmaconutrient that provided 1500 mg ascorbate per 24 hours. However, there was a wide range of plasma ascorbate levels recorded in response to enteral supplementation, and 7 days of treatment were required before all patients were above the lower threshold.

**Dosage and pharmacokinetics**

Pharmacokinetic data indicate that maintaining plasma ascorbate levels requires increased frequency of dosing when compared with healthy individuals (eg, four times a day or 4-hourly in the critically ill or continuous infusion) due to both increased clearance and altered volume of distribution that arises during acute illness. There is currently no upper limit of intravenous dosage clearly identified, although case reports describe calcium oxalate nephrolithiasis at doses exceeding 100 g sodium ascorbate intravenous daily in two patients with burn injuries. Doses as substantial as 1.5 g/kg intravenous vitamin C three times weekly have been used in the outpatient oncological and alternative medicine settings and are reportedly well tolerated. Administration rates of up to 1 g intravenous ascorbate per minute via a central catheter have been described in the oncological literature. In an observational study of 9328 patients receiving an average of 28 g (range, 1–200 g) of intravenous ascorbate administered on average every 4 days (range, 1–7 days), 1% of patients reported adverse events, including fatigue, mood changes, and irritation (phlebitis) at the site of injection. No other major adverse effects were reported in patients with normal renal function and no history of glucose-6-phosphate dehydrogenase deficiency.

Thus far, no serious adverse events have been described as related to intravenous ascorbate in any recent study when administered to critically ill patients, although experience is relatively limited. It has also been suggested that because of the increased clearance and volume of distribution during acute inflammation and inability of the body to store ascorbate, the use of a loading dose followed by a continuous infusion is likely to be a superior method of achieving adequate plasma concentrations. Doses of ascorbate used in studies of pharmacological administration vary widely between 4 to over 20 g per day, and the dose being given as either bolus doses every 4–12 hours or via continuous infusion. Dose regimens are typically continued for between 2 and 7 days. Timing of initial dose administration, likely to be an important factor given the mechanisms involved, has not been specifically examined to date.

Oxalate nephropathy has been well described in non-critically ill patients receiving regular oral and/or intravenous supplements, with one study showing an unexpected delayed rise in oxalate levels 7 days after ascorbate supplementation was completed. However, oxalate nephropathy with intravenous ascorbate replacement in the acute setting appears to be of minor significance as mole to mole conversion of intravenous ascorbate to oxalate has been shown to average between 0.3% and 2.3% of the administered dose. In addition, during their single centre sequential period trial, Marik and colleagues noticed improvement in biomarkers of renal function with the administration of a total 6 g daily intravenous ascorbate with concomitant 200 mg intravenous thiamine 12-hourly administration. Further clarification of the effect of intravenous ascorbate on oxalate production in critically ill patients over the days and weeks after ascorbate administration is needed.

An additional pragmatic consideration when administering ascorbate is the structural similarity between glucose and ascorbate — which bedside glucometers and fluorine-18 fluorodeoxyglucose positron emission
tomography and computed tomography scanners are unable to discriminate — and presents a risk of erroneous blood glucose readings.\(^{83-89}\)

As ascorbate is a highly water-soluble molecule and structurally similar to glucose, it has been recommended to increase the administered dose by 100% during continuous renal replacement therapy.\(^{90}\) There is currently insufficient information to guide dosing during episodes of acute kidney injury not requiring renal replacement therapy. Further research in this area is required.

**Conclusion**

During acute inflammation, there is a rapid and substantial reduction in plasma levels of ascorbate. It is plausible that acute ascorbate deficiency triggers a variety of deleterious responses, including enhancement of the inflammatory response, activation of a series of genes involved in the hypoxic response and alterations in neuroendocrine activity. While ascorbate is known to play a central role in these biological processes, the extent to which ascorbate administration can ameliorate or mitigate these responses is currently unclear. To date, studies with intravenous administration of ascorbate to critically ill patients to either replenish endogenous concentrations or to achieve pharmacological effect have not reported harm. It remains to be clarified if replacement of ascorbate is beneficial (or harmful) and, if it is of benefit, whether normal endogenous or pharmacological plasma levels should be targeted. Several studies conducted in the critically ill have used dosing strategies, which, based on current understanding of pharmacokinetics, would not achieve plasma concentrations representative of normal values. If ascorbate truly has any impact on patient outcomes (either beneficial or harmful), such underdosing of vitamin C may explain why investigators did not observe any signal (either beneficial or harmful) in previous trials.\(^{55,66,91-93}\) The optimal dose, timing of first dose and duration of ascorbate administration in the critically ill is presently unknown. There is also uncertainty as to guide dose adjustment in patients with acute kidney failure. We believe that, because of uncertainty regarding dosing and inconsistent effects observed across studies, off-label use cannot be supported. Rather, robust evidence provided by prospectively registered, multicentre randomised controlled trials of adequate doses of ascorbate is required to inform clinical practice.

**Competing interests**

None declared.

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