Vitamin C: a wound healing perspective

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Wound healing requires a variety of macronutrients and micronutrients, each of which varies according to the stage of healing (Lansdown et al, 1999; Patel, 2005). During the proliferation phase of wound healing, fibroblasts produce collagen fibres, a process dependent upon an adequate availability of dietary nutrients such as vitamin C, iron and copper (Flanagan, 1997). Demling (2009) explains how wounds such as pressure ulcers or acute burn injuries can lead to a hypermetabolic and catabolic state, thereby increasing nutritional needs. Of the micronutrients involved in wound healing, Anderson (2005) advocates vitamin C, more commonly known as ascorbic acid (AA), as the most essential due to its influence on collagen synthesis and angiogenesis. Humans cannot synthesise AA and must therefore ingest it in sufficient quantities from their diet (Pauling, 1970). Scurvy, or scurvy as it is more commonly known, is a disease caused by a deficiency of AA which may hinder healing.

Physiology of ascorbic acid
AA (chemical name 2,3-didehydro-L-threo-hexano-1,4-lactone) is an acidic, water-soluble antioxidant and cofactor of several enzymes, which humans are unable to synthesise due to L-gulonolactone oxidase (GLO) deficiency (Linster and van Schaftingen, 2006). When AA acts as a donor of ions to cell reactive free radicals, it is oxidised to semi-dehydroascorbate and then dehydroascorbic acid (DA) (Kalay and Cevher, 2012). Strehle et al (2011) discuss the reconversion of DA via enzymes to AA, although Kasahara et al (2009) maintain that DA is unable to irreversibly decompose to di-keto-L-gulonic acid, suggesting monodehydroascorbic acid (MDA) has a more relevant role to play in AA recycling. AA is ubiquitous throughout the body and is present in varying degrees in serum, plasma, the cellular immune system and bone cells (Jacob, 1996). Carr et al (2013) suggest that, in general, plasma levels relate to dietary intake and tissue levels reflect the bioavailability of AA. The concentration of AA within immunocompetent cells is between 10 and 100 times that of plasma levels (Strohle et al, 2011). Graumlich et al (1997) identified, from their experimental study of the plasma and urinary pharmacokinetics of AA, that with increasing levels, the intracellular concentration reached a saturation point of AA, and that doses of AA to replete individuals via their diet beyond 100mg per day were excreted in the urine.

More recently, Kasahara et al (2009) determined from their laboratory study that homeostasis of AA levels in rats, regardless of their ability to synthesise AA, was rapidly maintained by a process of reductive regeneration from MDA. This was achieved by administering varying doses of an enzyme, an ascorbate oxidase derivative, and monitoring the subsequent levels of AA in vitro and in vivo. The authors indicated that the speed with which AA is decomposed and recomposed from its oxidised metabolites reflects the necessity for maintenance of AA levels within cells and organs. Interestingly, it was also observed that the plasma levels of AA were consistently lower in hyperglycaemic rats, suggesting ramifications for wound healing in diabetic patients, due to the increased generation of reactive oxygen species and consequent oxidative stress. Villacorta et al (2007) had previously identified that this sort of stress occurs when there is oxidative stress. Antioxidants such as vitamin C, more commonly known as ascorbic acid (AA), are therefore particularly useful in the management of chronic wounds due to hyperglycaemia. The authors in this study indicate the importance of vitamin C in reducing oxidative stress and improving wound healing in diabetic patients.

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ABSTRACT
Vitamin C, also known as ascorbic acid (AA), is involved in all phases of wound healing. In the inflammatory phase it is required for neutrophil apoptosis and clearance. During the proliferative phase, AA contributes towards synthesis, maturation, secretion and degradation of collagen. Deficiencies affect the maturation phase by altering collagen production and scar formation. The body strives to maintain homeostasis of AA, thereby ensuring availability for collagen synthesis. After wounding, plasma and tissue levels of AA diminish and, as a consequence, supplements may be useful for healing, although levels beyond saturation are excreted. Clinicians need to be aware of both the nutritional status of patients with either acute or chronic wounds and the possibility of any AA deficiency which may hinder healing.

