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EDUCATIONAL ARTICLE

A Proposed Guide for Interpretation of Plasma Ascorbate Concentrations

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ABSTRACT

Vitamin C, Ascorbic acid (AA) is an essential water-soluble nutrient involved in many physiologic functions such as energy metabolism, neurotransmitter synthesis, tissue repair, immune function, and the regeneration and the recycling of many molecules. The physiological need for this nutrient can vary widely and is increased in the presence of physiological stress such as trauma, infection, inflammation, increased toxin load, chemotherapy, radiotherapy, and diseases such as diabetes and cancer. It has been found that low plasma levels of ascorbate often correlate with increased severity of disease and symptoms. In addition, it has been reported that when ascorbic acid is given at supraphysiologic levels, a variety of pharmacologic effects can be observed. In-vitro and in-vivo clinical research has allowed us to understand some of the mechanisms involved and even correlate concentrations to effect. Recent reports suggest that, even in high-income countries like the United States, a considerable proportion of the population have inadequate levels. Given the prevalence of ascorbate insufficiency and its role in maintaining homeostasis and tissue repair, it is important to evaluate plasma levels to properly assess the patient's health. This assessment could be useful to optimize outcomes by providing the nutritional and pharmacologic benefits of AA as needed. We present a guide to the interpretation of plasma ascorbate. This guide includes seven levels with the corresponding descriptor, intake levels, and plasma concentrations to facilitate clinical decisions pertaining to intravenous Ascorbic Acid therapy.

Keywords: Vitamin C, ascorbic acid, ascorbate, plasma concentration, therapeutic, deficient, insufficient

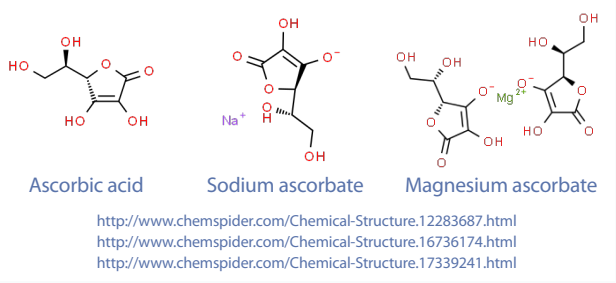
INTRODUCTION

Vitamin C, or Ascorbic acid (AA), is a nutritional factor in food that serves as an important cofactor for essential metabolic processes. Given in large quantities, AA has also been evidenced to execute favorable pharmacological activities in varied diseases.

AA is a neutral molecule with 6 carbon atoms, a ring structure with 6 oxygen, and 8 hydrogens, some of them forming hydroxyl groups with oxygen. When a hydrogen ion is lost (removed) from the hydroxyl group, the molecule becomes negatively charged and is called ascorbate. The ascorbates have a negatively charged anion associated with a positively charged cation. The anion component of the ascorbate donates electrons and is responsible for most of the biological effects. The cation can also have biological effects. Some of the ascorbate forms are sodium ascorbate, calcium ascorbate, magnesium ascorbate, potassium ascorbate, manganese ascorbate, and zinc ascorbate (Figure 1).

AA can be ingested in the form of ascorbic acid ascorbate. Regardless of the form received by the body, it will interact with other molecules and membranes in a physiological dance that will transform one form into another through perpetual cycles of simple oxidation-reduction reactions (RedOx) or complex conformational changes in DNA (Yoshikawa et al., 2003). This AA molecule's versatility is important for the process of photosynthesis and mitochondrial ATP production (Gonzalez et al., 2010).

Figure 1. Ascorbic acid (AA), sodium ascorbate and magnesium ascorbate



Research has demonstrated that AA is exceptionally safe in humans at very high doses and has relevant pharmacological and physiological pleiotropic effects (Padayatty et al., 2010; Magrì et al., 2020; Luchtel et al., 2020; Guo et al., 2022; Lee et al., 2019; Ried et al., 2016; Vollbracht et al., 2018; Emadi et al., 2019; Doseděl et al., 2021; Carr and Maggini 2017). These effects explain its wide array of clinical uses that improve patient outcomes in burns (McGregor & Biesalski, 2006), infections (Schencking et al., 2012; Chen et al., 2019; Gonzalez et al., 2018; Marcial-Vega et al., 2017; Mikirova et al., 2014), sepsis, and many other conditions (Holford et al., 2018; Fowler et al., 2019; Mahmoodpoor, 2021; Schencking et al., 2012). Despite its proven importance in physiological and pharmacological processes, most clinical curriculums fail to teach the importance of assessing ascorbate sufficiency (Mandl et al., 2009).

