Pharmacokinetic Model of Ascorbic Acid in Healthy Male Volunteers During Depletion and Repletion

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Purpose. To develop a new pharmacokinetic model for ascorbic acid (vitamin C) since no previously published model describes ascorbic acid absorption and disposition over a broad physiologic range of doses and plasma concentrations.

Methods. A new model was developed through exploratory simulations. The model was fitted to pharmacokinetic data obtained from seven healthy volunteers who underwent ascorbic acid depletion then gradual repletion. Concentrations of ascorbic acid were measured in plasma and urine. Final pharmacokinetic model parameter estimates were obtained using nonlinear regression analysis.

Results. The new model included saturable absorption, distribution and renal tubular reabsorption parameters. The model described ascorbic acid concentrations in plasma, cells, and urine during depletion and gradual repletion phases with a residual error less than 15%.

Conclusions. The model was useful for obtaining a new understanding of the likely causes for the complex concentration-time profile observed during gradual repletion. At doses of 200 to 2500 mg per day, the plateau in pre-dose concentrations is largely due to apparent saturation of tissue uptake and less a function of oral bioavailability and renal excretion than previously thought.

KEY WORDS: ascorbic acid; pharmacokinetics; human; models—theoretical; models—nonlinear; bioavailability; ascorbic acid deficiency.

INTRODUCTION

Despite the previous publication of several pharmacokinetic models for ascorbic acid, there is no consensus about the best way to mathematically describe the plasma concentration-time profile and disposition of ascorbic acid in humans. Previous models assume one (1), two (2,3), or three (4) pharmacokinetic compartments with saturable oral absorption (5,6), linear disposition, (1,2,4) or zero-order renal tubular reabsorption (5). Many of these models were derived from data obtained over rather narrow dose and concentration ranges. None of these models simultaneously accounts for all the likely complexities of ascorbic acid absorption and disposition, including saturable intestinal absorption (5–8), active uptake into the intracellular space of at least some cells and tissues (9–12), saturation of proximal renal tubular reabsorption, (5,13) and possible saturable nonrenal clearance (1). Therefore, we have developed a pharmacokinetic model which accounts for the principal physiologic aspects of ascorbic acid disposition based on plasma, intracellular, and urine data obtained during gradual repletion of ascorbic acid following depletion in healthy male volunteers (14).

METHODS

Subjects

Seven healthy men aged 20–26 years who weighed 74.6 ± 6.7 kg (mean ± SD) were included in the study. The studies were performed with informed consent and were approved by the institutional review board of the National Institute of Diabetes and Digestive and Kidney Diseases of the National Institutes of Health. Research followed the tenets of the Declaration of Helsinki promulgated in 1964.

Design

The study had an open, prospective, dose escalation design. The study proceeded in two successive phases: a depletion phase and a dose escalation, repletion phase. To reduce in-hospital depletion time, subjects were placed on an out-patient diet providing 60 mg ascorbic acid daily for three weeks prior to admission. Subjects were then admitted as in-patients to a metabolic ward for 146 ± 23 days (range: 114–179 days). The day of admission was time zero of the pharmacokinetic study. At time zero, subjects had a fasting plasma concentration of ascorbic acid of 23.1 ± 7.5 (mean ± SD) micromolar. Once admitted, subjects consumed a diet containing less than 5 mg ascorbic acid daily for the entire study (15). Ascorbic acid intake from every in-patient meal was calculated. Other vitamins and minerals were given as supplements. During the depletion phase, volunteers received no supplemental ascorbic acid. Plasma ascorbic acid concentration at the end of the depletion phase was 7.2 ± 2.3 micromolar (1.3 ± 0.4 mg/L). During the repletion phase, subjects were dosed successively to steady-state with ascorbic acid doses of 15, 30, 50, 100, 200, 500, and 1250 mg orally twice daily. Doses were formulated as a solution (14). Not all subjects completed the entire escalation scheme. One subject received a maximum dose of 200 mg twice daily and three received a maximum dose of 500 mg twice daily. Pre-dose plasma ascorbic acid concentrations were obtained from blood samples taken before breakfast every 1 to 4 days throughout the study. Steady-state was assumed to have occurred when the mean of ≥ (greater than or equal to) five plasma concentrations over at least 7 days had less than or equal to 10% standard deviation. Eighty-five per cent of steady-state calculations were based on ≥6 plasma concentrations.

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The earliest plasma concentration included in the calculation of the steady-state value was ≥90% of the mean. White blood cells were obtained by phlebotomy and apheresis. The first new ascorbic acid dose at each escalation was administered orally and blood samples were obtained before and then 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 18, 21 and 24 hours after dosing. The second ascorbic acid dose after each escalation was an intravenous dose and samples were obtained before and then 0.033, 0.083, 0.167, 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, and 9 hours after dosing. The intravenous dose was administered at a rate of 250 mg per minute. Subjects collected all spontaneously voided urine during the pharmacokinetic profiles. Aliquots from each urine sample were saved for ascorbic acid assay. Further details about study subjects, methods, and formulation were published (14–17).