KEY WORDS
- Vitamin C
- Ascorbic acid
- Wound healing
- Deficiency
- Supplements
are more free radicals than can be eliminated, leading to tissue injury and inflammation. The use of male laboratory-bred rats may be considered a limitation of the study, since, according to Ames et al (1993), rats have a basic metabolic rate approximately seven times higher than that of humans, which is likely to have affected the metabolised rate of AA. In addition, wound healing in male rats is impeded by androgens (Gilliver et al, 2007) and laboratory breeding may introduce genetic abnormalities, potentially affecting the reliability and validity of the study.

There is ongoing research regarding the biological mechanisms of AA. For example, the precise transport route of AA into the tissues was investigated by Corpe et al (2005). The authors characterised a probable membrane transporter using a similar compound to AA in vitro (6-bromo-6-deoxy-L-ascorbic acid), a transporter substrate which is completely specific to the Na+–dependent vitamin C transporters SVCT1 and SVCT2. Whether these results can be translated in vivo remains unclear as the authors concede that oxidised dehydroascorbic acid is difficult to detect and may be involved to a greater degree in membrane transportation than is currently predicted. Previously, Runsey and Levine (1998) had proposed that substrate affinity and substrate availability can affect transport, which in turn can be adversely affected by glucose in the plasma. This evidence suggests a possible link between AA transport, effect of glucose and the role of neutrophils. Padayatty and Levine (2001) identified the process whereby neutrophils recycle and concentrate extracellular AA internally via transportation when exposed to bacteria, a depletion of which Jacob (1996) suggests causes a type of intracellular scurvy. Neutrophils primarily phagocytose bacteria in the late inflammation phase of wound healing and release proteinases to degrade surrounding components in order to prepare for tissue deposition (Hart, 2002). Therefore, a disruption of this process in relation to a serum deficiency in AA may result in a reduced somatic ability to deter infection.

**Role of ascorbic acid**

AA is involved in many somatic processes and indirectly in a variety of enzymatic activities, which include the degradation of tyrosine, synthesis of epinephrine from tyrosine, bile acid formation, absorption of iron and neurotransmitter synthesis (Jacob, 1999; Murray et al, 2000; Villacorta et al, 2007). AA also acts as a direct and indirect antioxidant by influencing the activity of the immune system via phagocytes, leucocytes and lymphocytes (Ströhle et al, 2011). Jacob (1999) states that the high levels of AA in neutrophils provide tissue protection against reactive oxidants and free radicals during the respiratory burst. This respiratory burst from neutrophils and monocytes is a necessary product of immune system phagocytosis to degrade internalised pathogens (Bürzle et al, 2013). A study by Vissers and Wilkie (2007) investigating the effect of AA deficiency in neutrophils using murine AA-depleted mice determined that in vitro testing of the neutrophils did not undergo timely apoptosis and did not appear to be phagocytosed by macrophages. The authors suggested that, in vivo, these neutrophils may not be cleared from the wound and indeed may degrade to spill the toxic contents into the wound bed, thereby degrading tissues and prolonging the inflammatory phase of wound healing.

A significant factor of AA with regard to wound healing is the synthesis and cross-linkage of collagen, which has an impact on vascular integrity and capillary bed strength (MacKay and Miller, 2003). Early research by Dunphy et al (1956) established a connection between the healing of acute wounds in vivo in an animal model and the necessity for adequate systemic availability of AA. Their study of wounds in guinea pigs deficient in AA paralleled an early wound healing model in that there was fibroblast and endothelial cell activity, but no synthesis of collagen fibres. The administration of AA prompted the catabolism of collagen formation.

Villacorta et al (2007) identified collagen as the principal protein of structures such as skin, bones, cartilage, tendons and blood vessels. Urban et al (2012) conducted an in vitro study to examine osteoblast activity in the presence of AA. Bovine medium was supplemented with increased concentrations of AA and digital images examined for cellular proliferation. Collagen type I of osteoblast-like cells increased in line with AA concentration, peaking at 200 µg/ml. While these results may not precisely reflect human osteoblast activity, it does suggest that AA positively influences collagen biosynthesis by acting as a cofactor in procollagen production (Baum and Arpey, 2005).

