REVIEW ARTICLE

Pharmacokinetics of Vitamin C: insights into the oral and intravenous administration of ascorbate

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There is a strong advocacy movement for large doses of vitamin C. Some authors argue that the biological half-life for vitamin C at high plasma levels is about 30 minutes, but these reports are the subject of some controversy. NIH researchers established the current RDA based upon tests conducted 12 hours (24 half lives) after consumption. The dynamic flow model refutes the current low-dose recommendations for dietary intakes and links Pauling's mega-dose suggestions with other reported effects of massive doses of ascorbate for the treatment of disease. Although, a couple of controlled clinical studies conducted at The Mayo Clinic did not support a significant benefit for terminal cancer patients after 10 grams of once-a-day oral vitamin C, other clinical trials have demonstrated that ascorbate may indeed be effective against tumors when administered intravenously. Recent studies confirmed that plasma vitamin C concentrations vary substantially with the route of administration. Only by intravenous administration, the necessary ascorbate levels to kill cancer cells are reached in both plasma and urine. Because the efficacy of vitamin C treatment cannot be judged from clinical trials that use only oral dosing, the role of vitamin C in cancer treatment should be reevaluated. One limitation of current studies is that pharmacokinetic data at high intravenous doses of vitamin C are sparse, particularly in cancer patients. This fact needs prompt attention to understand the significance of intravenous vitamin C administration. This review describes the current state-of-the-art in oral and intravenous vitamin C pharmacokinetics. In addition, the governmental recommendations of dose and frequency of vitamin C intake will also be addressed.

Key words: Vitamin C; Pharmacokinetics; Intravenous; Mega-Dose; Dynamic Flow.

A well-known article published in 1979 by Cameron, Pauling and Leibovitz established the scientific basis to support the use of vitamin C as a therapeutic weapon in the battle against cancer (1). As early as 1949, the use of ascorbate in cancer treatment was documented (2). Another publication from 1952 describes the use of ascorbate as a chemotherapeutic agent (3). Hundreds of articles describing an array of related clinical and preclinical studies have been published in peer-reviewed journals, including the paper by Cameron and co-workers, which is considered to be the first comprehensive review on this topic (1, 4-10).

Although certain discrepancies have arisen since then, there are a number of reports in the literature that suggest a relationship between the presence of cancer and the metabolism of ascorbate (11). The evidence for this relationship hinges on two types of data: the relative levels of ascorbate in normal versus tumor tissues, and the utilization of ascorbate by the cancerous organism.

Consequently, the question whether the concentration of ascorbate is altered in tumors has been the subject of considerable controversy. There is evidence that high doses of vitamin C are required to yield systemic concentrations that in turn could become cytotoxic to tumor cells (12). However, how much vitamin C needs to be administered via either the intravenous or per-oral route to achieve the desired concentration as well as how long does it take are questions that remain to be answered. Further assessment of the pharmacokinetics of vitamin C is needed to address these questions.

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Several studies in the literature show that the urinary excretion of ascorbate is reduced in patients with cancer when compared to healthy individuals. Spellberg and Keeton have reported that after continued daily oral administration of 400 mg of vitamin C, healthy persons excreted 56 to 80% of intake, whereas cancer patients excreted only 34 to 48% (13). Likewise, Bodansky reported a daily urinary excretion of vitamin C in two cancer patients that ranged from 5 to 10% of the intake (11). This decreased percentage of ascorbate excretion in urine after daily oral intake under a controlled metabolic regimen, reflects a higher consumption of ascorbate by cancerous patients. This is probably related to certain metabolic differences between patients and healthy individuals. Studies by Holmes and co-workers indicated that healthy persons excrete about 30 to 50% of a daily intake of about 100 mg ascorbate and even higher percentages at greater intakes (14-15).

In view of the conflicting nature of the evidence that is described in the specialized literature about the role of disease-state (i.e., cancer) on vitamin C turnover, as well as the fact that relatively few reports on the intravenous pharmacokinetics of vitamin C are available, a review on the pharmacokinetics of vitamin C will help direct future research and provide a better guidance to vitamin C dosing in cancer patients.

Background

The controversy about vitamin C requirements continues to this day. The mega-dose hypothesis, popularized by Pauling and Stone, suggests that individuals need 4-20 grams of oral ascorbate per day (16-17). They based their hypothesis largely on evolutionary arguments. Most animals synthesize large amounts of ascorbate internally or, less commonly, obtain equivalent gram-level intakes from their diets. Animals also increase their production of vitamin C when they are diseased. Therefore, it was proposed that higher doses provide increased resistance to many, if not all, diseases.

Shortly after Pauling's death, researchers from the National Institutes of Health (NIH) published various papers on the pharmacokinetics of oral ascorbate (18-20). The results led to a widespread assumption that the mega-dose hypothesis, at least for cancer, was wrong. In fact, it was reported that doses of vitamin C as low as 200 mg per day saturate the body and, therefore, higher doses seem to be unnecessary. This saturation claim was highly influential, becoming a cornerstone of the Recommended Dietary Allowance (RDA) (21-27).

Reports in the literature suggest that a minimal intake (i.e., 90 milligrams a day) may not be optimal because low doses could result in degenerative diseases, a compromised immune system and reduced ability to respond to stress (16-17).

Pharmacokinetic reports

Recently, a dynamic flow model for vitamin C has been postulated by Hickey, Roberts and Cathcart (28). They bring together evidence from both sides of the argument, seeking to answer the apparent contradictions. According to these authors, if interpreted correctly, the NIH data supports the claim for higher daily intakes of ascorbate (28). In their paper, it is stated that the plasma ascorbate levels corresponding to varying intakes of ascorbate exhibit dual-phase pharmacokinetics. The first phase occurs when blood levels are below 70 µM (0.123 mg/dl). In this stage, the kidney's sodium-dependent vitamin C transporters (SVCT) reabsorb ascorbate (28-30). While the ascorbate concentration remains relatively low, the transporters pump ascorbate back into the blood stream preventing its loss by renal clearance. The switch toward a second stage begins when the blood ascorbate concentration becomes higher (over 70 µM), and the body excretes ascorbate more rapidly (31).