AA is a cofactor in hydroxylation reactions in the formation of various neurotransmitters, hormones, and in collagen stabilization, the protein that comprises 30% of human cellular protein mass (Ballaz et al., 2019; Gallie et al., 2013; Pandipati et al., 1998; Kishimoto et al., 2013). Ascorbate is also fundamental in RedOx homeostasis in the mitochondria and endoplasmic reticulum (Boel et al., 2019; Pozzer et al., 2021; Singh-Mallah et al.,. 2019). The RedOx potential of AA has a central role in the regeneration of vitamins within the body, such as alpha-tocopherol (Niki, 1987) and ubiquinone (Beyer, 1994). In addition, AA also Is involved in epigenetic regulation of genomic stability and has modulatory effects on nucleic acids and histones with implications for carcinogenesis and other relevant biological processes (Young, 2015, Brabson et al., 2021).

This article will discuss the physiological effects and response of the human body to a spectrum of concentrations of AA ranging from pathologically low to pharmacologically high and proposes a novel guide to its interpretation.

PHYSIOLOGIC ASCORBIC ACID

The Human Metabolic Disadvantage in Ascorbic Acid Synthesis

Contrary to humans, a vast majority of vertebrates can synthesize AA in accordance with physiologic demands. Typically, they convert D-glucose to AA through a biochemical pathway mediated by the enzyme l-gulono-g-lactone oxidase (GLO), which catalyzes the last step of AA biosynthesis. When sufficient AA is present, it helps control excess inflammation, support leukocyte function, inhibit microbial pathogen growth and neutralize harmful reactive oxygen species (ROS). However, humans lack a functional gene and, therefore, the GLO enzyme (Nishikimi et al., 1991). Homo sapiens are not able to synthesize AA and acquire the necessary amounts of this molecule by administering nutritional or pharmacologic doses, according to the current physiological demands.

AA Absorption and Plasma Concentrations in Health and Disease

In its reduced form, AA has a high electron-donating potential. Once oxidized to dehydroascorbic (DHAA) acid it may be converted back to the active reduced form. This gives the molecule the capacity to neutralize excess damaging reactive oxygen species and serve to provide stores/transports of the cell metabolic antioxidant potential (Cite). AA concentrations in body fluids and tissues are largely regulated through absorption, tissue accumulation, utilization, and renal reabsorption.

Inter-individual differences in genetics, metabolism, physiology, absorption, activity level, and body size affect the optimal amount of AA needed to maintain health. An individual's AA needs may also vary depending on the conditions that create an intense change in physiology such as (Long et al., 2003), infection (Tanzer, 1993; Li et al., 2006), as well as exposure to certain substances, all of which create excess oxidative stress, inflammation, and other increased metabolic demands. The utilization of AA increases during stress-inducing experiences, such as surgery Fukushima & Yamazaki, 2010), diabetes (Pecoraro & Chen, 1987), critically ill (Berger, 2015) patients after severe burns (McGregor & Biesalski, 2006), exposure to tobacco (Preston, 2006), and cancer (Mayland, 2005).

PHARMACOKINETICS OF ASCORBIC ACID

Absorption and Distribution

The absorption of AA is dose-dependent. Low, single oral doses of 30–180 mg/day are approximately 70-90%

absorbed. As single oral doses approach 1 g/day and higher, absorption falls to less than 50% and unmetabolized AA is excreted in the urine (Jacob, 2002).

The absorption of reduced AA or dehydroascorbic acid (DHAA oxidized form) in the intestinal membrane can occur via a family of sodium-dependent AA active transporters (SVCT) or through facilitated diffusion via Glucose (hexose) transporters (GLUT1 or GLUT3 transporters), respectively (Lykkesfeldt, 2019). The pH level in the intestine is also a regulatory mechanism of AA absorption (Sobala, 1989). The pharmacokinetics of AA is highly regulated by the transporters SVCT. Because there tSVCT1 is responsible for in whole-body dynamics of AA, while SVCT2 activity provides protection against oxidative stress in metabolically active cells (Savini 2008). There are many types of SVCTs distributed in the body and these can be tissue-specific. Therefore, the metabolic activity of cells and the density membrane transporters in a tissue facilitate the accumulation and storage of AA in tissues depending on primary need or susceptibility to depletion. This means that the distribution pattern of AA differs between and within organs and tissues. For example, the normal concentration of AA can vary from 0.2 mM in the muscle and heart, and up to 10 mM in the brain and adrenal gland (Lykkesfeldt &Tveden-Nyborg, 2019).