Kleinsmith and Kish (1988) and Alberts et al (2002) explain the mechanism by which collagen synthesis is affected by lack of AA: collagen triple-helix α-chains are bonded by hydrogen and stability is provided in part by hydroxyproline residues. AA is responsible for the activation of the enzyme prolyl hydroxylase which catalyses proline hydroxylation, the absence of which causes immediate degradation of the triple helix and resultant defective collagen synthesis. Blood vessels, tendons and skin are consequently affected by becoming friable (Lodish et al, 2004). A deficiency therefore results in a defect in the structure of collagen, thereby impeding the proliferative phase of wound healing (Sanders and Emery, 2003).

**Deficiency and sufficiency**

In 1753, James Lind became aware of a disease that afflicted sailors in particular. He consequently undertook one of the first clinical experiments, namely, to examine potential causes of scurvy (Patel, 2005). He examined the effects of a variety of diets on sailors and determined that ‘oranges and lemons were the most effectual remedies for this distemper at sea’ (Lind, 1983). Scurvy is now known to be a deficiency of AA, the major symptoms of which include easy bruising, pinpoint haemorrhages, bleeding.
gums and poor wound healing (Coffee, 1998). However, Dickerson (1993) argues that AA deficiency is difficult to establish clinically, given the time taken for the diverse symptoms to manifest.

The optimum amount of AA for health is currently unknown (Padayatty and Levine, 2001). Previously Ames et al (1993) identified that, in the United States, the recommended intake of 60 mg per day of AA is only effective in preventing observable deficiencies—although this was increased to 75 mg for women and 90 mg for men more recently (Padayatty and Levine, 2001). The body pool is thought to be around 1500 mg when 75 mg of AA are consumed daily, and maximum metabolic turnover is approximately 40 mg per day in healthy individuals (Graumlich et al, 1997). Currently, deficiency is defined as a leucocyte AA level below 0.01 mg per 10^8 cells (Food Standards Agency (FSA), 2003). Subsequently, the FSA (2006) recommended a dietary intake of 40–50 mg of AA for a healthy adult.

AA in wound healing
Wound healing is adversely affected by dietary deficiencies, and the subsequent metabolism profile of macro and micronutrients changes (Collins et al, 2005).

According to Brown and Phillips (2010), there are no specific guidelines for AA in wound healing, although Demling (2009) recommends between 500 mg and 2 g for support of energy production in the hypermetabolic state, which constitutes more than 10 times the recommended daily intake as suggested by FSA (2006).

In order to provide insight into the nutritional status of patients with wounds, Pitt et al (2007) investigated the diet of those admitted for diabetic foot complications. AA was not deemed to be deficient, but it is difficult to determine how well the methods of data collection and analysis accurately reflected the true levels. This is because AA levels were not determined by chemical methods; rather, the amount consumed was deduced from a short-term retrospective recall and the method of data analysis did not allow for the increased nutritional needs required for wound healing.

Gupta et al (2002) undertook a study to understand the wound healing antioxidant profile in immunocompromised rats. Tissue samples were collected from wounds on specific days and investigated for antioxidant enzymatic activity in vitro. The authors’ observations indicated statistically
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significant increased rates of superoxide dismutase (SOD) and reduced rates of the neutralising and detoxifying enzymes against levels in the immunocompetent rats (p<0.05). Of note were the depleted levels of AA in the skin, as well as antioxidants. This suggests that beneficial AA-influenced antioxidant activity is hampered by the altered chemical profile of immune deficiency, thereby delaying wound healing, particularly in the inflammatory phase.

Use of supplements in wound healing

Ter Riet et al (1995) were unable to establish any improvement in pressure ulcer healing using AA supplements. The intervention group received 1000 mg of AA per day compared to 20 mg in the control group. No significant differences in healing outcomes were noted between the groups after 12 weeks. However, in a study by Long et al (2003), increasing levels of AA supplements were administered to trauma patients. With lower levels of AA supplements at 300 mg and 1000 mg per day, plasma levels remained insufficient. It was not until the administration of 3000 mg per day that normal plasma levels of AA were detected. Baseline levels of AA on admission were on average 0.11 mg/dl, which the authors suggest was considerably lower than the normal range of 0.45–1.2 mg/dl, indicating that radical depletion of AA is a physiological response to injury. This suggests that immediate high supplemental doses of AA may be beneficial in replacing initial losses in order to ensure adequate antioxidant levels, thereby protecting cells and tissues from oxidative damage in the inflammatory phase of wound healing (Padayatty and Levine, 2001).