The plasma half-life of ascorbate is widely reported to be between 8-40 days (24, 32). However, this applies only to periods of deficient intake, when the renal transporters actively reabsorb the vitamin to prevent acute scurvy. When intake levels are higher, more rapid excretion occurs. During this phase, ascorbate has a half-life of about 30 minutes. The previously discussed NIH pharmacokinetic data clearly shows a rapid excretion decay slope after intravenous doses (18, 20).

The NIH group performed a series of pharmacokinetic experiments suggesting that after oral administration of vitamin C, plasma becomes saturated at approximately 70 µM (18, 20). Nonetheless, subsequent papers suggested sustained and higher plasma levels of at least 220 µM, following oral administration, which are consistent with other reports in the literature (33-36). As a matter of fact, the Hickey group has measured peak plasma levels of approximately 250 µM after a single 5-grams oral dose of vitamin C (personal communication; 2007).

Interestingly, Padayatty and co-workers recognized that intravenous doses can produce plasma concentrations 30- to 70-fold higher than the maximum tolerated oral doses. Although patient data were not available to confirm pharmacokinetic modelling at high doses and in patients with cancer, these results led to a reconsideration of the role of vitamin C in cancer treatment. In fact, data showed that intravenous administration of vitamin C produces substantially higher plasma concentrations than those that can be achieved by oral administration (33). That is, the long-lasting dogma of no benefit from mega-doses of

vitamin C can no longer be sustained. This dogma is highly based on some placebo-controlled trials in cancer patients after high dosage (e.g., 10 grams daily) of vitamin C given orally. Two randomized, double blind, placebo-controlled studies from the Mayo Clinic found no benefit of high dose oral ascorbate on terminal cancer patients (37-38). These studies included 200 patients who were treated with 10 grams of vitamin C daily. The Mayo Clinic studies were considered to be definitive even though vitamin C was only given orally once a day. Therefore, the Mayo Clinic studies neither support nor refute possible effects of intravenously administered vitamin C on cancer. Moreover, the Mayo Clinic reports also have little relevance in terms of repeated oral doses that may result in sustained plasma ascorbate levels. Keep in mind that the larger the oral vitamin C dose, the more incompletely absorbed from the gut. In addition, the dosing interval was too long with respect to the half-life of ascorbate.

Based on the conclusions in Padayatty's paper, intravenous vitamin C may have a role in the treatment of cancer as a result of the plasma concentrations that can be achieved only by this route. According to the report by Padayatty et al., with consumption of 5 to 9 servings of fruits and vegetables daily, steady-state plasma concentrations are 80 µM or less, and peak values do not exceed 220 µM, even after maximum oral administration of 3 grams given every 4 hours (i.e., 6 times daily) (33). Notice that Padayatty and coworkers used their own pharmacokinetic model for a computer-aided extrapolation in order to estimate these figures. However, others have obtained higher values after a single 5 grams oral vitamin C dose (Steve Hickey, personal communication; 2007).

By contrast, intravenous vitamin C may produce plasma concentrations as high as 15,000 µM. It has been determined that at extracellular concentrations greater than 1,000 µM, vitamin C is toxic to cancer cells, although exact mechanisms and interpretation are still controversial. In fact, the actual minimum ascorbate concentration to kill cancer cells will depend on the cell line because some cancer cells have increased sensitivity. As was confirmed by data from the NIH study, apoptosis is induced even when ascorbate concentrations as low as 200 – 300 µM are maintained for one hour (39). The vitamin C free radical species, ascorbyl radical, is detectable in animals only when they receive an intravenous vitamin C dose equivalent to 10 grams in humans (33).

Riordan and co-workers built a two-compartment model with four adjustable parameters in order to fit their experimental data after intravenous vitamin C, as depicted in Figure 1. They postulated a structural two compartment pharmacokinetic modeling approach using a first-order rate constant that represents the excretion of ascorbate out of the blood by renal mechanisms, and two inter-compartmental rate constants to account for the rate of diffusion of ascorbate between the blood and peripheral tissues. According to the authors' report, the excretion constant was found to be remarkably uniform, as was the ratio between both inter-compartmental distribution constants. In fact, they observed that the tissue uptake rate constant did vary, but not in a systematic way, being compensated by the variation in the other distribution constant for ascorbate's return to the blood stream from tissue compartments. Notably, they also suggested that the ascorbate pharmacokinetics for subjects under study were the same over at least three months of treatment. This further supported their conclusion that ascorbate transport parameters for a given subject remain relatively constant during the treatment period (40).

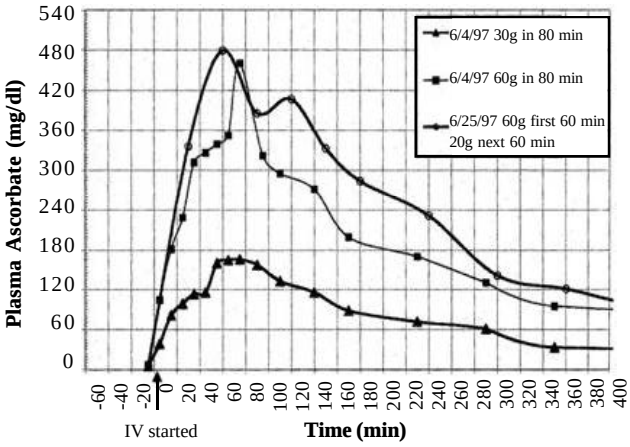

Figure 1. Vitamin C disposition profiles after intravenous infusions in a 72 years-old patient. This figure is electronically reprinted with permission from the International Society for Orthomolecular Medicine (ISOM), the publisher of Journal of Orthomolecular Medicine (taken from Riordan NH, Riordan HD, Casciari JP. Clinical and Experimental Experiences with Intravenous Vitamin C. J of Orthomolecular Medicine 2000; 15(4): 201-213)

Overall, Riordan and co-workers showed evidence that the concentrations of vitamin C to kill tumor cells can be achieved in humans following intravenous infusion scheme. That is, a short-term intravenous bolus before a slow infusion rate of vitamin C can result in sustained plasma ascorbate concentrations that will be therapeutically effective in humans. Modeling vitamin C pharmacokinetics the authors accurately predicted plasma concentrations of ascorbate using varied infusion protocols. In addition, they reported that vitamin C in blood concentrations achievable through oral supplementation was capable of increasing collagen production by tumor cells.

