ORIGINAL ARTICLE

Pharmacokinetics of oral vitamin C

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Abstract
Purpose. To test whether plasma vitamin C levels, following oral doses in supplemented volunteers, are tightly controlled and subject to a maximum in the region of $220\,\mu M\,L^{-1}$, as suggested by previous researchers for depleted subjects. To determine plasma levels following single, variable-sized doses of standard and liposomal formulations of vitamin C and compare the effects of the different formulations. To determine whether plasma levels above $\sim 280\,\mu M\,L^{-1}$, which have selectively killed cancer, bacteria or viruses (in laboratory experiments), can be achieved using oral doses of vitamin C. *Design.* This was a single blind study, measuring plasma levels in two subjects, in samples taken half-hourly or hourly for 6 hours, following ingestion of vitamin C. Data were compared with published results and with data from 10 years of laboratory plasma determinations. *Materials and methods.* Standard 1 gram tablets of vitamin C; liposomal vitamin C. Plasma levels were analysed using the method of Butts and Mulvihill. *Results.* Preliminary investigations of the effects of liposomal and standard formulation ascorbate showed that blood plasma levels in excess of the previously assumed maximum of $220\,\mu M\,L^{-1}$ are possible. Large oral doses of liposomal ascorbate resulted in plasma levels above $400\,\mu M\,L^{-1}$. *Conclusions.* Since a single oral dose can produce plasma levels in excess of $400\,\mu M\,L^{-1}$, pharmacokinetic theory suggests that repeated doses could sustain levels well above the formerly assumed maximum. These results have implications for the use of ascorbate, as a nutrient and as a drug. For example, a short *in vitro* treatment of human Burkitt's lymphoma cells with ascorbate, at $400\,\mu M\,L^{-1}$, has been shown to result in $\sim 50\%$ cancer cell death. Using frequent oral doses, an equivalent plasma level could be sustained indefinitely. Thus, oral vitamin C has potential for use as a non-toxic, sustainable, therapeutic agent. Further research into the experimental and therapeutic aspects of high, frequent, oral doses of ascorbic acid either alone or (for cancer therapy) in combination with synergistic substances, such as alpha-lipoic acid, copper or vitamin K3, is needed urgently.

Key words: *Vitamin C, ascorbic acid, nutrition, liposomes, cancer, pharmacokinetics*

Introduction

The amount of vitamin C required for good health represents a challenging aspect of scientific nutrition. Although authorities agree that a few milligrams of ascorbate will

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prevent acute scurvy, estimates of the optimal intake vary widely. Various experts have proposed doses, ranging from ~40 milligrams [1] to several grams a day [2]: a difference more than 100-fold. The debate has been vigorous, especially since Linus Pauling popularized the hypothesis of higher dose requirements.

In an attempt to set vitamin C requirements on a more rigorous scientific basis, the National Institutes of Health (NIH) conducted a series of pharmacokinetic depletion and repletion experiments. These studies were based on seven male [3] and 15 female [4] young, adult subjects [5]. Results indicated that the kidney selectively retains a minimum plasma level of $\sim 70 \mu M L^{-1}$; which varies slightly among subjects. This baseline is protected by ascorbate re-uptake pumps in the kidney, leading to an excretion half-life of 8–40 days, for low (sub-baseline) levels of vitamin C [6].

Single, daily 200 mg doses of vitamin C were shown to maintain this baseline plasma concentration at what the NIH described as a maximal (or 'saturation') level [4,5]. The NIH reported that single doses higher than ~200 mg did not substantially increase the baseline level, which appeared to be limited by the capacity of the re-uptake transporters. The NIH data shows that single, higher, oral doses (up to 1250 mg) raise plasma levels transiently.

The shape of the vitamin C response curve results primarily from the rate of oral absorption, combined with an excretion half-life of ~30 minutes. A secondary effect concerns its absorption and release from body tissue compartments. Oral absorption of vitamin C is a two-phase, non-linear process; bodily levels are dependent on current intakes. Low intakes produce plasma levels below $\sim 70 \mu M L^{-1}$, with a long-half life, consistent with resistance to acute scurvy [7]. Higher single doses produce transient increases in plasma concentration, which the NIH group predicted to be limited to a maximum of $220 \mu M L^{-1}$ [8].

