International Journal of Pharmaceutics, 31 (1986) 83–89
Elsevier

IJP 01031

Dose-dependent absorption and excretion of vitamin C in humans

Srikumaran Melethil, William D. Mason and Chian-Jo Chang

Schools of Pharmacy and Medicine, University of Missouri at Kansas City, Kansas City, MO 64108-2792 (U.S.A.)

(Received December 27th, 1984)
(Modified version received July 30th, 1985)
(Accepted January 16th, 1986)

Key words: vitamin C – ascorbic acid – absorption – excretion – bioavailability

Summary

Four subjects ingested 500, 1 000 and 2 000 mg of ascorbic acid daily for one week according to a three-way crossover design. Following the last dose, serial urine and plasma samples were obtained over a 12-h period. The ascorbic acid content of these samples were determined by an HPLC method employing electrochemical detection. The plasma concentration–time profiles are similar at all 3 doses, with the area under the curve values (mean ± S.D.) being 206.0 ± 50.5, 212.1 ± 40.7, and 231.8 ± 52.6 mg · h/l for the 500, 1 000 and 2 000 mg doses. The corresponding percents (mean ± S.D.) of dose recovered in urine are 73.2 ± 25.7, 46.9 ± 21.7 and 35.8 ± 12.4. This decrease in recovery is significantly different ($P < 0.05$) between the 500 mg dose and the two higher doses. Renal clearance increases in proportion to plasma ascorbic acid in the concentration range (10–40 mg/l) encountered in the study. Results from this study indicate that both gastrointestinal absorption and renal tubular reabsorption of vitamin C are saturable processes. Therefore, (1) the validity of previous studies which have used linear pharmacokinetic analyses and (2) the systemic effects to be derived from megadoses of the vitamin administered orally are open to question.

Introduction

For humans, vitamin C is an essential nutrient which must be supplied from exogenous sources. The pharmacokinetics of the vitamin appear to be quite complicated. As early as 1938, it was shown that a sigmoidal relationship exists between urinary clearance of ascorbic acid and its plasma concentration in humans (Ralli et al., 1938; Friedman et al., 1940). This indicates the renal reabsorptive process is saturable. More recent evidence shows gastrointestinal absorption of the vitamin may also be saturable in man (Mayersohn, 1972). These two aspects, dealing with absorption and excretion of the vitamin, raise the possibility that increasing the oral dose of the vitamin may not proportionately increase its plasma concentration. Since this aspect had not been studied in the past, the objectives of this study were: (1) to examine steady-state plasma concentration of the vitamin following daily doses of 500, 1 000 and 2 000 mg in humans, and (2) to re-investigate the relationship between plasma ascorbate and its renal clearance using a specific analytical method (Mason et al., 1980) for the determination of vitamin C in plasma and urine.

Correspondence: S. Melethil, Schools of Pharmacy and Medicine, University of Missouri at Kansas City, 2411 Holmes Street, Kansas City, MO 64108-2792, U.S.A.

Materials and Methods

Four healthy non-smoking subjects (3 male, 1 female; ages: 22–29 years), participated in the study in which a complete three-way crossover design was used. They were selected following a medical history, physical examination and a complete laboratory evaluation and gave informed consent. To avoid ascorbic acid-depleted subjects, a pre-study plasma concentration of 8 mg/l was part of the inclusion criteria. This criterion was determined in a preliminary study showing steady-state was achieved in less than one week in such subjects. The subjects did not ingest other drugs for seven days prior to the study. The dietary intake of high vitamin C content foods and meal schedules were controlled during the study. Details of the protocol are given in Table 1. On study days (days 7, 14 and 21; see Table 1) the subjects did not eat high vitamin C foods. Following an overnight fast (10 h), the dose of vitamin C (500, 1 000 or 2 000 mg) was ingested with 200 ml of water in the morning. The subjects drank 200 ml of water every 2 h during the next 12 h. No food was ingested for the first 4 hours, post-administration. Plasma samples were drawn at 0, 1, 3, 5, 7, 9 and 12 h and urine samples were collected at 2-h intervals over the 12-h period. The procedures previously reported were used for sample collection and analysis of vitamin C in urine and plasma (Mason et al., 1980). Area under the plasma concentration–time curve (AUC) was estimated using the linear trapezoidal method. Areas were computed on the seventh day of each treatment to 12 and 24 h (AUC_{0-12} and AUC_{0-24}). For the AUC_{0-24}, the 24-h concentration was estimated on the concentration at time 0, consistent with steady-state. Renal clearance of vitamin C was calculated by dividing the urinary excretion rate by the plasma vitamin C concentration at the midpoint of the collection interval. Also, an average 12-h renal clearance was estimated by dividing the total ascorbate recovered in urine in 12 h by AUC_{0-12}.