Vitamin C in doses up to 50 grams per day, infused slowly, was not toxic to cancer patients and, even more importantly, some patients had complete remission after high doses following intravenous infusion. Although concentrations that kill most tumor cells (i.e., 200 - 400 mg/dl) were not achieved after infusion of 30 grams of vitamin C, remissions were still observed in patients treated with this dose level, which was assumed by the authors to occur as a result of vitamin C induced biological response modification rather than its cytotoxic potential (40). It could also result from the subject simply having a high level of coper or iron that enhances hydrogen peroxide production by ascorbate in a Fenton-like reaction. The cytotoxic action of vitamin C is greatly enhanced by the presence of synergistic substances such as vitamin K3 or other quinones, lipoic acid, oxygen and futile redox cycling substances. The RECNAC team at Whichita, KS have tested the anticancer efficacy of vitamin C and other antioxidants using the hollow fibre solid tumor model at clinically achievable concentrations finding that ascorbate efficacy was enhanced by lipoic acid and K3 (41). As an example, Evens and co-workers recently reported that Motexafin Gadolinium, a redox cycling quinone, shifts the ascorbate requirement downward to a lower selective cytotoxic concentration of only 100 μM (42).

Certainly, determination of the appropriate model for ascorbate has been particularly difficult because of saturability in both absorption and renal excretion, the latter being the major elimination pathway at high doses (32, 43). Ralli *et al.* summarized the relation between renal clearance and plasma concentrations at steady-state for ascorbate by using a model developed for glucose, which fits the observations well at higher concentrations (≥45.4 μM) but did not predict the virtually zero renal clearance at low ascorbate concentrations (< 45.4 μM) (44).

Blanchard et al. presented their "ceiling effect" hypothesis for ascorbate pharmacokinetics after megadoses (45). They argued that as the daily oral dose is increased, the background concentration of ascorbate in the plasma and other body compartments does not increase proportionally, but instead tends to approach an upper limit. For example, when the daily dose is increased from 200 to 2,500 mg (1.1 to 14.2 mmol) the peak plasma response increases, but the background concentration increases only from 12 to 15 mg/L (68.1 to 85.2 μM).

Blanchard and co-workers based their arguments on the observed renal ascorbate clearance changes, which rose sharply with increasing plasma ascorbate concentrations as a result of saturable tubular reabsorption. Their analysis indicated that both saturable gastrointestinal absorption and nonlinear renal clearance act additively to produce the ceiling effect in plasma concentrations. As a consequence of this phenomenon they concluded that there was no pharmacokinetic justification for the use of megadoses of vitamin C in therapy. However, these authors only focus their analysis on pharmacokinetic data from young, healthy male volunteers instead of cancer patients. That is, the implications of the disease-state condition on ascorbate pharmacokinetics must be considered as a factor at the moment of analyzing the pharmacokinetic perspective of megadose use.

In a recent work, we conducted a pharmacokinetic characterization of six different intravenous vitamin C infusion dosage scheme given at high doses, following dose-escalation protocols (15 to 65 g), in a 75 year-old prostate cancer patient. The results for this pilot pharmacokinetic study of vitamin C at high dose infusions in a cancer patient suggest a dual-phase kinetic behaviour of ascorbate (Figure 2). This disposition pattern depends on the actual infusion-generated plasma ascorbate concentrations with respect to the saturation cut-off level (ca. 70μM = 0.123 mg/dl). That is, the rapid ascorbate clearance we observed at the administered high dose could be assumed to be a result of saturation of kidney's ascorbate reabsorption after renal excretion and absorption into tumor. A physiological-based pharmacokinetic model including the renal reabsorption-associated non-linear (capacity-limited) clearance component will be highly recommended for further characterization of vitamin C disposition in cancer patients (46).

Nonetheless, considering that many anti-neoplastic agents are highly schedule dependent, caution must be taken while interpreting results from *in vivo* experimental studies, and their comparison to one another, if different dosing schedules were used. Reportedly, the plasma ascorbate level after infusion of 75 grams, over 75 min, for a 60 year-old, 105 lb. female breast cancer patient was 5.21 μg/dL. Strikingly, the same patient one month later was infused with 50 grams in 30 minutes, and then continued with another 25 grams over the next 90 minutes, the resulting plasma ascorbate concentration at steady-state was 4.23 μg/dL. According to Dr. Warnock, the results suggest that once blood levels of ascorbate are established, it is possible to maintain them over a period of time with a much lower amount. The infusion rate over the first 30 minutes was about 1.6 grams per minute and over the next 90 minutes was about 0.3 grams per minute. Dr. Warnock is currently planning to extend the lower infusion rate out to 3 hours if the patient consents and take another ascorbate level. Previously, this patient had been receiving 75 grams of vitamin C three times a week for about 2 months, but she did not seem to be