Assay

Samples were analyzed for ascorbic acid using high pressure liquid chromatography with coulometric electrochemical detection (14,17). The lower limit of detection is 20 nanomolar.

Model Development

A model-independent assessment of the results was published (14). Attempts to fit previously published models to the complete individual data sets were unsuccessful. None of these available models adequately described the overall behavior of ascorbic acid concentrations during dose escalation. As part of our modeling design, we explored the number of kinetic compartments and the inclusion of saturable absorption, saturable nonrenal clearance and saturable reabsorption from the renal tubule. A series of simulations using STELLA™ (version 2.2.2, High Performance Systems, Hanover, New Hampshire, USA) was then undertaken to explore other possible models and to examine the sensitivity of the model-based predictions to parameter values. Model formulation was influenced by the following facts: white blood cells and fibroblasts accumulate ascorbic acid in a saturable manner, and ascorbic acid within white blood cells may leak back into plasma or undergo metabolism (9–12,14). Therefore, a saturable tissue uptake component was included. Initial values for some model parameters came from previously published data, for example, initial estimates of the V_{max} and K_M values for absorption (5), renal tubular reabsorption (13), and saturable tissue uptake (9–12). Likewise, the initial apparent metabolic rate constant for ascorbic acid was derived from a study of depletion kinetics (18) and from data from our 7 subjects. The initial amount of ascorbic acid in the tissue compartment was 8.5 millimoles (1500 mg), the size of the body pool estimated for subjects consuming ascorbic acid 75 mg per day (18). Plasma protein binding of ascorbic acid was assumed to be negligible (17). Delivery of ascorbic acid to the proximal renal tubule, the site of renal reabsorption, was based on glomerular filtration rate (19). Other estimates, such as the apparent volume of distribution of the central (rapidly equilibrating) compartment and other linear rate constants, were obtained directly from the data or by trial and error.

Nonlinear Regression Analysis

Simulations performed with STELLA™ are extremely useful for identifying potential models and for obtaining approximate parameter values. However, simulations did not provide optimized parameter estimates nor statistical measures of goodness-of-fit. Therefore, each subject's entire data set was analyzed using nonlinear regression analysis and a maximum likelihood objective function as implemented in the NONMEM™ software package (20).

RESULTS

Ascorbic acid concentrations were measured in the plasma, cells and urine of seven healthy men (14). Plasma concentrations demonstrated marked nonlinear kinetics with lack of dose-proportionality (Table I). In depleted subjects, oral ascorbic acid 15 mg twice daily produced a mean pre-dose plasma ascorbic acid concentration plateau of 8.7 micromolar (1.5 mg/L). After doubling the dose to 30 mg twice daily, the mean plateau concentration almost trebled. Ascorbic acid doses of 100–1250 mg twice daily failed to produce proportional increases in pre-dose ascorbate plasma concentrations (Table I).

Subjects demonstrated saturable intracellular accumulation of ascorbic acid as a function of plasma concentration (Figure 1A). At or above oral doses of 50 mg twice daily, there was a plateau in the intracellular concentration of ascorbic acid. Urinary excretion of ascorbic acid also exhibited saturable kinetics as a function of plasma concentration (Figure 1B). There was no measurable ascorbic acid in urine following either oral or intravenous doses of 15 or 30 mg. At and above a threshold dose of 100 mg, all subjects excreted ascorbic acid in the urine.

Pharmacokinetic profiles with frequent sampling were performed after oral and intravenous doses at each dose escalation. Representative pharmacokinetic plots appear in Figure 2.

Attempts to describe the plasma and urinary pharmacokinetic data using one, two or three compartment linear models or previously published nonlinear models were unsuccessful. Therefore, a model was developed which included saturable distribution as well as saturable absorption and saturable renal tubular reabsorption parameters. The simulations using the STELLA™ program helped demonstrate that the inclusion of saturable tissue uptake and first-order metabolism in the tissue compartment could account for the highly nonlinear behavior

Table I. Plateau Plasma Ascorbic Acid Concentrations at All Doses

Ascorbic acid dose rate (mg per day)	Plateau plasma ascorbic acid concentrations (micromolar)	
	Mean	Standard deviation
0 (depletion nadir)	7.6	1.6
30	8.7	1.7
60	24.8	14.1
100	56.0	4.5
200	65.8	7.3
400	70.0	6.9
1000	76.9	5.3
2500	85.0	5.4

Note: Dose rate is the total, administered, daily amount of ascorbic acid in mg per day given as two, equal, divided doses. Concentration values are the means of plateau (apparent steady-state) plasma ascorbic acid concentrations from all subjects. Plasma samples were obtained from fasting subjects, pre-dose.