In lower physiological concentrations, AA time concentration curve follows a linear dose-dependent or constant (zero-order) behavior.However, at higher pharmacological concentrations it follows first-order pharmacokinetics, which means that at higher concentrations, the higher the clearance. Thus, the aggregate half-life of AA at pharmacologic concentration is about 2 hours after infusions 50 grams and higher and volume of distribution 0.19 L/kg (Padayatty, 2004).

This was observed in studies in patients with advanced cancers using 60 grams of intravenous (IV) AA (Nielsen, 2015; Stephenson, 2013). In a recent study in critically ill patients with septic shock, using 1.5 gm IV every 6 hours, AA volume of distribution was 23.3 L, and the half-life 4.3 h (Hudson, 2019). Although in these studies the authors have reported biological half-lives of AA that ranged from 2-4.3 hours/ This is likely an aggregate effect of tissue AA redistribution. Therefore, the actual elimination half-life of AA seems to be shorter (approximately 30 minutes) following a rapid IV administration, as can be seen in Figure 2 of the publication discussing AA Pharmacokinetics and its implications for oral and intravenous use (Padayatty et al., 2004)

Metabolism

AA takes part in a myriad of physiological reactions as a cofactor or as an electron donor. The reduced form AA donates two electrons to produce the oxidized form (DHA) serving its antioxidant function.

AA itself is oxidized to an intermediate, ascorbyl free radical, which at the systemic can convert to AA and DHA. DHA's biological half-life is a few minutes brief (Bode, 1990) because it is efficiently reduced intracellularly by a variety of cell types. DHA is generally reduced back to AA by enzymatic means, an efficient intracellular process in healthy individuals. However, smoking and disease states increase the turnover of AA requiring more intake to meet physiologic demands (cite).

Turnover of AA is associated with the catabolism of DHA, starting with hydrolysis through a series of enzymes with products entering the pentose phosphate pathway for further degradation (Banhegyi, 1997).

Excretion

AA is quickly eliminated through glomerular filtration with no significant reuptake. Following an intravenous high-dose AA, a biological half-life of about 2 hours has been reported (Padayatty, 2004). However, based on prior data from Levine and Padayatti, the actual elimination half-life of AA could be shorter than that (approximately 30 minutes) following a rapid IV administration (add citation date) [Padayatty, 2004]. The apparent discrepancy appears to come from the fact that biological half-live estimations do not rid of the impact that some concomitant events (e.g., distribution delays, lag time) have on drug disposition kinetics as the method to estimate the elimination half-life does (Nerella, 1993).

Therefore, it is expected that after achieving millimolar plasma concentrations by intravenous infusion, blood plasma levels are normalized to physiological levels in approximately 16 hours. However, disease states may alter excretion dynamics. Animal data support the hypothesis that tumor tissues maintain an elevated level for as much as 48 h (Campbell, 2016). This could be caused by increased tissue uptake related to metabolic use and increased tumor GLUT expression (cite) [Blaszczak et al 2019]. Increased tumor ascorbate was associated with slowed tumor growth, reduced tumor microvessel density, and decreased hypoxia. The hypoxic tumor environment does not appear to causally affect AA concentration.

Genetic Polymorphisms

Genetic polymorphism refers to allelic variations which alter the DNA sequence at a given locus. This variation can result in changes in proteins that may have functional or structural implications, such as the reduced affinity of an enzyme for its cofactor (Ames, 2004).

SVCT (sodium-dependent vitamin C transporters) are involved in the tissue distribution of AA. Thus, SVCT variants can result in reduced AA saturation in a specific tissue (Michels, 2013). Additionally, genetic variants of proteins can suppress oxidative stress or detoxify damaged biomolecules. The antioxidant enzyme glutathione peroxidase (GPx1) has an important role in determining the oxidative stress of individuals. It has been found that individuals with a less active genetic variant (GPx1 rs1800668 genotype) produce significantly less total glutathione, reduced/oxidized glutathione, and ubiquinone when compared to healthy individuals (Gugliandolo et al. 2016). Patients with more active glutathione or taking glutathione supplements will reduce oxidative stress; those that have a less active variant or are exposed to chemicals that produce oxidative stress. These effects have an impact on AA use and therefore its concentration level in the human body. The higher the level of reducing agents the lower the oxidative stress and therefore the lower the consumption of AA in redox functions.

Genetic variants in HP (Human plasma haptoglobin) GST, (glutathione-s-transferase) and SOD2 (superoxide dismutase) have known roles in oxidative stress (cite) (Sitar et al. 2013, Levy et al. 2010, Pintér et al. 2017, Manivasagam et al. 2020). Single nucleotide polymorphisms in each of these genes were found to be related to AA status. This suggests that genetic variations of other antioxidant-related genes could alter AA by utilizing less AA in redox activity. In summary, here is an indication that genetic alterations, in the form of single-nucleotide polymorphisms, gene duplications, or gene deletions, alter AA levels in the human body (Michels, 2013).