Statistical analysis (SAS Users Guide, 1979) consisted of analysis of variance (ANOVA) by subject and drug followed by Duncan's multiple range test to determine which differences were significant. Power calculations were performed as previously suggested (Heck et al., 1979) to determine the probability $(1 - \beta)$ of rejecting a false hypothesis (type II error).

TABLE 1

STUDY PROTOCOL AND SAMPLING SCHEDULE FOR PLASMA AND URINE

Phase	Study day(s)	Dose [a] (mg)	Sampling schedule	In/out visit
I	1–6	500	–	Out
II	7	500	12 h profile [b,c]	In
III	8–13	1000	–	Out
IV	14	1000	12 h profile	In
V	15–21	500	–	Out
VI	22–27	2000	–	In
VII	28	2000	12 h profile	In

[a] Vitamin C tablets, 500 mg, Skaggs lot 2C781.

[b] Plasma sampling times were 0, 1, 3, 5, 7, 9, and 12 h post-administration.

[c] Urine collection intervals were 0–2, 2–4, 4–6, 6–8, 8–10 and 10–12 h post-administration.

Results

Mean plasma concentration–time profiles at steady-state for the three dosage levels are shown in Fig. 1. While concentrations increase slightly with dose, there are no significant differences ($P > 0.05$) in the plasma concentrations at any of the sampling times related to dose. Peak concentrations occur consistently at 3 h post-dosing. Mean peak values increase only slightly with dose (23.2, 24.2 and 26.9 mg/l at the 3 doses in increasing order) and are also not significantly different ($P > 0.05$). Table 2 lists the steady-state AUC_{0-24} values for the individual subjects. Only subject 3 exhibits a consistent increase in AUC_{0-24} as a function of dose, and even this increase is small (a 16% increase when the dose is doubled from 500 to 1 000 mg and a 14% increase when the dose is again doubled from 1 000 to 2 000 mg). Changes in 12-h areas, AUC_{0-12}, are similar to those observed with AUC_{0-24}. However, the percent of ascorbic acid dose recovered in the urine over the 12-hour collection interval at steady-state decreases as the dose increases (see Table 2). Analysis of variance

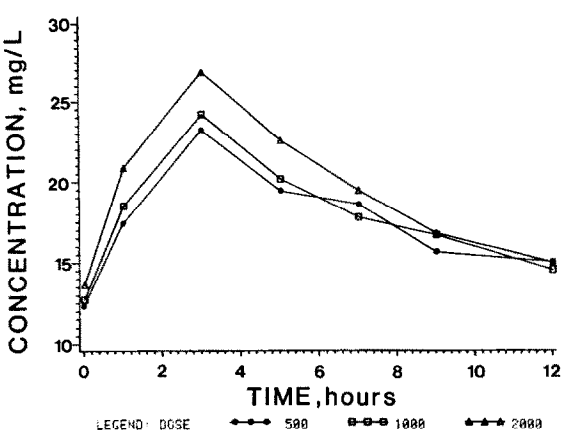

Fig. 1. Mean steady-state plasma concentrations of vitamin C following various oral doses. Values for doses are expressed in mg.