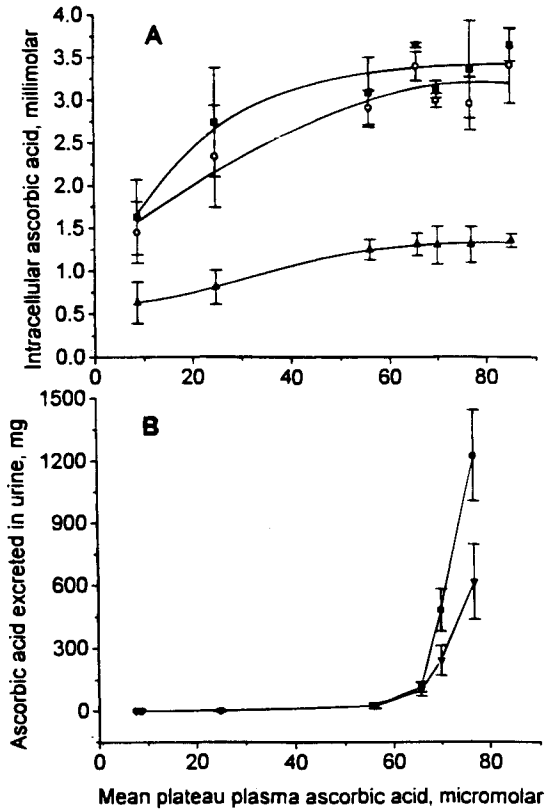

Fig. 1. Ascorbic acid in cells and urine as a function of plasma ascorbic acid concentration. X-axis values (micromolar) are the means of plateau (apparent steady-state) plasma ascorbic acid concentrations from all subjects. Plasma samples were obtained from fasting subjects, predose. Panel A shows intracellular concentrations on the y-axis (millimolar, mean ± SD). Lymphocytes (squares), monocytes (circles), and neutrophils (triangles) were collected from subjects at each plateau of plasma ascorbic acid concentration. Panel B depicts amount of ascorbic acid excreted in the urine on the y-axis (mg, mean ± SD). Urine was collected from subjects after administration of single doses of ascorbic acid, 15–1250 mg, either intravenously (circles) or orally (triangles).

of ascorbic acid kinetics over the range of 15–50 mg twice daily. In addition, these simulations provided good initial parameter estimates for the nonlinear regression analyses.

The model that adequately describes the concentration-time data is depicted in Figure 3. Oral input is into compartment 1. The central compartment (compartment 2) equilibrates rapidly. Conceptually, the central compartment correlates most likely with the plasma space and the rapidly equilibrating portion of the extravascular, extracellular space. Compartment 3 is a slowly-equilibrating, peripheral compartment called the tissue compartment. The intracellular space and the slowly-equilibrating portion of the extracellular space are possible physiological correlates of the tissue compartment. Precise correlation between model compartments and physiological spaces is conditioned by our observations of ascorbic acid concentrations in only plasma, urine, and white blood cells. The behavior of ascorbic acid in white blood cells (Figure 1A) was employed as a general model for behavior in the slowly-equilibrating tissue compartment. The renal tubule and urine compartments are described in Figure 3. The Appendix contains the differential equations and associated parameters for the model.

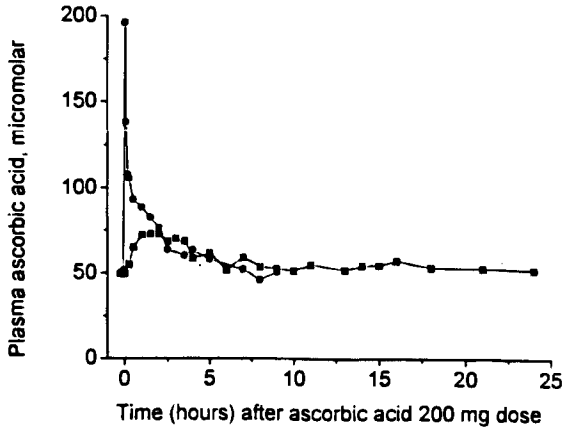

Fig. 2. Pharmacokinetic profile after oral and intravenous administration. These are representative plots of plasma ascorbic acid concentrations (micromolar) versus time (hours) for subject 6 after intravenous (circles) and oral (squares) doses of ascorbic acid 200 mg.

During the course of data analysis, simpler models were tested. These simpler models included first-order absorption with dose-independent bioavailability instead of saturable absorption, first-order nonrenal elimination from the central compartment instead of from the saturable peripheral compartment, zero-order instead of "Michaelis-Menten" renal tubular reabsorption, and rapid equilibration between renal tubular fluid and urine (compartments 4 and 5). All of these simpler models