Continuous supplementation, in combination with specific administration technologies, can produce levels greater than the NIH prediction. Here, we provide evidence that plasma levels following oral administration of liposomal ascorbate can reach approximately twice the predicted maximum. These findings have previously unrealized implications for the use of oral vitamin C as a therapeutic agent for various diseases, including cancer.

Methods

Ethical approval for the study was obtained from the ethics committee at Manchester Metropolitan University and the UK Department of Health. Two subjects, one female (52) and one male (55), had previously taken a minimum of 6 grams per day of vitamin C and an average exceeding 10 grams per day, in divided doses, for a period of 12 months. Both subjects had apparently normal renal function, with no history of kidney stones or renal disease. At the start of the study, each subject fasted for 12 hours, with no intake of vitamin C before the first (background) measurement. This interval was chosen to ensure almost complete return of the plasma level to baseline, consistent with published pharmacokinetic data [4,5,8] and known absorption and excretion characteristics [9].

On three sequential mornings, a nurse drew a background blood sample from each subject. Immediately after this, the subjects consumed single 5, 20 or 36 gram doses of vitamin C, in a liposomally encapsulated formulation. Control measurements employed 5 gram doses of a standard ascorbic acid tablet (Table I). The dosing procedure was single blind: neither nurses nor biochemists were aware of the dose taken. Subsequently, blood samples were taken at half-hourly or hourly intervals by individual venipunctures, to avoid the use of anticoagulants.

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Table I. Protocol for doses.

	Day 1	Day 2	Day 3
Subject 1 (M)	5 g (stn)	20 g (lip)	36 g (lip)
Subject 2 (F)	5 g (lip)	5 g (stn)	36 g (lip)

The standard commercial preparation of vitamin C employed nominally 1 gram tablets with bioflavonoids (Holland and Barrett, Nuneaton, UK). This provides a comparison with existing formulations, since the short-term plasma response to oral ascorbic acid has been quantified previously [4,5,10]. The response to these doses was compared with published pharmacokinetic responses to oral doses of ascorbic acid. In addition, one author (NM) had access to the results of plasma ascorbate measurements from Biolab Laboratories. Biolab has made plasma measurements since 1984 and NM has made ~30 plasma determinations per week (1500 per year) since 1997; overall ~15 000 measurements.

The liposomal formulation employed nominally 1-gram doses, encapsulated in phosphatidylcholine liposomes (Livon Laboratories, Henderson, NV). The liposomal vitamin C was subject to an external high performance liquid chromatography (HPLC: Varian ProStar PDA) validation, which measured 1055 mg of ascorbate per (1 g) sachet. The measured pH was 6, consistent with the fact that the liposomes contained vitamin C in a salt form, sodium ascorbate.

Blood plasma measurements were obtained over the first 6 hours of absorption. Plasma vitamin C was measured by the method of Butts and Mulvihill [11], adapted for the Cobas Mira analyser (F. Hoffman-La Roche Ltd., Diagnostics Division, CH–4002, Switzerland). The technique depends on reduction of ferric to ferrous ions by ascorbate, which is simultaneously converted to dehydroascorbate. This method had been calibrated against the 2,4-DNPH method [12] and the fluorimetric method [12]. Calibration standards were freshly prepared for each analytical run and three different control samples were included with each batch of samples, giving a between-batch coefficient of variation of ~3%.

We analysed the data using standard pattern recognition techniques [13] in terms of outlier determination [14,15] and computed the probability that the z-value of an observed pattern was consistent with a normal distribution of values by direct numerical integration, using Simpson's method [16].

Results

The Biolab plasma ascorbate measurements are entirely consistent with the reference interval of $34–114 \mu M L^{-1}$ [17], which corresponds to the 95% interfractile interval of the population studied. Approximately 2.5% of values are below that reference interval and 2.5% are above the reference interval. Biolab's lowest recorded value for serum vitamin C was $8.0 \mu M L^{-1}$, at which level the subject was in danger of entering acute scurvy. Of the 15 000 measurements, the highest recorded serum value in a subject not receiving intravenous vitamin C was $220 \mu M L^{-1}$. This is consistent with the published maximum value. With intravenous administration, Biolab have recorded plasma values in excess of $5000 \mu M L^{-1}$, in subjects under ascorbate treatment for osteosarcoma.

Baseline levels in the two long-term supplemented subjects, measured at the start of each day, ranged from $100–150 \mu M L^{-1}$. The subjects were thus consistently in the short elimination half-life phase of plasma response.