A randomised control trial by Blass et al (2012) to measure oral supplementation of antioxidants including AA in trauma patients with wound healing disorders failed to establish the exact mechanism by which the appreciable difference in wound healing occurred in the treatment group. However, the level of AA supplements used in the control group conformed to the recommended daily dose of 40 mg by the FSA (2006). Rather than using supplements, Lima et al (2009) conducted a study on wound healing in rats using a topical AA cream containing 10% AA. The results indicated a consistent acceleration in wound response in the treatment group at specified days post-wounding. The number of macrophages was reduced, which may have reflected the anti-inflammatory property of the cream. Statistically significant increases in the density of collagen fibres were noted throughout the healing period in the treatment group, with wound closure on day 8 as opposed to day 12 with the control group (p<0.05). However, the results may not be clinically significant as both wound groups ultimately healed, and without infection.

Clinical implications

Edmonds (2007) explains how wound healing is impaired in nutrition deficiency and that attempts at wound healing without addressing nutritional needs will result in a delayed wound response, which in turn may demoralise the patient.

Jacob (1999) suggests that as there are no reliable functional tests for AA deficiency, levels must be determined by leukocyte, or, more preferably, plasma measurements, as the results are more easily interpreted. However, in a clinical setting, ready access to this form of chemical analysis is unlikely and easier methods to determine nutritional status and the likelihood of AA deficiency must be utilised.

Visual clues of AA deficiency such as inflamed hair follicles, coiled hairs on the arms and back and bleeding, swollen gums (Landsdown, 2004) are easily noticed and may be an indicator for nutritional investigation. The Department of Health (DH) (2010) suggests that screening for nutritional status should take place at the patient’s first clinical appointment using a recognised tool such as the Malnutrition Universal Screening Tool (MUST) (Malnutrition Advisory Group, 2011). Despite available evidence, De Tullio (2012) claims much of the relevance of AA metabolism is ignored, resulting in little translation to clinical practice.

Conclusion

A review of the literature suggests that AA plays a comprehensive role in all phases of wound healing with regard to cellular apoptosis, antioxidant processes, collagen synthesis and bone formation (Anderson, 2005). In the inflammatory phase, AA is required for timely neutrophil apoptosis and clearance (Vissers and Wilkie, 2007). During the proliferative phase, AA differentially interacts in the integral processes of synthesis, maturation, secretion and degradation of collagen (Ronchetti et al, 1996).

Deficiencies hinder the maturation phase by interfering with the integrity of collagen production, consequently affecting scar formation (Burns et al, 2003).

The body strives to maintain essential circulating levels of AA by rapid regeneration of the metabolites that are oxidised after scavenging free radicals. Subsequently, levels of the necessary catalyst for collagen synthesis are constantly available (Kasahara et al, 2009).

The use of initial high-dose AA supplements appears to be useful in healing as plasma and tissue levels are rapidly depleted in response to wounding, particularly if the individuals are already scorbutic (Long et al, 2003). Collins et al (2002) suggest considering AA supplements in those at risk of pressure ulcers or established wounds. However, supplements beyond cellular—and therefore beyond plasma—saturation point are simply excreted (Long et al, 2003).

It is incumbent upon clinicians to be aware of the nutritional status of patients with wounds, and to refer for a nutritional assessment where appropriate. As exact levels of AA can only be determined by laboratory testing, an element of pragmatism must be adopted and clinicians...
must be vigilant for any visual clues of deficiency in patients with either acute or chronic wounds.

Malnutrition Advisory Group (2011) Malnutrition Universal Screening Tool. BAPEN, Redditch
Wounds 4(1): 1159–210

KEY POINTS

- Ascorbic acid is an essential micronutrient for wound healing
- Ascorbic acid contributes to collagen synthesis and angiogenesis
- Ten times the recommended daily intake has been advocated for wound healing
- Clinicians must be aware of patients' nutritional status