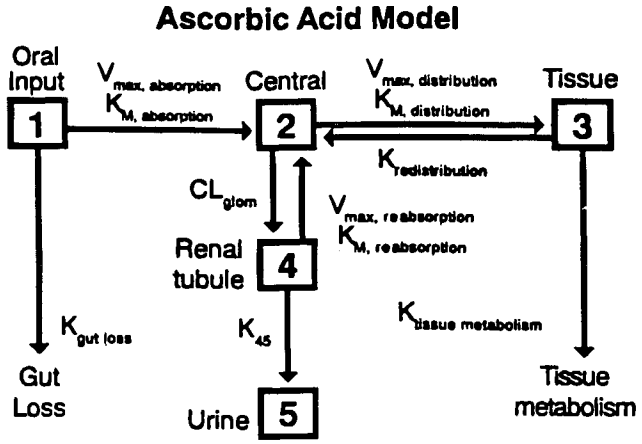

Fig. 3. Pharmacokinetic model of ascorbic acid in healthy humans. Abbreviations and differential equations appear in the Appendix. Oral input is into compartment 1. The actual amount of drug reaching the oral absorption compartment (compartment 1) is adjusted by a parameter for initial availability. There are two simultaneous losses from compartment 1, a saturable absorption process into the central compartment (compartment 2) and a first-order loss (gut loss) to the distal gut or lumen metabolism/degradation. Ascorbic acid leaves the central compartment via a saturable distribution process into compartment 3 (tissue) where there is first-order metabolic elimination (tissue metabolism). Ascorbic acid returns to the central compartment by a first-order process from the tissue compartment (redistribution). Ascorbic acid also exits the central compartment into renal tubular fluid (compartment 4) by glomerular filtration (CL_{glom}). A saturable, dose-dependent, reabsorption process returns ascorbic acid from renal tubular fluid to the central compartment. There is also a first-order loss of ascorbic acid from the renal tubular fluid to urine (compartment 5).

yielded a relatively large increase in the NONMEM™ objective function (>10 units) in a majority of subjects, indicating that these simpler models were inferior to the more complex model (Figure 3).

The summary parameter values estimated by nonlinear regression appear in Table II. Some model parameters compare well with observed values. For example, the volumes of the central compartment were calculated after all intravenous ascorbic acid doses from 15 to 1250 mg. The observed volumes of the central compartment remained similar, 10.93 ± 1.56 (mean ± SD) liters, across this wide dose range. The model-predicted volume of the central compartment, 11.8 liters (Table II) compares favorably with the observed value. These values also compare with the estimated extracellular volume for adult men, 15 liters (21).

In certain cases the model parameters are not informative. For example, the estimates for the K_M for renal tubule reabsorption ($K_{M\text{-ren}}$) and the elimination rate constant from renal tubule to urine (K_{45}) are imprecise. The lack of observed ascorbic acid concentrations in the renal tubule compartment and the apparent variability in ascorbic acid urinary excretion rate contribute to the imprecision in $K_{M\text{-ren}}$ and K_{45} parameter estimates. Future studies to better elucidate the renal parameters could include control of urine flow rate and urine pH.

In some cases, parameters of interest may be derived from the model. These derived parameters (Table II) offer further insights into ascorbic acid pharmacokinetics. For example, the K_M for renal tubular reabsorption, estimated in the model, is relative to the amount of ascorbic acid in compartment 4. Of more practical interest is the approximate K_M for renal tubular reabsorption relative to plasma concentration, $K_{M\text{-ren-p}}$. The $K_{M\text{-ren-p}}$ is derived from a plot of the amount in the tubule compartment versus the simultaneous plasma concentration. From this plot, a plasma concentration, expressed as the apparent $K_{M\text{-ren-p}}$ value, corresponds to a K_M value, expressed as the amount in the renal tubule compartment. The median apparent $K_{M\text{-ren-p}}$ value was approximately 33.7 micromolar (Table II).

Additional derived parameters of interest are the oral bioavailability values for ascorbic acid doses. Using the model, oral bioavailability was defined as a function of the initial availability of the dose in the absorption compartment, the parameters describing saturable absorption and the first-order loss from the gut. Bioavailability as a function of dose was calculated from the individual's parameters and summarized in Table II. Estimated bioavailability of oral doses declined from approximately 89% for the 15 mg dose to 47% for the 1250 mg dose.

Further derived parameters are estimated initial amounts of ascorbic acid in the central and tissue compartments (Table II). The model-predicted amount of ascorbic acid in the central compartment at time zero ($A_{2,0}$) is 212 micromoles. After dividing the predicted amount by the predicted volume (11.8 liters), the derived concentration of ascorbic acid in the central compartment at time zero is 18 micromolar which is similar to the observed concentration of 23.1 ± 7.5 (mean ± SD) micromolar at time zero.

The model demonstrates good performance when predicting concentration-time profiles for ascorbic acid over the entire experimental period. Figure 4 shows the overall plasma concentration-time profile, observed and predicted, for subject 3. Figure 5 demonstrates the correlation between observed and model-predicted urinary concentrations of ascorbic acid for all seven subjects. The quality of the agreement between observed and model-predicted values is similar for all seven subjects. The residual variability in plasma concentrations not explained by the model was 10–15%.