Epigenetic reprogramming in cancer cells involves DNA hypermethylation and histone modification (Yun, 2012). It has been found that TET (Ten-eleven Translocase) proteins can be activated by the AA as a cofactor. TET proteins are enzymes that can demethylate DNA. Neomorphic mutations of Isocitrate dehydrogenase expression can produce reduced TET activity increasing DNA methylation and promoting the expression of tumor-associated genes (Lu et al., 2012). In some lymphomas, AA enhanced mutated TET activity, leading to DNA demethylation, increased expression of tumor suppressor genes, and chemosensitivity (Shenoy, 2017).

Emerging evidence has suggested that the epigenetic mechanisms by which AA may enhance gene reprogramming in somatic cells are due to its cofactor role in Fe (II) and 2-oxoglutarate-dependent dioxygenases, including the TET and histone demethylases (Kuiper et al., 2014). Recently, Liu et al. examined the available evidence concerning the postulated role of AA in DNA and histone demethylation and highlighted its potential involvement in regulating N6-methyladenosine demethylation (2021). Liu et al. also indicated an affiliation of demethylases with AA-facilitated epigenetic reprogramming and a contribution of AA to epigenetic regulation (2021).

Prior studies have also shown that AA administered at high intravenous doses can suppress cancer cell growth through epigenetic mechanisms, namely DNA demethylation (Mastrangelo et al., 2018). Steers et al. have proposed that the co-administration of high IV-AA doses and DNA methyltransferase inhibitors may offer a therapeutic advantage in the treatment of pancreatic cancer through both direct cytotoxic mechanisms and epigenetic alterations (2021).

TOXINS AND DISEASE

Toxins, injury and disease create oxidative stress in various tissues, subsequently increasing AA body utilization and depletion if AA consumption is inadequate.

Tobacco smoke is a toxic substance consumed by humans that significantly impact AA dynamics Research has demonstrated that active smoking typically diminishes AA plasma by 25–50% (Lykkesfeldt, 2006), while passive tobacco smoke exposure reduces plasma AA concentrations by approximately 12-25% (Preston, 2006).

It has been proposed that critically ill patients can tolerate higher/ supratherapeutic doses of orally ingested AA without experiencing significant gastrointestinal upset. This method was coined "titrating to bowel tolerance". Cathcart reported that at least 80% of adult patients will tolerate 10 to 15 grams of AA per day without having diarrhea when AA was dissolved in water and given in divided doses (1981). The absorbed dose is proportional to the severity of the illness with intakes over 100 grams being tolerated. In the case of very toxic diseases, doses may have to be taken every half hour. Short delays in taking these doses may prolong the disease (Cathcart, 1981).

Absorption and distribution of AA into the diseased tissues occur at an accelerated rate, presumably because of increased AA metabolism. More specifically, it is a change in signaling and controls that open up transport channels. Therefore, to supply the metabolic demand, this frequent

dosing provides an adequate amount at an adequate rate (Cathcart 1981). Some conditions known to be associated with low levels of AA include cancer (Mayland 2005; Fritz et al., 2014), and viral illnesses (Tomasa-Irriguible & Bielsa-Berrocal, 2021), sepsis (Marik, 2018), and diabetes (Ali et al., 1989). Previously, a study had demonstrated that plasma total AA was significantly lower in individuals with diabetes compared to age and sex-match controls (N=100). In addition, in patients with diabetes and diabetic retinopathy, the plasma total AA was significantly lower than that of uncomplicated diabetics (Ali et al., 1989). Another controlled study showed that low AA levels in diabetes appear to be a consequence of the disease itself and not due to inadequate dietary intake of AA (Sinclair, 1994). Furthermore, the presence of complications seems to be an important prognostic factor in AA depletion. For example, diabetic patients with microangiopathy have lower levels of AA than age-matched diabetics without microangiopathy (Sinclair, 1991).

CANCER, INFLAMMATION, AND BACTERIAL/VIRAL INFECTION

A study of 50 patients recruited from a large hospice with advanced cancer from different types (i.e., brain, breast, bronchial, urogenital, gastrointestinal, prostate, etc.) found that 30% of individuals were deficient (<11 µM) and 42% were low (11.1 -23 µM) in plasma AA. Additionally, low plasma AA was found to be significantly associated with low albumin, low PLT, high CRP, and shorter survival (Mayland,2005).