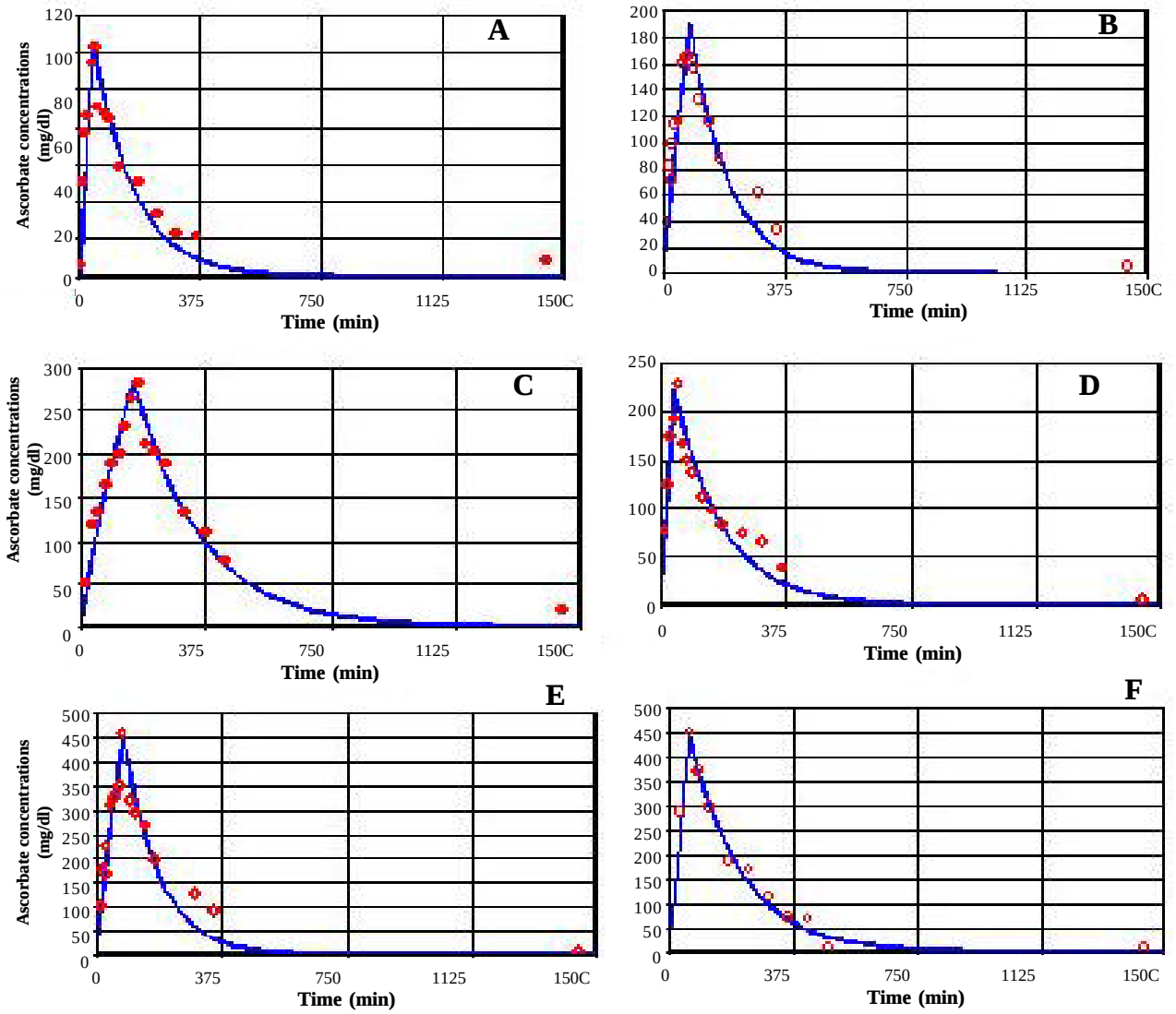

Figure 2 Plasma vitamin C disposition exponential profiles in a male 75-years old prostate cancer patient after curve-fitting by using a mono-compartment model (intravenous infusion input). Panels represent different IV infusion protocols: **A**, 15g of AA in 45 min (day 0, R_0=20 g/hr); **B**, 30g of AA in 80 min (day 5, R_0=22.5 g/hr); **C**, 60g of AA in 160 min (day 6, R_0=22.5 g/hr); **D**, 30g of AA in 40 min (day 11, R_0=45 g/hr); **E**, 60g of AA in 80 min (day 12, R_0=45 g/hr); **F**, 65g of AA in 60 min (days 17, R_0=65 g/hr). Note: F panel is a single profile representing all the three R_0=65 g/hr infusion protocols given on days 17, 18 and 24.

making significant progress with her cancer condition (personal communication, W Warnock; 2006).

Bioavailability

Hickey, Roberts and Cathcart's paper suggested that the NIH's misinterpretation of oral ascorbate bioavailability has an impact on the proposed RDA for vitamin C (28). According to the NIH viewpoint, the ascorbate bioavailability is maximized at a dose of 200 mg. This statement assumed a complete absorption of ascorbate by the body at this dose, or at lower intakes, whereas a smaller proportion (although a higher absolute amount) will be absorbed after higher doses.

Notably, as Hickey and co-workers pointed out, the short half-life of vitamin C during the rapid excretion phase is sometimes ignored (28). Plasma levels above 70 µM have a half-life of approximately 30 minutes, so large doses taken several hours apart should be considered independent, as should be their bioavailability. This cumulative pattern means that splitting a single large dose into several smaller ones, taken a few hours apart, increases the effective bioavailability of the large dose. This schedule-

dependence phenomenon needs to be taken into consideration for any further interpretation of ascorbate pharmacokinetic and pharmacodynamic results.

Cathcart's bowel tolerance method indicates that individual bioavailability can vary by a factor of at least two orders of magnitude, thus confirming that any variation depends upon the individual's health status (47). Accordingly, bioavailability is not a static property of ascorbate, but is subject to individual differences and varies with the timing of the dose. It follows that the appropriate intake will vary widely, both between individuals and also over time for the same person, depending on factors such as state of health and intake patterns, among others.

Blanchard and co-workers published various ascorbate bioavailability values calculated from different literature reports (45). The estimated dose-dependent absorbed fractions (bioavailability) using either the mean plasma ascorbate concentrations or the urinary recovery data were observed at daily vitamin C doses ranging from 200 mg (1.1 mmol) to 2,500 mg (14.2 mmol) and from 1 (5.7 mmol) to 12 g (68.1 mmol), in several reference sources.