Table II. Summary of Estimated and Derived Subject Parameters for Ascorbic Acid Model

	Estimated parameters				Derived parameters		
	Mean	SD	Median		Mean	SD	Median
$V_{max\text{-}abs}$ micromole/hr	1011	707	715	$K_{M\text{-}ren\text{-}p}$ micromolar	33.1	4.33	33.7
$K_{M\text{-}abs}$ micromole	1744	1205	1164	F-15 mg	0.856	0.201	0.891
$K\text{-}_{gut}$ (1/hr)	0.175	0.123	0.247	F-30 mg	0.847	0.201	0.873
$V_{max\text{-}ren}$ micromole/hr	715	207	602	F-50 mg	0.837	0.202	0.850
$K_{M\text{-}ren}$ micromole	0.677	1.26	0.271	F-100 mg	0.815	0.206	0.801
$V_{max\text{-}tiss}$ micromole/(L*hr)	305	77.7	349	F-200 mg	0.778	0.218	0.720
$K_{M\text{-}tiss}$ micromolar	15.8	7.33	15.2	F-500 mg	0.748	0.241	0.633
V_c (L)	11.8	1.84	11.8	F-1250 mg	0.623	0.337	0.465
$CL\text{-}_{glom}$ (L/hr)	10.9	3.51	9.71	$A_{2,0}$ micromole	212	88.9	190
$K\text{-}_{redist}$ (1/hr)	0.313	0.101	0.259	$A_{3,0}$ micromole	7949	5751	5530
$K\text{-}_{met}$ (1/hr)	0.00172	0.000827	0.00153				
K_{45} (1/hr)	53.8	111	0.574				

Notes: Abbreviations: $K_{M\text{-ren-p}}$ = the apparent K_M value for renal tubular reabsorption relative to plasma concentration. F − X = the bioavailability of the X mg oral dose calculated from the model. $A_{i,0}$ = amount in compartment i at time = 0. Explanations of other abbreviations appear in the Appendix.

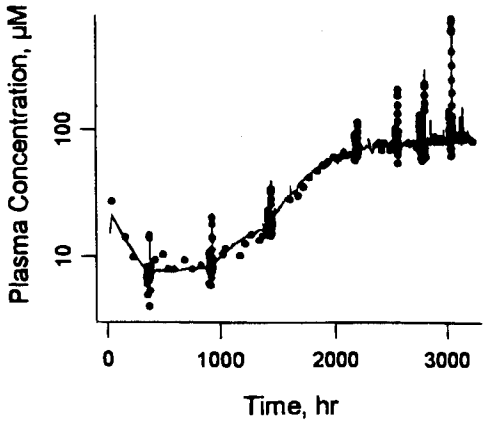

Fig. 4. Pharmacokinetic model predictions of ascorbic acid for Subject 3. On the log-linear plot, the y axis indicates ascorbic acid plasma concentration (micromolar). Dark circles are observed plasma concentrations. The line plot shows model-predicted concentrations in the central compartment. The x axis shows the number of hours from the start of ascorbic acid depletion at time zero. Pharmacokinetic profiles after oral and intravenous doses occurred at 356, 908, 1412, 2180, 2540, 2756, and 3020 hours. Pharmacokinetic profiles corresponded with successive ascorbic acid oral dose escalations: 15, 30, 50, 100, 200, 500, and 1250 mg twice daily. During the depletion phase, there was minimal ingestion of ascorbic acid (less than 5 mg per day) and plasma concentration and model-estimated tissue amounts declined. After initiating ascorbic acid 15 mg twice a day (392 hours), plasma levels stabilized. After the dosage increased to 30 mg twice a day, pre-dose plasma and tissue levels rose during the period from approximately 908 to 1412 hours. When the dose further increased from 30 to 50 mg twice a day, there was a four-fold increase in pre-dose plasma concentration while tissue levels increased. As dose further increased to 100 mg or more twice daily, pre-dose plasma and tissue levels increased very little.

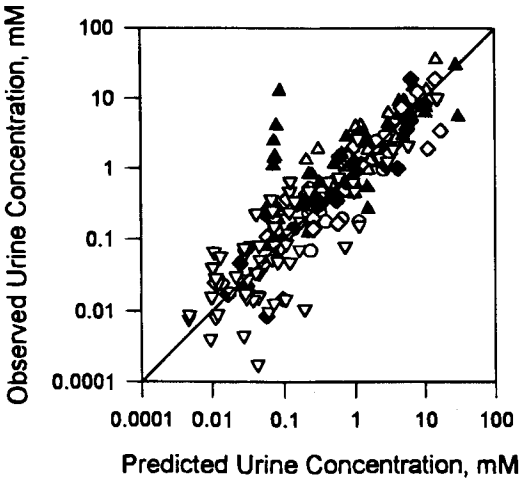

Fig. 5. Correlation between observed and model-predicted urinary concentrations of ascorbic acid for all seven subjects. The log-log scale for millimolar concentration was used because of the broad concentration range to avoid obscuration of low concentration values.

DISCUSSION

The present model describes ascorbic acid concentrations in healthy humans during depletion and repletion. The proposed model is necessarily complex. Reduced forms of the present model do not adequately predict plasma and urine concentration data over broad dose and concentration ranges. The proposed model is unique. Although previous models included nonlinear absorption or elimination, the present model is the first to combine these processes with nonlinear tissue distribution.