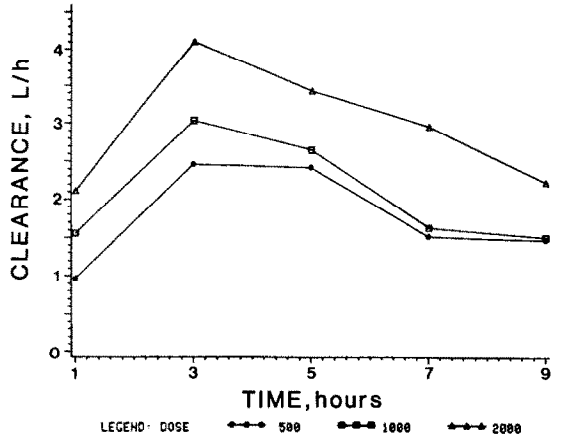

Fig. 2. Average renal clearance of vitamin C as a function of time.

in conjunction with Duncan's test showed the percent urinary recovery at the 500 mg dose is significantly higher ($P < 0.05$) than those recovered at the two higher doses. The analysis also revealed that urinary recovery in subject 2 is significantly lower than that in the other three subjects.

Plots of renal vitamin clearance versus the midpoint plasma concentration and time (Figs. 2 and 3) illustrate clearance is dependent on plasma concentration. Figs. 1 and 2 show increases and decreases in renal clearance with time coincide with similar fluctuations in plasma concentration. Multiple regression analysis which tested the relationship between midpoint clearance and plasma concentration, urine flow rate and urine pH for individual subjects showed correlation exists only between midpoint clearance and plasma vitamin C concentration. The linear regression slope values for plots similar to Fig. 3 for individual subjects ranged from 0.144 to 0.289. While the Y-axis intercept is not significantly different from zero in subjects 1–3, it is -3.107 for subject 4 ($P < 0.05$). In principle, such an intercept suggests that renal

TABLE 2

STEADY-STATE PLASMA CONCENTRATION AND URINARY EXCRETION DATA FOR VITAMIN C AT VARIOUS DOSES

Subject number	AUC_{0-24} (mg · h/l)			% dose excreted [a]			Average clearance [b] (l/h)		
	500 mg	1 000 mg	2 000 mg	500 mg	1 000 mg	2 000 mg	500 mg	1 000 mg	2 000 mg
1	440.5	425.8	400.5	98.5	72.2	33.8	2.02	2.94	2.92
2	340.1	299.1	327.3	37.5	19.6	19.1	1.10	1.16	2.10
3	301.0	349.6	399.0	80.9	51.6	44.0	2.34	2.48	3.79
4	491.3	472.7	525.8	76.1	44.2	46.6	1.36	1.65	2.92
Mean ±S.D.	393.2 ± 87.9	386.8 ± 77.4	413.2 ± 82.5	73.3 [c] ± 25.7	46.9 ± 21.7	35.9 [c] ± 12.5	1.71 [d] ± 0.574	2.06 ± 0.802	2.93 [d] ± 0.690

[a] 0–12 h urine collection.

[b] Average renal clearance was calculated as: $\frac{\text{amount excreted in 12 h}}{AUC_{0-12}}$

[c,d] Significantly different ($P < 0.05$).

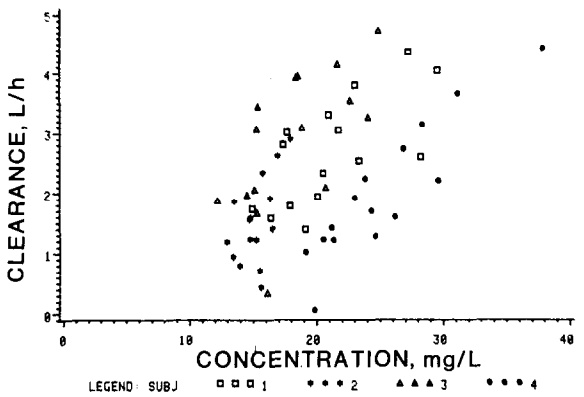

Fig. 3. Renal clearance of vitamin C versus corresponding plasma concentration in 4 human subjects.

excretion occurs only when plasma vitamin concentration exceeds a threshold value (i.e. X-axis intercept).

Average 12-h clearances for the 500, 1 000 and 2 000 mg (mean ± S.D.) are 1.71 ± 0.574, 2.06 ± 0.802 and 2.93 ± 0.690 l/h, respectively. The differences between the 500 and 2 000 mg doses are statistically significant ($P < 0.05$).