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Figure 1 shows the response of the female subject to single 5 g doses of liposomal and standard formulation vitamin C; both produced similar response curves. These results are comparable in form and magnitude to those expected for oral vitamin C in previously depleted subjects. However, peak values exceeded $220 \, \mu M \, L^{-1}$, which has been reported as the maximum value attainable with repeated oral doses of 3 g six times daily [8]. The subjects were experienced users of high-dose vitamin C and neither suffered any gastrointestinal effects at this dose level.

Increasing the dose of liposomal vitamin C to 20 g gave a broader response, with a delayed maximum, as shown in Figure 2. In this graph, the 20 g liposomal dose is compared with a 5 g standard dose (male subject). With a 20 g intake, the peak plasma level was delayed and the response was broader, indicating a greater absorption of vitamin C. The 5 g data set shows a marked outlier (peak): this is attributed to the fact that one of the (5 g) blood samples was difficult to extract, with inflammation at the puncture site, providing only a small sample. The subject experienced no bowel tolerance effects at either of these intakes.

Figure 3 shows plasma levels following a 36 g dose of liposomal vitamin C, for both subjects. This resulted in peak plasma levels, in the region of $400 \, \mu M \, L^{-1}$. A 95% interfractile range (34–114), which contains 95% of the distribution with a mean of 74 corresponds to a calculated standard deviation of 17.4. We note that, under these conditions, an outlier measurement of $400 \, \mu M \, L^{-1}$ would correspond to a deviation of 10.3 σ with a theoretical p-value of 1.6×10^{-13} (i.e. $p < 0.000\,000\,000\,000\,1$). With this high dose, both subjects exceeded their bowel tolerance, leading to diarrhoea. This intolerance presumably arose from the high intake of phospholipid, without food buffering, in fasting individuals. However, our observations using hourly doses suggest that daily intakes of this magnitude are tolerable without bowel effects, as long as the dose is spread throughout the day.
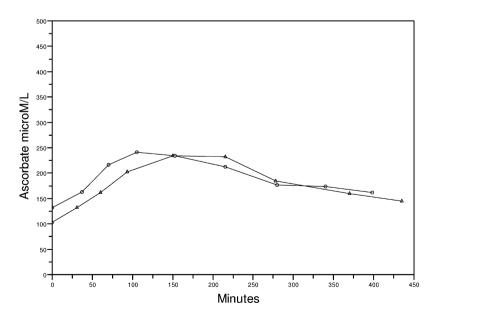

Figure 1. Plasma response curve following 5 g oral doses of standard vitamin C tablets (o) and liposomal ascorbate (Δ) in the same subject.
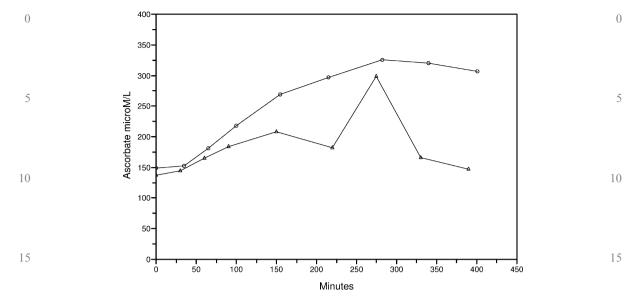
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Figure 2. Plasma response to single doses: 5 g standard (Δ) and 20 g liposomal (o) ascorbate. (NB: Sampling problem—only a small blood sample was able to be taken at 275 minutes, corresponding to the outlier.)

Discussion

This is the first published report of the effects of a liposomal formulation on the absorption of vitamin C. This preliminary study covers two subjects, which is ~10% of the number used by the NIH to recommend a dietary allowance for the general population. While results from two subjects do not provide an indication of the underlying biodiversity, they demonstrate that previous expectations for vitamin C plasma levels may have been based
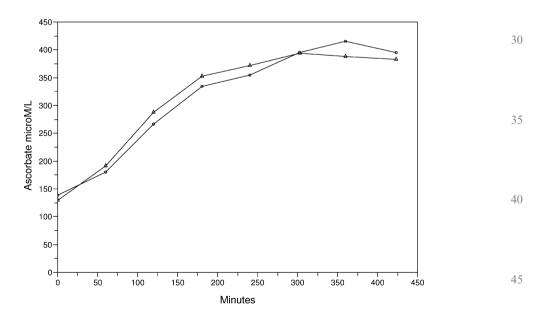

Figure 3. Plasma response to a single 36 g dose of liposomal ascorbate in two subjects.