The proposed model expands the concept of saturable oral absorption presented by others (5–8). During exploratory data analysis, we examined the ratios of oral and intravenous AUC (area under the plasma-concentration-versus-time curve). Oral bioavailability appeared to be decreased for the 500 mg and 1250 mg oral doses (14). Although these data supported the concept of saturable oral absorption, we interpreted the actual values for AUC ratios with caution because of possible violation of the assumption of constant average clearance during comparable intravenous and non-intravenous doses. We tried various linear models of oral absorption, with and without lag times. No linear model of absorption adequately described the concentration data obtained from our subjects.

Saturable renal tubular reabsorption is necessary to explain the full range of concentration-time data. Simpler models assuming constant fractional renal tubular reabsorption adequately explain only portions of the data. The mean V_{max} for renal tubular reabsorption from our subjects, 715 micromoles per hour (126 mg/hr), is comparable with previous data (19). The time of ascorbic acid appearance in the urine compartment generally corresponds to the time of increased concentration of ascorbic acid in the central compartment. The mean K_M value for tubular reabsorption relative to plasma is 33.1 micromolar (5.8 mg/L). Thus, when plasma concentrations are at the upper plateau region of the profile, tubular reabsorption is approaching saturation. Concentrations observed during the dosing interval can be much higher than pre-dose values. At these times, tubular reabsorption would be expected to be even closer to saturation. As tubular reabsorption approaches saturation, the renal clearance of ascorbic acid will approach the CL_{glom} value shown in Table II. The mean value for CL_{glom} is about 11 liters/hour whereas glomerular filtration for the healthy male volunteers would be expected to be about 7.5 liters/hour. This would suggest that at high concentrations of ascorbic acid there might be net tubular secretion in humans, a finding previously reported in canines (22).

Using parameters from the model, maximum metabolic turnover was calculated. Assuming median K_{met} is 0.00153/hour and maximum amount of ascorbate in tissue is 6.5 millimole, then 0.00153/hour × 24 hour/day × 6.5 millimole × 176 mg/millimole equals 42 mg/day. The value for maximum metabolic turnover, calculated from the proposed model, agrees with a previous report of approximately 40 mg per day (4).

Maximum metabolic turnover is useful when discussing mass balance of ascorbic acid. For example, study subjects ingested single oral doses of ascorbic acid, 200 mg, and excreted 103 mg of ascorbic acid in the urine during the subsequent 24 hours (14). Assuming the oral bioavailability of ascorbic acid 200 mg dose is 72% (Table II), then the following mass balance equation pertains:

(oral dose) × (bioavailability) = (urinary turnover) + (metabolic turnover)

$200 \times 0.72 = 103 + 42$

$144 \approx 145$

Maximum metabolic turnover calculated from observations in healthy subjects should be interpreted with caution. Ascorbic acid metabolism (oxidation) in tissues or plasma could change in disease states or as a function of extracellular oxidants (23). In the proposed model, metabolic loss occurs from the tissue compartment. Attempts to describe the data from our healthy subjects with a first-order metabolic loss from the central compartment were not successful. After data accumulate in patients, the possibility of simultaneous first-order or saturable losses from the central compartment will be explored.

The tissue distribution component was a necessary innovation for the present model. Reduced models lacking saturable tissue distribution fail to fit the nonlinear behavior of plasma concentration when subjects ingest ascorbic acid 30–50 mg twice daily. The physiologic counterpart to the saturable tissue compartment remains to be determined. One potential explanation is that the tissue compartment represents the sum of all tissues known to accumulate ascorbic acid against a concentration gradient. Examples of cells and tissues which concentrate ascorbic acid include neutrophils, monocytes, lymphocytes, fibroblasts, liver, pituitary, spleen and adrenals (9–12,14,24).

It is tempting to attribute the plateau in pre-dose concentrations to increased renal clearance and decreased oral bioavailability. However, the same plateau in pre-dose concentration is observed for subjects who did not exhibit a dose-related decrease in oral bioavailability. Simulations based on the proposed model and various dose regimens, including multiple-dose intravenous regimens, clearly suggest the plateau in pre-dose plasma concentrations is more a result of the plateau in tissue levels than the changes in renal clearance and oral bioavailability. With an intravenous regimen there is a predicted increase in AUC with an increase in dose size, but little or no increase in pre-dose concentrations (Figure 6). Given the twice per day dosing schedule, the assumed model and the estimated parameters, pre-dose concentrations appear to be largely a function of the relatively slow return (leakage) of ascorbic acid from tissue storage sites.

The proposed model provides an excellent representation, on a macroscopic time scale, of the time course of plasma and urine ascorbic acid levels during depletion and gradual repletion. The model describes the average behavior of individual dosing events and is able to account for consistent dose-related changes in absorption and disposition. The proposed model is based on reasonable simplifications of physiologic factors known to influence ascorbic acid absorption and disposition. However, it is possible alternative models might provide an adequate description of the data. The present model was defined from data acquired from seven healthy young men. We do not know if the model will accurately describe ascorbic acid dose-concentration relationships in other populations, for example women, smokers, elderly, or patients with renal failure. The model does not attempt to address events occurring over the span of minutes such as recycling of ascorbic acid by activated neutrophils (23). Future modeling efforts may explore the roles of oxidative stress and ascorbic acid recycling as new pharmacokinetic data accumulate from patients. When designing future studies, the proposed model may help to explore optimal strategies for depleting, dosing, and sampling subsequent volunteers and patients.