Discussion

Results from this study clearly show renal clearance of ascorbic acid to be dependent on its plasma concentration with increases in clearance corresponding to increases in plasma concentration. Time dependency of clearance (Fig. 2) corresponds with time dependency of plasma vitamin concentration (Fig. 1). The apparent linear relationship observed between renal clearance and plasma concentration in all 4 subjects is not in agreement with the sigmoidal relationship reported between these 2 variables (Friedman et al., 1940; Ralli et al., 1940). This discrepancy can be easily explained by comparing the concentrations encountered in this study with those in the previous studies. The sigmoidal relationship reported by Ralli et al. and Friedman et al. were observed in the 0–250 mg/l range. Concentrations observed in this study (10–40 mg/l) fall in the "linear" segment of the sigmoidal curve; and, therefore, a linear relationship between renal clearance and plasma concentration would be expected on data analysis. The most likely mechanism for the observed relationship between clearance and concentration is due to saturability of tubular reabsorption, as has been reported for another water-soluble vitamin, riboflavin (Jusko and Levy, 1970). At low plasma concentrations of the vitamin, its renal clearance is essentially zero due to complete reabsorption. As plasma vitamin concentrations increase, there is a corresponding increase in its clearance, due to progressive saturation of the reabsorptive process. Finally, when plasma concentrations are high enough to provide renal tubular concentrations much greater than the Michaelis constant for reabsorption, only an insignificant fraction of the filtered amount is reabsorbed and renal clearance becomes essentially independent of plasma concentration. Zetler et al. has reported that vitamin C is bound only to a small extent (~ 25% in the 12–18 mg/l range) to plasma proteins in humans. Hence, it is unlikely that observed concentration-dependent increase in renal clearance is due to increased filtration resulting from saturation of protein binding.

There is a trend for average renal clearances to increase with dose in all 4 subjects (Table 2). It is interesting that while AUC_{0-24} does not show a statistically significant increase with dose, average renal clearance shows a significant increase between the 500 and 2 000 mg doses. If renal clearance is related to plasma concentration, how can clearances increase without a corresponding increase in plasma concentration? This apparent inconsistency can be explained as follows: The mean value of the slopes of the regression lines between renal clearance and plasma concentration in the 4 subjects is 0.207 — that is, a 1 mg/l increase in plasma concentration results in a 0.207 l/h increase in renal clearance. This increase in clearance will dampen the tendency of plasma concentrations to rise with increasing dose. Since AUC value represents changes over a dosing interval, it is even less sensitive to transient increases in concentration that may have occurred. Fig. 1 does show that plasma concentrations increase slightly with dose, particularly during the first 6 hours after dosing. The power ($1 - \beta$) of ANOVA used to test for differences in AUC_{0-24} was 0.92. There-

fore, though only 4 subjects were used, the probability of detecting a significant difference, if it existed, is 0.92. Hence, large increases in dose (4-fold in this case) may only result in relatively small increases in AUC_{0-24}.

Data obtained from this study (Table 2) show the percent of dose excreted in urine over 12 h decreases with dose. This is suggestive of saturable absorption. Since vitamin C was dosed at 24-h intervals, the amount excreted in the 0–12-h period can underestimate the actual amount absorbed. However, in a previous unpublished study in our laboratory, mean plasma vitamin concentrations in 12 human subjects at 12 and 24 h following a 500 mg oral dose were 9.1 and 7.9 mg/l (a drop of only 13.2% over 12 h), respectively. In the present study, similar results are also observed. Plasma concentrations at the end of 12 h are essentially equal to the predose values at all 3 doses (15.1 ± 4.0 vs 12.3 ± 3.9 mg/l for the 500 mg dose, 14.6 ± 3.6 vs 12.8 ± 2.4 mg/l for the 1 000 mg dose, and 15.0 ± 2.7 vs 13.7 ± 1.6 mg/l for the 2 000 mg dose). The mean ratios of Cp (t = 12) to Cp (t = 0 or 24) at the 3 doses are 0.81, 0.89, and 0.92, respectively. Friedman et al. has shown that renal clearance of the vitamin is negligible up to about 12 mg/l. The mean value of $46.9 \pm 21.7\%$ for the extent of dose absorbed at the 1 000 mg dose (Table 2) is higher than the values of $29.8 \pm 17.6\%$ reported by Yung et al. (1982), where the investigators compared urinary excretion following 1 000 mg administered via oral (as tablets) and intravenous routes. Therefore, the excretion during the 12–24-h period was assumed to be negligible in comparison to the first 12 h (i.e. 0–12 hour collection) because of complete reabsorption, and the 12-h urinary vitamin excretion was taken as a good estimate of its absorption.