The tendency for bioavailability to decrease with increasing dose is clearly evident from the literature values cited. However, the magnitudes of the bioavailability values reported in the literature for daily doses higher than 200 mg were considerably greater than the values calculated by Blanchard and co-workers (45). One reason for this discrepancy was proposed by the authors who argued that bioavailability is commonly calculated from the ratio of oral to intravenous AUC, assuming that the AUC is directly proportional to the amount absorbed, which implies constancy of clearance. But plasma ascorbate concentrations are often considerably higher soon after an intravenous dose than after oral administration and consequently the excretion rate of ascorbate would be greater after intravenous dosing than after an equivalent oral input. Because of the nonlinear renal tubular reabsorption, a direct comparison of an oral AUC with an intravenous AUC will produce an overestimation of oral bioavailability.

In addition, the oral bioavailability estimates reported in the literature may be somewhat biased because they are frequently based on the assumption that the rate of ascorbate absorption is linear and therefore the nonlinear carrier-mediated absorption is often ignored. Likewise, multiple oral doses are expected to produce fluctuations in the absorption rate. Despite the uncertainty in the estimation of oral bioavailability, the analysis suggests that plasma ascorbate concentrations largely depend on limited absorption, probably as a result of a saturable transport mechanism.

Because ascorbate absorption is mediated by transport sites in the proximal small intestine, factors that reduce the rate at which the concentration of ascorbate builds up at these carrier sites (e.g., a delay in the rate of stomach emptying or a modified drug delivery system) would be expected to increase the fraction absorbed. Indeed, the bioavailability of ascorbate will be increased when given in divided doses or concurrently with food (48). It even could be assumed if slower *per oral* ascorbate release is achieved after modifying the delivery system by using, for instance, controlled release matrices or protective liposome-formed multilamellar vesicle cores.

Model-derived overprediction of plasma ascorbate concentrations with respect to the observed ones in several different literature reports could be the result of incomplete absorption of administered vitamin C at daily doses higher than 200 mg (1.1 mmol). Otherwise, it could also be a result of inappropriate timing of actual measurements.

Targeting drugs into the lymph has certain advantages that arise mainly as a result of the unique anatomy and physiology of this process. These advantages could include enhanced absorption through an alternative mechanism other than the saturable carrier-mediated transport located at the intestinal villi. The lymphatics of the small intestine are characterized by the presence of a plexus of lymphatic capillaries that drain, via the mesenteric lymph vessel, into the general circulation. Various lymphotropic delivery systems such as liposomes have been used to target the lymphatic system. The mechanism of the enhanced lymphatic transport is complex and not fully understood. However, many studies have reported the effect of lipid-based vehicles and the uptake of compounds following oral administration by the M-cells in the Peyer's patches. It has been suggested that the stimulation of lipoprotein synthesis by the enterocyte and the subsequent association of the drug with the chylomicrons is fundamental to the process. Depending on size, charge and composition, a proportion of the particles that enter the lymphatic system will eventually reach the general circulation (49).

Dr. Hickey's group has investigated different oral liposomal doses of vitamin C (5 to 36 grams), attaining plasma ascorbate levels greater than 400 µM from large single 36-gram oral dose of liposomal vitamin C (Figure 3). The results show a plasma response particularly broad and for the larger dose the broad peak occurred about 6 hour (360 minutes) after the dose. Accordingly, we can speculate that steady-state blood ascorbate levels of at least 500 µM could be achieved and sustained through repeated oral liposomal doses.

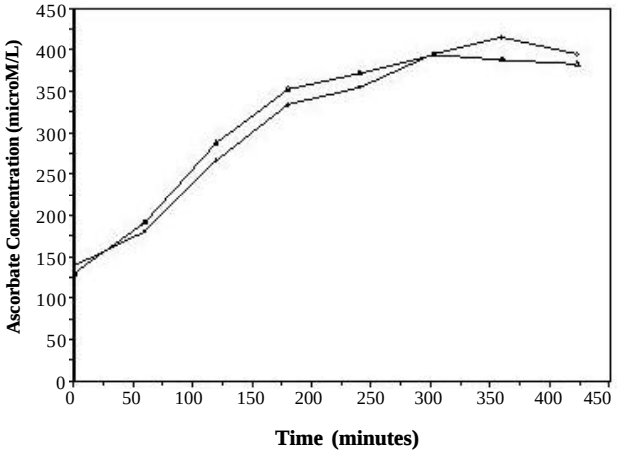

Figure 3. Truncated plasma pharmacokinetic profiles (absorption phase) after a single 36-gram dose of liposomal ascorbate in two subjects. Note both peaked about 6 hours (360 minutes) after the dose. Data supplied by courtesy of Steve Hickey.

Metabolism and Distribution

Vitamin C is one of the most important water-soluble antioxidants in mammalian tissues. Its role as a free radical scavenger and its redox relationship with other antioxidants has been the focus of continuous interest. However, less attention has been paid to the metabolism of ascorbate, whose mechanisms are not yet fully understood, and sometimes the fact that humans are unable to synthesize this vitamin diverted the interest in this area. Strikingly, some authors believe that the exploration of ascorbate metabolism and its regulation may contribute to the understanding of the evolutionary events leading to the loss of ascorbate synthesizing capability.

Reports in the literature refer to a relationship between cancer and ascorbate metabolism, which rests either upon the relatively biased ascorbate concentrations in normal to neoplastic tissue or the utilization of ascorbate by the cancerous organism, as determined by the urinary excretion of ascorbate at varying levels of intake. In fact, the question whether tissue ascorbate amount is altered in cancer has been the subject of considerable controversy.

Certainly, several studies show that the urinary excretion of ascorbate is less in cancer patients than in normal people (11, 13). These results have been considered to reflect higher utilization of ascorbate by cancer tissues leading to an alteration of its renal excretion pattern.

A considerable proportion of cancer patients have low values for ascorbate concentration in both plasma and white cells (11). On the basis of results presented by Bodansky and Lowry, it can be suggested that at least partially the depletion of tissue ascorbate in cancer and other chronic diseases is associated with an excessive catabolism of proteins (11, 50).

The ascorbate utilizing reactions are based on its property of being easily oxidized to dehydroascorbate. According to the bibliography, ascorbate can be regenerated from its oxidized dehydroascorbate form (NADPH-dependent ascorbate recycling) in any mammalian cell of species (51). Economically, it is more advantageous for the cell to reduce back the oxidized form of ascorbate than to synthesize it *de novo*. This recycling mechanism could therefore be interpreted as a phylogenetically gained adaptive benefit in superior mammalian species.