AA at high doses (7,500 and over mg/day), especially when producing high micromolar or millimolar concentrations has been shown to exhibit anticancer (antineoplastic), anti-inflammatory, antioxidant, immunomodulatory, and antiviral effects among others (Levine et al., 2011; Sun et al., 2019; Nakajima et al., 2019; Cheng et al., 2012; Kim et al., 2013; Sorice et al., 2014; Feigen et al., 1982). There are many documented anticancer mechanisms described for AA, most notably, the preferential promotion of hydrogen peroxide and oxidative stress in cancer cells, AA-mediated downregulation of HIF transcriptional activity, and AA-regulation of epigenetic changes such as DNA demethylation. This DNA methylation process is facilitated by the ten-eleven translocation enzyme activation which results in the re-expression of tumor suppressor genes in cancer cells (Vissers & Das, 2018).

Intravenous AA at doses of 7.5-50 g can reduce inflammation by as much as 44%, as measured by C-reactive protein or CRP (Mikirova et al., 2012). Several studies describe the various mechanisms by which AA enhances the function of leukocytes. These include chemokinesis and chemotaxis (Schwager et al., 2015), phagocytosis (Shilotri, 1977), the production of lysosomal enzymes (Anderson 1982), the generation of reactive oxygen species (Sharma et al., 2004), microbial killing (Vilchèze et al., 2018), up-regulation of the antibody response (Mitsuzumi et al., 1998), and increased interferon production (Stone, 1980).

In addition, studies have shown many clinical benefits, including lowering infection risk (Vorilhon et al., 2019; Kim et al., 2018). Studies in septic mice suggest that an rate in septic mice occurs by activating Nrf2/HO-1 signals (Kim et al., 2015).

In-vitro observations with pharmacologic concentrations of AA (millimolar range) suggest a direct antiviral effect (Furuya et al., 2008; Shatzer et al., 2013), consistent with clinical observations of patients with Epstein-Barr viral (EBV) infection (cite). Moreover, intravenous AA has demonstrated clinical benefits against different viral infections, including SARS-Cov-2 (Schencking et al., 2012; Chen et al., 2019; Gonzalez et al., 2018; Marcial-Vega et al., 2017).

The use of AA as an effective antiviral has been documented as early as 1949 when Frederick R. Klenner reported the ability of AA to potentially cure many different acute infectious diseases and to neutralize toxins (Klenner, 1949; Klenner 1971). The caveat was that the AA needs to be provided in sufficient doses, repeated at short intervals, and continued for a long enough period. Klenner claimed that AA is a powerful oxidizer and when given in massive amounts such as 50 grams to 150 grams, intravenously (Klenner, 1971). Klenner's report detailed that supratherapeutic doses (1000 -2000 mg) of AA, administered orally or intramuscularly led to the resolution of poliomyelitis in 60/60 (100%) patients (Klenner 1949). He also reported the cure of advanced polio and its associated flaccid paralysis with AA in 1951. Other clinicians supported Klenner's reports of AA's therapeutic effect on polio (Greer, 1955; Baur, 1952).

These results are consistent with previous in-vitro and in-vivo research that has shown that AA inactivates polio, herpes, vaccinia (Kligler, 1937; Turner, 1964), tobacco mosaic (Lojkin, 1936), bacteriophage (Lominski,1936; Murata, 1975; Morgan, 1976; Richter, 1982), enteroviruses (Salo, 1978), influenza (Cheng, 2012; Chen, 2014), and rabies (Amato, 1937) viruses.

Intravenous AA administration has been successfully used (complete clinical recovery) in the treatment of viral encephalitis (Klenner, 1949; Klenner, 1951, Klenner 1953; Klenner, 1971), viral pneumonia and bronchitis (Dalton 1962), measles (Joffe, 1983), mumps (Karam, 1953), Herpes

(Zureick, 1950), influenza (Cai, 2015) and rabies in guinea pigs (Banic,1975). Human case reports have also supported that the intravenous administration of AA is useful in the treatment of influenza (Vilchèze et al., 2018) mononucleosis (Mikir ova, 2014), chikungunya (Marcial-Vega, 2015, Adrover, 2015), Zika (Gonzalez, 2016), and SARS COV-2 (Gonzalez, 2020).