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on a restricted sample of depleted individuals and do not likely cover the range of responses to oral vitamin C formulations.

It is apparent that the full range of responses in the human population was not represented by the NIH study, as the current data are well outside their published maxima for depleted subjects. These values also exceed statistical expectations [17], derived from 15 000 subjects measured at Biolab in London. The estimated probability is too small ($p=1.6 \times 10^{-13}$) to be considered accurate but provides a quantitative indication of the observed discrepancy. Additional research, to include populations not represented in the published data on depleted subjects, is clearly needed.

At over $100\,\mu M\,L^{-1}$, the subjects' baseline vitamin C levels, following a 12-hour washout period, were higher than those reported in the NIH studies used for the RDA [4,5]. A possible explanation is that high vitamin C levels redistribute from the blood plasma into other body compartments and *vice versa*. Thus, subjects who habitually consume large amounts of vitamin C might carry a reserve in their tissues. Plasma vitamin C, which is depleted through kidney excretion, has a half-life of only 30 minutes. However, while it is being excreted, it can be replaced by absorption from the gut or by redistribution from other body compartments.

The elevated baselines observed in these subjects are inconsistent with the NIH's pharmacokinetic modelling of the plasma response of gram level doses of vitamin C [3–5]. Regular use of high dose vitamin C, given at intervals of 12 hours or less, produces baseline plasma levels greater than those the NIH study described as 'saturated'. Since 'saturation' implies a maximum level, which has been refuted by the higher levels reported here, use of this term (as employed by the NIH in the context of vitamin C plasma levels) should be considered obsolete, inappropriate and inaccurate and the word returned to its conventional meaning.

The plasma levels reported here are higher than is usually seen with oral administration of vitamin C, although the doses were also higher. Biolab noted that the plasma levels attained with 20 g and 36 g doses of liposomal vitamin C were higher than any they had measured previously, following oral doses. Biolab's previous experience and measurement of plasma vitamin C levels has been consistent with the reference interval for human plasma ($34–114\,\mu M\,L^{-1}$). Neonates and supplemented subjects with impaired renal function routinely record plasma ascorbate concentrations of up to $200\,\mu M\,L^{-1}$.

Since plasma levels in this study were outside the normally reported range, they were communicated to Dr Ron Hunninghake, Clinical Director of the International Center for the Improvement of Human Functioning, who carried out an independent replication. Hunninghake, who acted as his own experimental subject, is a habitual high-dose vitamin C supplement user (10 grams per day). Using repeated doses of liposomal and standard vitamin C, at lower intakes than our 36 g maximum, Hunninghake determined vitamin C levels in excess of $300\,\mu M\,L^{-1}$. This level was achieved with 10 g of standard C, followed by an additional 12 or 18 g of liposomal C, divided at intervals through the day. Through this procedure, high vitamin C plasma levels were sustained, rather than peaking as in our single dose study. Following a washout period (no vitamin C intake), Hunninghake found he retained between $100–150\,\mu M\,L^{-1}$, which supports our findings.

Hunninghake's laboratory (Bio-Center, Wichita) has averaged ~ 2000 plasma C determinations each year since 1994; in 2006, they performed 6634 plasma measurements. Their background results (from $37–120\,\mu M\,L^{-1}$) provide a reference range that is consistent with Biolab's ($34–114\,\mu M\,L^{-1}$). The maximum value recorded at the Bio-Centre (from a cancer patient, taking ~ 100 g per day orally) was $318\,\mu M\,L^{-1}$ [18].

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The results of the present study, together with Hunninghake's replication, clearly refute the NIH group's suggestion that the blood is saturated with vitamin C at $\sim 70\,\mu M\,L^{-1}$.

The similarity in the plasma response curves for liposomal vitamin C and the standard commercial formulation, shown in Figure 1 (5 g doses), is interesting. There is a hint that the liposomal form has a slower onset to peak level and a broader profile. Liposomes are absorbed from the gut and into the liver, before being released into the blood stream. This response can be seen more clearly in the 20 g dose, in Figure 2. It is apparent that sustained levels of plasma ascorbate, above the previously assumed maximum of $220\,\mu M\,L^{-1}$, are possible with oral intakes of liposomal vitamin C.