As per Banhegyi *et al*., the fact that the mutation in the gulonolactone oxidase gene did not remain an enzymopathy affecting only a minority of the population but spreading widely and becoming exclusive, should mean that this change was advantageous. Another hypothesis based on biochemical data is that hydrogen peroxide production and the consecutive glutathione consumption during ascorbate synthesis are in the background of the event. Thus, the loss of gulonolactone oxidase activity saved the reduced GSH, the main defense against oxidants, while the access to ascorbate was not hindered (51).

NADH-dependent semi-dehydroascorbate reductase activity has been described in cellular membranes. Dehydroascorbate can be reduced by either enzymatic or non-enzymatic reactions. The non-enzymatic recycling occurs at the expense of GSH, a process that has already been described decades ago. In contrast, the existence of an enzymatic reaction was doubtful until the middle of 90's. In fact, it has been observed that gluta-redoxin and protein disulphide isomerase exhibit GSH-dependent dehydroascorbate reductase activity. During normal conditions, the redox status of the cells probably permits that the majority of oxidized ascorbate can be reduced back following one of the above-mentioned mechanisms, but under prolonged oxidative stress, or pro-oxidant pathophysiology, the in-vivo dehydroascorbate accumulation can prevail.

Ascorbate can generate hydrogen peroxide upon oxidation in biological systems. This action can be enhanced by divalent cations such as iron and copper. Hydrogen peroxide may further generate additional reactive species and secondary products of oxidation. In general, the cytotoxicity induced by ascorbate seems to be primarily mediated by hydrogen peroxide formation. It has been shown that the amount of hydrogen peroxide generated by the cells is proportionally dependent on the ascorbate concentration and inhibited by serum (i.e., serum contains antioxidants). Hydrogen peroxide is most likely

generated intracellularly during ascorbate metabolic oxidation to dehydroascorbate. The hydrogen peroxide reduces cellular levels of thiols and can initiate membrane lipid peroxidation (10). Also, as mentioned earlier, it should be emphasized that ascorbate cytotoxic action can be enhanced by the inclusion of synergistic substances (e.g., futile redox cycling quinones)

Like many redox molecules, ascorbate can act either as a pro-oxidant or a reducing (i.e., anti-oxidant) agent depending on the redox status of the cell and its environment, as well as the concentration of ascorbate at the specific time (10, 52). Cancer cells can absorb high levels of ascorbate (that is, at millimolar range) and unlike normal tissues their disturbed metabolism produces redox cycling and Fenton-type, Fe^{2+}-mediated, free radicals (i.e., *Reactive Oxygen Species*). Since tumor cells are often catalase deficient, they are more sensitive to cytotoxic levels of hydrogen peroxide than normal cells (53).

If dehydroascorbate is not recycled to ascorbate, it is rapidly decomposed since this compound is highly unstable at physiological pH. The main product of hydrolysis is 2, 3-diketo-L-gulonate, which does not possess biologic action. Finally, this 2, 3-diketo-L-gulonate is decarboxylated to L-xylonate and L-lyxonate, which can enter into the pentose phosphate pathway and the D-conversion is suggested to occur. Another minor pathway of ascorbate catabolism is a carbon chain cleavage yielding oxalate and 4-carbon intermediates. At doses higher than 180 mg (1 mmol), carbon dioxide formation has also been reported (54).

In general, the metabolism of ascorbate has been reported to be saturable. This conclusion comes from the observation of an upper limit to the rate of excretion of ascorbate metabolites as the oral dose of vitamin C is increased. However, re-analysis of the relation between the metabolite excretion rate and plasma ascorbate concentration indicates that this ratio remains essentially constant and that there is no saturation of ascorbate metabolism (47).

In species unable to synthesize ascorbate, the breakdown of the exogenous ascorbate can fuel the Cori cycle both in the liver and in the periphery. The uptake mechanism can be different in various cells, but some features seem to be constant: First, considering the water-soluble ionized nature of ascorbate and dehydroascorbate, simple diffusion through plasma membrane is often considered to play a minor role in tissue uptake. A facilitated mechanism involving a protein transporter has been postulated by Banhegyi et al (51).

Sodium-dependent and a glucose transporter mechanisms have been described for ascorbate uptake, whereas dehydroascorbate is considered to be transported by bidirectional facilitated diffusion using members of the family of mammalian hexose transporters (e.g., GLUT-1). The transport of both entities seems to be independent and subsequently not competitive with each other, but could be inhibited by high concentration of glucose and glucose transport inhibitors. Notably, the rapid reduction of dehydroascorbate on the internal side of plasma membrane by various reducing systems prevents the efflux of this compound and allows the effective accumulation of ascorbate against a concentration gradient as it has been observed in several cell types. Based on observations, a complex, but poor understood, metabolic regulation is assumed by scientists (51).

Vitamin C is accumulated in some cells in part by the sodium-dependent transporter with saturable kinetics. The transporter achieves V_{max} at approximately 70 mM, the same background or minimum plasma ascorbate concentration achieved by ingestion (orally) of 200 mg daily in healthy volunteers. The RDA yielded a plasma concentration of approximately 24 mM, similar to transporter K_M of 5-30 mM (18). Small changes in concentration of transporters yield large changes in the amount of ascorbate transported, behavior predicted by Michaelis-Menten kinetics. Kinetic and biochemical data imply that ideal vitamin C ingestion should yield a plasma ascorbate concentration above the K_M of the transporter. An intake of 200 mg daily produced this minimum (or background) plasma concentration in healthy young men, but for cancer patients, higher doses might be required. Notably, the saturable kinetic for ascorbate distribution into cells calls for slower intravenous infusion of high vitamin C dose, and this scheme matches our hypothesis of a schedule-dependence phenomena (i.e., prolong drug administration over a critical infusion time).