INTRAVENOUS ADMINISTRATION

Klenner's extensive work with intravenous AA infusions in 1949 significantly influenced the development of the Riordan Clinic, founded in 1975 (under the name The Center for the Improvement of Human Functioning). By 2015, Riordan Clinic had delivered over 70,000 infusions (in a period of 40 years) with a low frequency of mild to moderate, and usually transient, side effects (Riordan Clinic, 2015). This is consistent with the study of FDA's Adverse Events Database and a survey of 172 practitioners who administered IV-AA to 11,233 patients in 2006 and 8,876 patients in 2008. The average dose was 28 grams every 4 days, with 22 total treatments per patient. Adverse events were reported in 101 patients, including lethargy/fatigue in 59 patients, change in mental status in 21 patients, and vein irritation or phlebitis in 6 patients (Padayatty et al., 2010).

High-dose intravenous AA (HDIVAA) has been used as therapy for a variety of conditions ranging from infectious diseases of bacterial and viral origin to adjuvant therapy for cancer, and many others. A clinical protocol developed over the past several decades utilizing HDIVAA summarizes principles of treatment, rationale, baseline workup, infusion protocol, precautions, and side effects (Riordan et al., 2003).

Precautions

Renal function and hydration – A prospective study of 157 patients receiving intravenous vitamin c supplementation (IVC) determined that IVC was not clearly associated with patient-reported renal stones (Prier et al 2018). Adequate renal function, hydration, and urine voiding capacity must be documented prior to starting high-dose IVC therapy. Calcium oxalate stones during or following IVC are rare (Riordan et al., 2005). In a later study conducted in a group of 16 healthy individuals with normal renal function, intravenous doses ranging from 0.2 to 1.5 g/kg body weight less

G6PD – Hemolysis has been reported in patients with glucose-6-phosphate-dehydrogenase (G6PD) deficiency when given a high dose of intravenous AA (Campbell et al., 1975). Therefore, an assessment of the G6PD level is necessary before beginning IVC.

Transient electrolyte disturbance – Due to the chelating effect of IVC, some patients may complain of shakiness due to low calcium or magnesium. An additional 1.0 mL of MgCl added to the IVC solution will usually resolve this. If severe, it can be treated with an IV push of 10 mL of calcium gluconate, 1.0 mL per minute (Riordan et al., 2003).

Venous irritation – IV irritation may occur at the infusion site. This can be caused by an infusion rate exceeding 1.0 gram/minute. The protocol suggests adding magnesium to reduce the incidence of vein irritation and spasm (cite) (Riordan et al. 2003).

Osmolarity and pH – To facilitate comfortable infusion, in addition to infusion rate and other additives previously mentioned, osmolarity and pH are important factors. Osmolarity refers to the concentration of the solute or the number of solute particles per 1 L of solvent. The pH is the concentration of hydrogen ions, H+, in a solution. Human studies of osmolarity-induced phlebitis have arrived at different conclusions, but the most often cited reference found the lowest risk of phlebitis occurred with solution osmolarities under 450 mOsm/L, moderate risk at 450 to 600 mOsm/L, and the highest risk over 600 mOsm/L (Gazitua et al 1979). Human trials measuring the impact of pH on peripheral veins found that neutralizing the pH to 7 – 7.4 significantly reduced the incidence of phlebitis (Eremin &Marshall 1977; Fujita et al 2000)

Table 1 lists the calculated osmolality of various amounts of fluid volume. Our experience has found that osmolality of less than 1200 mOsm/kg H2O is tolerated by most patients. A low infusion rate (0.5 grams IVC per minute) also reduces the tonicity, although up to 1.0 grams per minute can be used in order to achieve higher post IVC saturation levels. (Pre and post serum osmolality measurements are advisable at this dose as per the Riordan Protocol (Riordan et al. 2003).

Table 1. Recommended Dilution and Osmolarity

AA grams per volume in the vial at 500 mg/mL	Recommended Dilution Dilute	Osmolarity mOsm/L
15 gm > 30 mL	250 mL Ringers	909
25 gm > 50 mL	5000 mL Ringers	7959
50 gm > 100 mL	500 mL H2O	1097
75 gm > 150 mL	750 mL H2O	1088
100 gm > 200 mL	1000 mL H2O	1085

THE NEED FOR NEW GUIDELINES

Since humans cannot synthesize AA, they are dependent on dietary intake and or supplementation. Contrary to expectations, vitamin insufficiency is common even in high-income countries. Since AA demands increase during stress, it is often depleted in patients with varied conditions, Understanding AA plasma concentration could be a useful tool for patient assessment and monitoring. The amount of AA needed to prevent acute scurvy is small and believed to be obtained in Western diets. However, the United States: 2003-2004 National Health and Nutrition Examination Survey (NHANES), indicated that the prevalence of low plasma AA concentrations (insufficiency) is as high as 22% to 33%, with 7% to 14% of people showing scorbutogenic deficiency (Schleicher, 2009; Cahill, 2009). However, data from subsequent National Health and Nutrition Examination Survey (NHANES) 2005-2016, revealed an increased prevalence of insufficient AA (inadequate) intake of 46% (Carroll, 2020). Studies in India, Malaysia, and China demonstrate similar or higher deficiencies of AA(Hughes, 1999). In Mexico,23% of children and 39% of women present with vitamin C deficiency (Villalpando, 2003). More recently, AA insufficiency among healthy people in the USA was reported to be 45% (Reider et al, 2020). Given these statistics, it may be presumed that the vast majority of people with certain risk factors or patients with acute or chronic conditions are depleted of this vitamin, which makes them more vulnerable to slow recovery or suboptimal clinical outcomes.