Cathcart [19] describes a mechanism of increased bowel tolerance to oral ascorbate. He reports a direct correlation between the severity of illness and the maximum amount of ascorbate absorbed by the oral route. These clinical observations indicate that the physiological response to ascorbate is variable; clarification awaits suitable pharmacokinetic studies in diseased patients.

The results of this preliminary study are consistent with the suggestion that continuous vitamin C supplementation facilitates increased absorption of large doses. The reference interval for vitamin C quoted for human plasma is $34–114\,\mu M\,L^{-1}$ [17]. In these experiments, the measured baseline levels for vitamin C were in the range $132–149\,\mu M\,L^{-1}$. This concentration is approximately twice the level found in previously depleted subjects. The mechanisms of absorption of ascorbate from the gut are not well understood. Our experience suggests that subjects who habitually consume high intakes of oral vitamin C and subjects that are ill may show a differing response to those who were previously depleted. Physiological adaptation to ascorbate intake may involve recruitment of glucose transporters (GLUT4) signalled by insulin, cellular contraction or reactive oxygen species [20,21].

Our observations suggest that poor oral absorption is related to carbohydrate intake. Glucose and other sugars compete with dehydroascorbate for absorption by GLUT transporters. The subjects in this study reported that they often consumed 10-grams or more of ascorbate in a single dose, without significant bowel effects, provided their carbohydrate intake was low. In the present study, the subjects reported no bowel effects or a slight constipation following the 5-gram doses. The 20-gram liposomal dose was tolerated well. In these fasting individuals, the bowel reaction to the 36-gram dose may have been caused by the large intake of phospholipids, without food buffering.

The pharmacokinetics that form a principal evidence base for determination of the RDA values for vitamin C rely on results from a small number of depleted or previously depleted individuals. Such individuals may not be representative of all members of the population. Well-nourished individuals, with Western levels of carbohydrate intake, who have been previously depleted of ascorbate, may have a low plasma response to oral ascorbate. In the present study, the levels attained from a single oral dose of five standard 1-gram tablets produced plasma levels above the predicted $220\,\mu M\,L^{-1}$ maximum level. Larger or repeated doses would have increased this level further.

The 'saturated' baseline level, determined as $70–80\,\mu M\,L^{-1}$ in the NIH pharmacokinetic studies, is not an upper limit. Baseline levels between 12–24 hours after a single oral dose are consistent with ascorbate transfer between blood plasma and other body compartments. In our study, the recorded background level after a 12-hour washout was in the range $132–149\,\mu M\,L^{-1}$, approximately double that found in depleted individuals. This is consistent with increased tissue levels or sustained absorption from the intestine. We suggest the NIH's assumption that plasma levels are tightly controlled and 'saturated' at $70–80\,\mu M\,L^{-1}$ was premature and thus the RDA for ascorbate could require revision.

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Our findings have important implications for therapy. The role of pharmacological levels of nutrients in cancer and other diseases is currently being re-evaluated [22]. In particular, the cytotoxic action of ascorbate on cancer cells suggests the possibility of a potentially non-toxic cancer therapy [23,24]. However, it has been assumed that cytotoxic levels of ascorbate could only be achieved with intravenous infusions of sodium ascorbate [8]. This would be an implication of the available data, if oral doses could achieve only $220\,\mu M\,L^{-1}$ in plasma. However, we have demonstrated that single doses of liposomal formulations can give levels above $400\,\mu M\,L^{-1}$. If given in a single dose to a fasting individual, such intakes might be impractical. However, these preliminary findings suggest that plasma levels of $500–600\,\mu M\,L^{-1}$ or more could be sustained indefinitely with smaller, but repeated, oral intakes.

Data for the cytotoxicity of ascorbate comes from laboratory experiments, performed over a timescale measured in hours. However, tumours can selectively concentrate ascorbate from plasma. Published results for a 1-hour *in vitro* treatment of human Burkitt's lymphoma cells, using ascorbate at $300\,\mu M\,L^{-1}$, show ~30% necrosis and apoptosis; $400\,\mu M\,L^{-1}$ increases the cell death to ~50% [25]. Moreover, ascorbate cytotoxicity is synergistic with other oral nutrients, including alpha-lipoic acid [26], vitamin K3 [27] and copper [28]. The implications of sustained high concentrations of ascorbate may thus be therapeutically significant.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper. [1]

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