The disposition kinetics of ascorbic acid in humans, like that of riboflavin (Jusko and Levy, 1970; Levy, 1976) appear to be complex in that both absorption and elimination may be saturable. Combined saturable absorption and renal tubular reabsorption appear to provide a powerful homeostatic mechanism for ascorbate in the body. The possibility that the absorptive process is saturable has been previously reported in the literature (Mayersohn, 1972; Stevenson, 1974). The urinary recoveries (1 000 mg dose) from solution and chewable tablet are similar to that of the tablet ($29.8 \pm 17.6\%$) but is considerably lower ($14.2 \pm 7.2\%$) from a time-released capsule. In a previous study, the same investigators (Yung et al., 1981) reported that administration of 1 000 mg dose, either in divided doses or with meals, increased urinary recovery to about 50% of the dose. These reports, along with data from this study clearly support the hypothesis that absorption is dose dependent and this dependency may be due to a specialized saturable absorption process. Results from this study, along with that of Ralli et al. and Friedman et al. show renal clearance increases with plasma concentration, suggesting tubular reabsorption of the vitamin is a saturable process. Therefore, areas under the plasma concentration–time curves cannot be used directly for bioavailability comparisons. This was verified by urinary excretion data which show that as the dose increases the actual amount excreted in the urine (0–12 h collection) increases less than proportionally (367 mg from the 500 mg dose, 469 mg from the 1 000 mg dose, and 718 mg from the 2 000 mg dose).

Due to the non-linear dependence of concentrations on bioavailable dose discussed above, the use of urinary excretion data to assess bioavailability of vitamin C preparations merits consideration. In principle, this approach is valid only if a constant (and preferably a large) fraction of the amount recovered in the urine is present as the intact vitamin. Following a 1 000 mg intravenous dose of vitamin C in 5 human subjects, 82.6% of the dose is recovered in urine and 84% of that amount is present as the unchanged vitamin (Yung et al., 1978). Kallner et al. have also shown that at oral doses of 1 000 and 2 000 mg, 82.4 and 87.5% of the amount recovered in urine represents ascorbic acid. This group also found that at low doses (60–180 mg) the corresponding values are much smaller (20.3–61.7%). Hence, the use of cumulative urinary excretion of the vitamin to assess bioavailability of doses equal to or larger than 500 mg appears valid.

In view of the dependency of renal clearance on plasma concentration, it is not valid to apply linear pharmacokinetic models to the analysis of

intravenous vitamin C data, unless the plasma ascorbate concentrations are in the 200–250 mg/l range (Ralli et al., 1938; Friedman et al., 1940). At these values, which reflect the upper plateau region of the sigmoidal curve, renal clearance is virtually independent of plasma ascorbate. Recently, it was reported (Yung et al., 1978) that using urinary excretion data, pharmacokinetics of intravenous vitamin C can be described by a 2-compartment model with first-order elimination in human subjects whose body stores were "saturated" with the vitamin by pretreatment of a daily oral dose of 1000 mg for a period that was less than 2 weeks. These investigators did not measure plasma ascorbate in their study. As shown in our study, 1000 mg doses of vitamin C do not increase plasma concentrations to a point where its renal clearance become independent of plasma concentration. An alternate explanation for the biphasic nature of the urinary excretion rate plot observed by Yung et al. (1978) can be given based on the saturation of the tubular reabsorptive process. Immediately following intravenous administration when plasma concentrations are the highest, excretion rates are also high due to capacity limited reabsorption. As plasma concentrations decrease, the corresponding excretion rates decrease more than proportionally, due to greater tubular reabsorption. Such excretion behavior can also be expected to result in a biphasic urinary excretion rate plot. Our data indicate that pharmacokinetic parameters available in the literature may be in error and that new studies are needed which take into account the capacity limited nature of the absorption and elimination of vitamin C.