These pharmacokinetic findings have implications for the recommended dietary allowance (RDA). The current RDA for ascorbic acid (vitamin C) of 60 mg daily is based on excretion threshold for urinary elimination and prevention of deficiency with a 30 day margin of safety (25,26). Based on these criteria, the RDA should be increased to 100 mg daily. New criteria have been proposed for the RDA (14,27,28) and include dietary availability, biochemical function in relation to concentration, steady-state concentration in plasma and tissues as a function of dose, urinary excretion, bioavailability, adverse effects, and beneficial effects in the population in relation to dose. Based on all of these criteria, a suitable RDA for ascorbic acid is 200 mg daily, which should be obtained from fruits and vegetables (14).

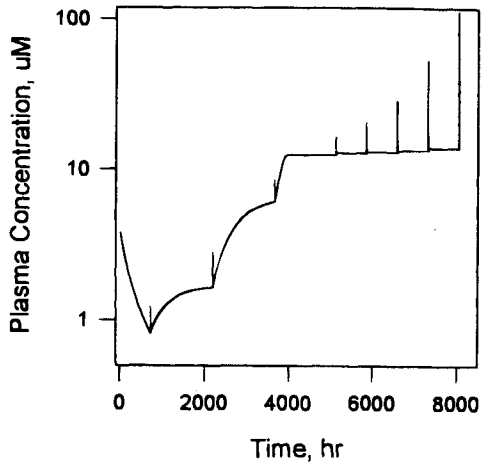

Fig. 6. Plasma ascorbic acid concentration-versus-time simulation of intravenous ascorbic acid doses to illustrate importance of saturable tissue distribution. The log-linear plot shows plasma ascorbic acid concentration (micromolar) on the y axis. The x axis shows the number of hours in the simulation. The model shows the plateau of pre-dose plasma concentrations is not dependent on a decrease in bioavailability at higher doses. The intravenous dosing schedule used in the simulation was 5 mg every 12 hours (0–720 hours, 61 doses), followed by 15 mg every 12 hours (732–2184 hours, 122 doses), then 30 mg every 12 hours (2196–3648 hours, 122 doses), then 50 mg every 12 hours (3660–5112 hours, 122 doses), then 100 mg every 12 hours (5134–5844 hours, 61 doses), then 200 mg every 12 hours (5856–6576 hours, 61 doses), then 500 mg every 12 hours (6588–7308 hours, 61 doses), then 1250 mg every 12 hours (7320–8040 hours, 61 doses). The parameters used for the simulation were those for Subject 3. The longer dosing periods for the 15, 30, and 50 mg doses were used to reasonably demonstrate achievement of steady-state. The plot shows only pre-dose plasma concentrations of ascorbic acid except the last dose of each dosing level. For the last dose, simulated plasma ascorbic acid concentrations are plotted for post-dose times of 0, 1, 2, 4, 6, 8, 10, and 12 hours.

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APPENDIX: PHARMACOKINETIC MODEL OF ASCORBIC ACID

Definition of abbreviations:

V_{max} is the theoretical maximum rate of a process.
K_M is the concentration or amount at which the rate of the process is equal to one-half the theoretical maximum rate.
K is the apparent first-order rate constant.
Asterisk (*) is the multiplication function and slash (/) is the division function.

Model Parameters

$V_{max\text{-}abs}$ = V_{max} absorption
$K_{M\text{-}abs}$ = K_M absorption
K_{gut} = K gut loss
$V_{max\text{-}ren}$ = V_{max} renal (tubular) reabsorption
$K_{M\text{-}ren}$ = K_M renal (tubular) reabsorption
$V_{max\text{-}tiss}$ = V_{max} (tissue) distribution
$K_{M\text{-}tiss}$ = K_M (tissue) distribution
V_c = volume of the central compartment
CL_{glom} = glomerular clearance by filtration from central compartment into renal tubule compartment
K_{redist} = rate constant for redistribution from tissue
K_{met} = rate constant for tissue metabolism
K_{45} = rate constant for elimination from renal tubule to urine
A_i = amount in compartment i

Gut absorption model	$X_1 = (V_{max\text{-}abs} * A_1)/(K_{M\text{-}abs} + A_1)$
Gut loss model	$X_2 = K_{gut} * A_1$
Renal tubular reabsorption model	$X_3 = (V_{max\text{-}ren} * A_4)/(K_{M\text{-}ren} + A_4)$
Tissue distribution model	$X_4 = (V_{max\text{-}tiss} * A_2)/(K_{M\text{-}tiss} + (A_2/V_c))$
Renal elimination model	$X_5 = (CL_{glom} * A_2)/V_c$
Tissue redistribution model	$X_6 = K_{redist} * A_3$
Tissue metabolism model	$X_7 = K_{met} * A_3$
Renal tubular elimination model	$X_8 = K_{45} * A_4$