Reports in the literature also suggest that tissue ascorbate levels can be substantially higher than plasma values, ranging from 3 to 6 mM level, in some of them (e.g., adrenal glands). This marked accumulation of ascorbate in peripheral store-type compartments is also reported in humans and could be associated with an evolutionarily acquired adaptive mechanism in order to avoid ascorbate deficiency in high-demand situations and tissues, especially in those species (e.g., primates) that are unable to synthesize the required amounts of ascorbate. Membrane-bound active pumps like those earlier described in the gut wall could mediate this mechanism. Interestingly, this preferential ascorbate accumulation/replenish process in the so-called store tissues is also evident in tumors, which may provide a therapeutic advantage, as these high ascorbate amounts may be enough for its cytotoxic action.

Casciari and co-workers published the effects of high ascorbate doses on tumor growth in Sewall-Wright strain-

Table 1 Pharmacokinetic parameters and metrics early reported by several authors in literature of Vitamin C given orally or intravenously. Interestingly, most data come from trials in healthy young volunteers.

Pharmacokinetic Parameters								
Dosing Rates and/or Dosage	CL renal (L/d)	CL total (L/d)	Peak Level	Vd	t½	F (%)	Modeling	Reference
90 – 12000 mg/d 0 – 100 mg/d[5] 200 – 2500 mg/d[6]	null[5] 173 at > 100 mg/L[6]	7.5[5] ~180[6]	12 – 15 mg/L[4]	-	-	16 – 90 [1] 8 – ~100 [2]	Clearance model $CL_R \bullet C_{ss} = GFR \bullet C_{ss} - Tr$ (excretion) (filtration) (reabsorption)	Blanchard et al., 1997 [45]
1500 – 6000 mg	3.1 at < 8 mg/L[3]	-	-	-	-	-	Clearance model	Ralli et al., 1940 [44]
Several infusion protocols (15 – 65 g; 40 – 160 min infusion time)	-	-	125.7 – 495 mg/dl	30 dl (fixed value)	27.8 min[8]	-	two compartment model	Riordan et al., 2000 [40][7]
-	-	-	10 mg/L[9]	-	-	-	-	Jacob et al., 1987 [59][9]
0.015 – 1.25 g[10] (oral and IV) 1 – 100 g[11] (simulation)	-	-	134.8 ± 20.6 µM[10] 885 ± 201.2 µM[10] 220 µM[11] 13 400 µM[11]	-	-	-	three compartment model	Padayatty et al., 2004 [33][12]
30 – 2500 mg daily dose, orally and IV	-	-	From 6.9 ± 0.5 µM to 91.8 ± 8.5 µM	-	-	100 (200 mg single dose) ~ 33 (1250 mg single dose)	Multi-compartment model with nonlinear renal tubule reabsorption incorporated	Levine et al., 1996 [18]
1 g IV over 3-4 min daily for several weeks	-	-	-	-	3.37 (± 0.78) hrs	82.6 (± 5.3)[13]	two compartment model, Non-linear regression analysis	Yung et al. 1978 [64][14]

[1] Percentage of dose absorbed estimated by Blanchard *et al* from data in literature (see references 18; 60-63)
[2] Percentage of dose absorbed. Values are approximations calculated by using the model developed by Blanchard *et al*. [1997]
[3] Expressed as the ratio of ascorbic acid to inulin clearance, the latter being a measure of glomerular filtration rate (GFR).
[4] Upper limit of plasma ascorbic acid concentration at steady-state (the so-called ceiling effect)
[5] The value of non-renal (metabolic) clearance at Vitamin C concentrations < 6-8 mg/L (daily dosing rates between 0 – 100 mg/d). This value is assumed to remain constant over the range of doses. At this plasma concentration range, renal excretion is virtually null.
[6] The value of renal clearance (excretion) at higher plasma Vitamin C concentrations > 100 mg/L. The total clearance is estimated as the sum of renal clearance and the above-mentioned non-renal clearance, 7.5 L/d, which remains constant over the dose range.
[7] Case study in a 72 years old cancer patient.
[8] Mean value calculated from the Kx parameter, rate of excretion of ascorbate out of the blood (renal excretion).
[9] Report in healthy, nonsmoking young adult male. Upper limit nearly 20 mg (0.1 mmol)/kg body wt.
[10] Mean peak plasma Vitamin C concentrations (±SD) after oral and intravenous input. Higher value belongs to IV administration.
[11] Pharmacokinetic modeling predicted peak values after oral dose of 3 grams every 4 hours (maximum tolerated oral dose), and 50 grams given intravenously (the higher value).
[12] Data from seven healthy men were used to simulate mega-doses. Plasma and urine vitamin C concentrations were measured after administration of oral and intravenous doses (up to 1.25 g), in 12 healthy young volunteers (3 men, 9 women, 19 – 27 years old).
[13] Percent of the dose recovered in urine (mean value ± SD).
[14] Study in 5 healthy volunteers (4 males, 1 female) ages 25-32 years old.

2 guinea pigs bearing intradermal L-10 hepatocarcinoma cell line, an animal model sharing the human necessity of obtaining its ascorbate requirements from the diet, in the form of vitamin C (lost the ability to synthesize their own ascorbate). According to the authors, subcutaneous daily 500 mg/kg injections of ascorbate were able to inhibit tumor growth up to 65%, with oral supplementation reducing it by roughly 50%. Interestingly, they observed an inverse correlation between tumor growth rate and intra-tumor ascorbate concentrations, which exceeded 2 mM in some cases supporting a possible role for high intravenous doses of ascorbate in the treatment of cancer (55).

Final Thoughts

The dynamic flow model of Hickey, Roberts and Cathcart provides a new paradigm that is consistent with the known pharmacokinetics of ascorbate (28). It is also consistent with claims for the health benefits of mega-doses. In fact, current knowledge of ascorbate pharmacokinetics and tissue physiology challenges the low-dose (or tissue saturation) hypothesis. In particular, according to Hickey et al, both white and red blood cells should be considered as specialized corporal reservoirs for ascorbate uptake from the blood supply, so that mean and minimum blood levels become of cardinal interest to its availability. In this context, Hickey et al. argued that the NIH data on ascorbate pharmacokinetics have been misinterpreted, resulting in both inappropriate recommended intakes and discrepancy with health benefit claims upon ascorbate mega-doses (28).