INGESTION, DOSING, AND PLASMA CONCENTRATIONS

Scurvy

Scurvy has been defined as a collection of symptoms related to deficient AA in the body (anemia, myalgia, edema, petechiae, gingivitis, poor wound healing, and others). These symptoms are associated with plasma AA levels below 1.5 mg/L (0.0085 mM/L, 8.5 µM/L) (Hage 2018) or below 1.9 mg/L [0.011 mM (11 µM/L)] [The unit formats µM µmol/L etc are varying I suggest µM/L for consistency] (Nyyssönen 1997; Food and Nutrition Board, 2000).

Marginal Hypovitaminosis

Marginal hypovitaminosis or low plasma levels is a state of minimal reserves which can lead to scurvy. Hypovitaminosis is characterized by AA concentrations below 23 µM/L (Smith, 1987; Carr, 2016; Carr, 2016; Jacob, 2002). It is assumed that adequate AA levels depending on the criteria, is likely anything above 23 µmol/L. More generously, as recommended by a group of European Countries, about 50 µM/L can potentially compensate for some normal metabolic losses (Krajcovicova-Kudlackova, 2007; Brubacher, 2000, EFSA NDA Panel, 2000). Consumption of 5-to 9 servings of fruits and vegetables daily or a 200 mg AA supplement has been estimated to produce near steady-state AA plasma concentrations of 70-80 µmol/L (Levine, 1996).

Oral Ingestion

Vigorous oral ingestion results in peak values that reportedly do not exceed 220 µmol/L in healthy volunteers (Padayatty, 2004). The dynamic flow model proposes restoring human physiology to nearly that of animals that synthesize their own AA. The mean and minimum plasma levels in dynamic flow are consistent levels of about 220 µM (Hickey, 2005).

Intravenous

Only when AA is given intravenously in multi-gram doses can a supraphysiological (millimolar) concentration can be achieved. A supraphysiological concentration of AA has been reported to have important pharmacologic properties and a significant impact on patient outcome (González, 2002; Verrax, 2009; Riordan, 2004; Chen, 2005; Takahashi, 2012; Raymond, 2016; Ma, 2014). Doses around 1.5 mg/Kg and up to 100 gm of intravenous AA have been shown to produce concentrations between 25-30 mM/L (Hoffer et al., 2008; Monti, 2012).

A NEW OPTIMAL CONCENTRATION AND DOSING SCHEME

Nearly 20 years ago, a previous guide for interpretation of plasma AA interpretation by Jacob and Sotoudeh (2002) proposed 3 levels: adequate (>23 µM), low (23 – 11.4 µM), and deficient (<11.4 µM) (2002). Levine et al. (1996) suggest 70-80 microM/L as a safe level when making dietary allowance recommendations. Despite being valuable, this guideline omits concentrations achieved when patients are receiving a clinically relevant range of oral doses and intravenous doses of AA. In our proposed table we include two levels of oral supplementation and two levels of intravenous dosing to serve as a guide for clinical decisions. This guide includes some physiological or pharmacological effects, dosing, and range in concentrations in both mg/L and µM units. However, chronic conditions such as cancer and diabetes, toxins, and trauma can be an important and dynamic source of AA turnover.

At this time, there is insufficient evidence to determine the optimal concentration and dosing regimen for each condition. A patient with a serious infection, cancer, or trauma

might need different frequencies and the optimal concentration might vary according to severity, comorbidities, and other factors. In the case of cancer, it is thought that effective distribution of AA is necessary throughout the tumor environment. (Vissers & Das, 2018).

A dosing regimen that is smaller in magnitude and more frequent will produce less fluctuation in AA plasma concentration but may be more difficult to achieve than single high dose administration. For the most severe cases, the preponderance of the data so far supports that robust intravenous doses are necessary to produce the best results.