Differential Equations

$$dA_1/dt = -X_1 - X_2$$
$$dA_2/dt = X_1 + X_6 + X_3 - X_4 - X_5$$
$$dA_3/dt = X_4 - X_6 - X_7$$
$$dA_4/dt = X_5 - X_3 - X_8$$
$$dA_5/dt = X_8$$

REFERENCES

1. J. Blanchard, K. A. Conrad, R. A. Mead, and P. J. Garry. *Am. J. Clin. Nutr.* **51**:837–845 (1990).
2. S. Yung, M. Mayersohn, and J. B. Robinson. *J. Pharm. Sci.* **67**:1491–1492 (1978).
3. G. Zetler, G. Seidel, C. P. Siegers, and H. Iven. *Eur. J. Clin. Pharmacol.* **10**:273–282 (1976).
4. A. Kallner, D. Hartmann, and D. Hornig. *Am. J. Clin. Nutr.* **32**:530–539 (1979).
5. S. Melethil, M. B. Subrahmanyam, C. J. Chang, and W. D. Mason. *Ann. NY Acad. Sci.* **498**:491–493 (1987).
6. V. K. Piotrovskij, Z. Kallay, M. Gajdos, M. Gerykova, and T. Trnovec. *Biopharm. Drug. Dispos.* **14**:429–442 (1993).
7. M. Mayersohn. *Eur. J. Pharmacol.* **19**:140–142 (1972).
8. S. Melethil, W. D. Mason, and C. J. Chang. *Internat. J. Pharmaceu.* **31**:83–89 (1986).
9. P. W. Washko, D. Rotrosen, and M. Levine. *J. Biol. Chem.* **264**:18996–19002 (1989).
10. P. Bergsten, G. Amitai, J. Kehrl, K. R. Dhariwal, H. G. Klein, and M. Levine. *J. Biol. Chem.* **265**:2584–2587 (1990).
11. R. W. Welch, P. Bergsten, J. D. Butler, and M. Levine. *Biochem. J.* **294**:505–510 (1993).
12. P. Bergsten, R. Yu, J. Kehrl, and M. Levine. *Arch. Biochem. Biophys.* **317**:208–214 (1995).
13. E. P. Ralli, G. J. Friedman, and S. H. Rubin. *J. Clin. Invest.* **17**:765–770 (1938).
14. M. Levine, C. Conry-Cantilena, Y. Wang, R. W. Welch, P. W. Washko, K. R. Dhariwal, J. B. Park, A. Lazarev, J. F. Graumlich, J. King, and L. R. Cantilena. *Proc. Natl. Acad. Sci. USA* **93**:3704–3709 (1996).
15. J. King, R. W. Welch, Y. Wang, K. R. Dhariwal, C. Conry-Cantilena, and M. Levine. *Am. J. Clin. Nutr.* (1997) in press.
16. M. Levine, K. R. Dhariwal, and C. C. Cantilena. *World Rev. Nutr. Dietetics* **72**:114–127 (1993).
17. K. R. Dhariwal, W. O. Hartzell, and M. Levine. *Am. J. Clin. Nutr.* **54**:712–716 (1991).
18. E. M. Baker, R. E. Hodges, J. Hood, H. E. Sauberlich, S. C. March, and J. E. Canham. *Am. J. Clin. Nutr.* **24**:444–454 (1971).
19. L. Berger, C. D. Gerson, and T. F. Yu. *Am. J. Med.* **62**:71–76 (1977).
20. S. L. Beal, and L. B. Sheiner. *NONMEM User's Guide Parts I–VI*, NONMEM Project Group, Division of Clinical Pharmacology, University of California, San Francisco, 1992.
21. M. Rowland and T. N. Tozer. *Clinical Pharmacokinetics Concepts and Applications, 3rd ed.*, Williams & Wilkins, Baltimore 1995.
22. S. Kleit, D. Levin, T. Perenich, and R. Cade. *Am. J. Physiol.* **209**:195–198 (1965).
23. P. W. Washko, Y. Wang, and M. Levine. *J. Biol. Chem.* **268**:15531–15535 (1993).
24. D. Hornig. *Ann. NY Acad. Sci.* **258**:103–118 (1975).
25. Food and Nutrition Board. *Recommended Dietary Allowances*, 10th ed., National Academy Press, Washington, DC, 1989.
26. Food and Nutrition Board. In Committee of Dietary Allowances (eds) *Recommended Dietary Allowances*, 9th ed., National Academy of Sciences, Washington, DC, 1980, pp. 72–81.
27. Food and Nutrition Board. *How should the Recommended Dietary Allowances be Revised*, National Academy Press, Washington, DC 1994.
28. M. Levine, S. Rumsey, and Y. Wang, et al. In L. J. Filer and E. E. Ziegler (eds), *Present Knowledge in Nutrition 7th ed.*, International Life Sciences Institute, Washington, DC, 1996, pp. 146–159.