Under disease-state or stress, dynamic flow states an increased ascorbate intake to sustain the desired plasma levels, so that an intravenous dose may be required to maintain the reducing status (56). Evidence shows that some cell types actively accumulate ascorbate amounts above the actual plasma levels due to the fact they have critical requirements for ascorbate and/or increased ascorbate transporters (GLUTs). Keep in mind that the higher ascorbate turnover rate at mega-doses could be assumed as a result of saturation of ascorbate reabsorption, but also because of its role in maintaining or re-establishing a reducing internal environment, the latter event being the main reason why high doses of ascorbate are justified at disease-state conditions.

Stress or the disease-state may be demanding a sudden increment of systemic redox molecules in order to recover the internal REDOX balance. Humans cannot synthesize ascorbate but as the blood levels drop he/she can start pumping more from those available in his/her intestine (up to 1,000-fold more) and/or from any other body reservoir such as white and red blood cells, as well as reabsorb the excreted ascorbate from urine. In addition, damaged tissues like cancer cells are highly demanding compartments that can absorb high levels of ascorbate (at millimolar range). Consequently, the consumption of higher ascorbate amounts by these tissues accounts for most of the systemic ascorbate in excess. Under such conditions, mega-doses may neither saturate the tubular ascorbate reabsorption nor the sodium-dependent transporters, which give rise to longer systemic half-life and residence times.

Levine et al. (1996) argued that the determinants of the recommended dietary allowance (RDA, based on the Food and Nutrition Board of the Institute of Medicine criteria) for vitamin C include, among others, the relationship between vitamin C dose, background or minimum plasma concentration, tissue store or cell compartments concentration/distribution, and urinary excretion. As they stated, these factors could account for the sigmoid shape of the dose/plateau plasma concentration curve they found in their vitamin C depletion-repletion pharmacokinetic study (18). Accordingly, in the event that a larger vitamin C distribution into cellular compartments occurs, like in disease-states or stress conditions, it is logical to expect a shift-to-the-right in the curve and, consequently, in the dosage recommendation (saturation cut-off). That is, the steep portion of the curve would occur at daily doses greater than what they found (100 mg). The first dose beyond the sigmoid portion of the curve might be larger than 200 mg daily and complete plasma saturation may occur at more than 1000 mg daily. These responses may be expected because of the kinetic connection leading to redistribution of ascorbate between plasma and tissues.

Some tissue stores are thought to be near saturation at an intake of 60 mg, and increased excretion would occur at higher doses. Nonetheless, there is only evidence that this occurs in some specialized tissues, such as white blood cells. In contrast, red blood cells have a completely different response and may be more representative of most body cells. Again, this information is supported by studies with healthy young humans; therefore, the conclusions are based on facts that need to be revised for patients with damaged tissues (e.g., cancer cells), which are demanding higher levels of ascorbate (at millimolar range) than healthy cells are.

Reviewing the current literature, most studies have used single large daily, or twice-a-day, doses of vitamin C. Occasional studies have used low-dose, slow release formulations. A large single daily dose will produce only a transient increase in plasma levels. Such once or twice daily mega-dose supplementation of ascorbate will not load tissues, such as red blood cells, or increase the body pool substantially; and therefore, would not be expected to show more than a minimal biological effect, when

compared with the dynamic flow model.

On the other hand, the intravenous pharmacokinetic of vitamin C may yield systemic disposition profiles showing a significant increment of up to two-orders of magnitude above those attainable following oral dosing. Published studies show that intravenous ascorbate dosing schemes can be properly used in order to achieve plasma levels equivalent to those early reported as necessary to kill tumor cells (56-58).

Accordingly, we are highly encouraged to conduct clinical pharmacokinetic studies in order to critically assess the vitamin C disposition pattern at high doses, given intravenously by continuous infusion.

Resumen

Recientemente, hay un fuerte movimiento en defensa de altas dosis de vitamina C para el tratamiento de cáncer y otras condiciones. Se ha reportado en la literatura científica que la vida media biológica en concentraciones supra-fisiológicas de vitamina C en plasma es alrededor de 30 minutos, aunque este dato debe ser confirmado. Los investigadores del NIH han establecido las recomendaciones actuales de consumo de vitamina C en la dieta (RDA) basadas en pruebas realizadas 12 horas posteriores a su consumo (24 vidas media). El modelo del flujo dinámico refuta las recomendaciones actuales de ingerir las dosis relativamente bajas recomendadas de vitamina C. Este modelo también establece un vínculo entre las sugerencias de Pauling respecto a ingerir mega-dosis de ascorbato con otros reportes sobre los efectos de altas dosis en el tratamiento de varias enfermedades. Aunque, dos estudios clínicos controlados realizados en la Clínica Mayo no mostraron beneficios significativos al tratar pacientes de cáncer terminal con 10 gramos diarios de vitamina C oral, otros estudios clínicos han demostrado que el ascorbato administrado intravenosamente sí puede ser efectivo contra los tumores malignos. Recientes estudios confirmaron que las concentraciones de vitamina C en plasma varían sustancialmente con el cambio en la ruta de administración. La administración intravenosa de vitamina C permite alcanzar niveles suficientemente altos en plasma y orina para desarrollar actividad anti-tumoral. Tomando en consideración que la eficacia de la vitamina C en el tratamiento de cáncer no puede ser establecida mediante estudios clínicos que sólo utilizan la ruta oral, el rol de la vitamina C en el cáncer debe ser reevaluado. Una limitación de los actuales estudios es la escasa disponibilidad de datos clinicos para establecer la conducta farmacocinética intravenosa de vitamina C administrada en altas dosis en pacientes con cáncer. Existe una necesidad imperiosa de estudios clínicos farmacocinéticos de vitamina C en altas dosis por vía intravenosa que ayuden a establecer los regímenes más eficaces y seguros de esta vitamina contra el cancer.

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