Table 2. Guide to AA Plasma Concentration Interpretation

Pathophysiologic and Pharmacologic Description	*AA Plasma concentrations
Deficiency (Scurvy), lowest plasma level – weakness, tiredness, anemia, gingivitis, poor wound healing, ecchymosis (Intake of AA < 10 mg/d oral)	< 0.2 mg/dL (<=17 µM) 1236 *Dose-concentration relationship altered by physiologic stress
Insufficiency, low plasma level (minimal reserves, also called hypovitaminosis) Nonspecific Symptoms i.e., fatigue, irritability) <75 mg/d	0.2- 0.49 mg/dL (11-28 µM) [1,4,5,5,7,8]
Nutritional plasma level (dietary AA produces physiologic concentrations) 5 to 9 servings of fruits and vegetables or low oral supplementation 100 - 200 mg/d	0.5-1.41 mg/dL (28 -80µM) [1,6,8.9,10,11] Only the upper part of this range is within RDA intakes/levels.
Oral Moderate Supplementation level (Preventive, risk reduction, for low level stressors; 500 mg – 3,000 gm/d)	>1.42-1.97 mg/dL (84-112µM) [11,12]
Oral High Supplementation level (Low Pharmacologic level) Oral > 3 g/d (i.e., 1.5 gm TID to 3 gm 6x d)	>2.0-3.9 mg/dL (112-220 µM) [12,13]
Intravenous Moderate level (Moderate Pharmacologic) 10-50 gm (uM = micromolar to mM = millimolar)	22.0 — 175 mg/dL, (1.25-10 mM) [12,14,15,16,17,18]
Intravenous High level (High Pharmacologic) >50 gm intravenous dose (over 100 gm may cause physiologic saturation and ADR's such as thirst)	194 - 528 mg/dL, (11-30 mM) [12,14,15,16,17,18]

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ACHIEVING ORAL HIGH SUPPLEMENTATION LEVELS

AA is available in liposomal formulation for oral consumption. Liposomes are a pharmaceutical delivery system consisting of microscopic sphere-shaped vesicles composed of phospholipid bilayers that encapsulate the active ingredient inside. The liposome can differ in particle size, composition, and charge, and drug carrier loaded with a variety of molecules and it is used for the purpose of protecting a compound from gastrointestinal degradation, reducing gastrointestinal adverse effects of the drug, and/or enhancing its absorption into the systemic circulation.

A bioavailability study conducted in the USA indicated that oral delivery of 4 g of AA encapsulated in liposomes produces circulating concentrations of AA that are 35% greater (AUC 0-4 h) than unencapsulated oral supplements and provides a similar level of protection from ischemia-reperfusion-mediated oxidative stress compared to unencapsulated oral and intravenous administrations (Davis et al., 2016). A different liposome increased half-life by 50% and elevated AUC 80%, and further evaluation of MTT tests in MCF7 cancer cell cultures demonstrated potency on the cellular level (Łukawski et al., 2020). Another clinical study of liposomal AA was demonstrated to be 1.77 times more bioavailable than non-liposomal AA (Gopi &Balakrishnan, 2020).

In summary, oral liposomes provide an enhanced bioavailability while improving tolerance. Presumably, the tissue distribution should be different because it may not entirely depend on the same transport mechanisms (glut, SVCT). These pharmacokinetic differences in distribution may impact the duration of action and may provide some therapeutic benefits.

CONCLUSION

In addition to the remarkable track safety record of intravenous and oral AA, there are a range of favorable physiological and pharmacological actions of AA in managing a variety of conditions.

In conclusion, AA is an essential nutrient responsible for an immense variety of physiological processes. National surveys in the USA and other countries have reported that 40% of the population has inadequate ingestion of this nutrient to meet the body's basic demands, which is exacerbated in physiologically stressful conditions Given these the body's needs are increased during physiologic stress and a large proportion of the population is presumably experienced transient or permanent AA insufficiency.

A vast body of research literature has demonstrated the pharmacologic activity of AA (antimicrobial, sepsis, anticancer, and others) when given at high levels especially intravenously. This notion that nutrients at higher concentrations can have additional pleiotropic actions is the essence of orthomolecular medicine and can be referred to as orthomolecular pharmacology. The proposed guide for plasma AA concentration can help the clinician to interpret the current condition of the patient and serve as a clinical guide, especially when applying intravenous AA as an adjunctive therapy.

DEDICATION

To the high dose vitamin C pioneers that gave us the light of understanding: Drs. Frederick R. Klenner, Robert F. Cathcart, Linus C. Pauling, Abram Hoffer and Hugh D. Riordan. Also, to our youngest and bravest inspiration that gave us the needed focus to never miss a step and the necessary courage to keep fighting: Gladys Isabel Rodriguez

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