There is continued interest in the clinical benefits of mega doses of vitamin C. For example, in 1985 Moertel et al. reported that large doses of the vitamin (10 g daily) are not effective against cancer. If the gastrointestinal absorptive and renal tubular reabsorptive processes of the vitamin are saturable, as existing data suggests, then the systemic exposure of the body to the vitamin may not increase with dose. Data from this study (Fig. 1) show that in the 500–2000 mg range, plasma concentrations are not significantly influenced by dose. A recent report by Yew et al. (1984), suggests that this may be true also at mega doses. After 3–4 weeks, the mean plasma concentration in 6 subjects who ingested 8 to 12 g of vitamin C is 19.5 ± 0.20 mg/l, while the corresponding concentration in 11 different subjects who ingested 1 to 3 g daily is 15.4 ± 0.16 mg/l. These values agree well with those observed in the present study described herein (Fig. 2). Therefore, the systemic value of mega doses of the vitamin is open to question. Smaller daily doses (0.5–1 g) may be just as effective systematically as large (8–10 g) doses. This hypothesis needs to be tested by future experiments.

References

Friedman, G.J., Sherry, S. and Ralli, E.P., The mechanism of the excretion of vitamin C by the human kidney at low and normal plasma levels of ascorbic acid. J. Clin. Invest., 20 (1940) 685–689.

Heck, H.D'A., Buttrill, E.S., Flynn, N.W., Dyer, R.L., Anbar, M., Carrns, T., Dighe, S. and Cabana, B.E., Bioavailability of imipramine tablets relative to a stable isotope-labeled internal standard: increasing the power of bioavailability tests. J. Pharmacokinet. Biopharm., 7 (1979) 233–248.

Jusko, W.J. and Levy, G., Pharmacokinetic evidence for saturable renal tabular reabsorption of riboflavin. J. Pharm. Sci., 59 (1970) 765–772.

Kallner, A., Hartmann, D. and Hornig, D., Steady-state turnover of body pool of ascorbic acid in man. Am. J. Clin. Nutr. 32 (1979) 530–539.

Levy, G., Pharmacokinetic approaches to the study of drug interactions. Ann. N.Y. Acad. Sci., 281 (1976) 24–39.

Mason, W.D., Amick, E.N. and Heft, W., Analysis of ascorbic acid in human plasma and urine by HPLC with electrochemical detection. Anal. Lett., 13B (1980) 817–824.

Mayersohn, M., Ascorbic acid absorption in man — pharmacokinetic implications. Eur. J. Clin. Pharmacol., 19 (1972) 140–142.

Moertel, C.G., Fleming, R.T., Creagan, E.T., Rubin, J., O'Connell, M.J. and Ames, M.M., High-dose vitamin C versus placebo in the treatment of patients with advanced cancer who have had no prior chemotherapy. N. Engl. J. Med., 312 (1985) 137–141.

Ralli, E.P., Friedman, G.J. and Rubin, S.H., The mechanism of the excretion of vitamin C by the human kidney. J. Clin. Invest., 17 (1938) 765–770.

SAS Users Guide (1979), SAS Institute, Raleigh, NC 27605, pp. 237–241.

Stevenson, N.R., Active transport of L-ascorbic acid in the human ileum. Gastroenterology, 67 (1974) 952–956.

Yew, S.M., Megadose vitamin C supplementation and ascorbic acid and dehydroascorbic acid levels in plasma and lymphocytes. Nutr. Rep. Int., 30 (1984) 597–601.

Yung, S., Mayersohn, M. and Robinson, B.J., Ascorbic acid elimination in humans after intravenous administration. J. Pharm. Sci., 67 (1978) 1491–1492.

Yung, S., Mayersohn, M. and Robinson, B.J., Ascorbic acid absorption in man: influence of divided doses and food. Life Sci., 28 (1981) 2505–2511.

Yung, S., Mayersohn, M. and Robinson, B.J., Ascorbic acid absorption in humans: a comparison among several dosage forms. J. Pharm. Sci., 71 (1982) 282–285.

Zetler, G., Seidel, G., Siegers, C.P. and Iven, H., Pharmacokinetics of ascorbic acid in man. Eur. J. Clin. Pharmacol., 10 (1976) 273–282.