Comparative Bioavailability to Humans of Ascorbic Acid Alone or in Citrus Extract

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Abstract

This study was performed to determine whether synthetic Ascorbic Acid (AA) alone or in a natural citrus extract containing bioflavonoids, proteins and carbohydrates was more bioavailable to human subjects. The effect of a single 500 mg ascorbate dose of the two forms and a placebo citrus extract on plasma concentration-time curves showed that the citrus extract was 35% more absorbed than AA ($p < 0.001$) and was more slowly absorbed than AA ($p < 0.001$). In six ascorbate-saturated male subjects the ascorbate in the citrus extract produced a greater ascorbate excretion than AA alone in 24-h post dose urine ($p< 0.05$). Citrus extract ascorbate was less excreted than AA ($p < 0.05$) in 12 non saturated subjects. Ascorbate in the citrus extract was found to be more bioavailable than AA alone in human subjects.

Introduction

There is now considerable general interest in vitamin C (Ascorbic Acid) supplementation because of recent popular books on the treatment of the common cold and cancer by synthetic ascorbic acid (AA). Also, certain populations such as smokers and elderly people are known to have low tissue levels of ascorbate (1,2) and might benefit from supplementation. Although natural and synthetic ascorbic acids are chemically identical, citrus fruits contain bioflavonoids such as naringin and hesperidin as well as carbohydrates and proteins that might affect the bioavailability of the ascorbate. However, individuals who drink frozen or reconstituted fruit juices are ingesting < 4 mg of total bioflavonoids/l of fruit juice (3), which are removed in processing due to their bitter taste.

The view that bioflavonoids might act as compounds that reduce the need for ascorbic acid and thus affect its tissue concentration was first postulated by a French group >35 years ago (4). They found that the amount of ascorbate in the organs of guinea pigs was increased by the addition of bioflavonoids to the diet. Our group determined that a citrus extract containing ascorbic acid was more bioavailable to guinea pigs than AA alone (5). The effect of bioflavonoids on tissue ascorbate was the subject of an extensive review (6).

Human studies, on the other hand, have been equivocal. A Canadian group recently found that the bioavailability of pure AA was slightly superior to ascorbate in orange juice (7). A study using interluminal perfusion of human small intestine showed no difference between ascorbate in reconstituted orange juice and AA (8). The objective

of the present study was to determine the relative bioavailability of a citrus extract to that of AA alone in humans.

Subjects and Methods

Test Solutions

The materials compared in this study were synthetic L-AA from Fisher Chemical Company (Pittsburgh, PA) and renatured vitamin C in citrus extract (CE), Citrus Fruit Media. The placebo CE was a light brown water soluble powder containing 0.2% AA, 19.5% citrus bioflavonoids (19.0% naringin, 0.34% naringenin and 0.17% hesperidin) and a minimum of 30% carbohydrates and 15% protein. The CE was identical to the placebo CE except that AA was added to a final concentration of 25.1g/100g as measured by a high pressure liquid chromatography (HPLC) procedure after protein precipitation by metaphosphoric acid (9). The bioflavonoids were identical and quantified by HPLC (10) and were the typical flavones of citrus fruit.

Subjects and Sampling

This study was approved by the Human Subjects Committee of the University of Scranton. All subjects gave their written informed consent before participation. Eight healthy, non-smoking individuals (five males and three females) volunteered for the plasma study. The subjects ranged in age from 18-41 years old with and average age of 22 ± 6 years. None of the subjects was taking vitamin supplements and none consume foods high in ascorbic acid on the day of the study. Each subject reported between 0800 and 0830 after an overnight fast for a base line drawing by finger prick sampling using an Autolet device (Owen Mumford Ltd. Oxford, UK). This technique was shown to give a sample ascorbate concentration equivalent to the more common venipuncture method (11) at much less risk and discomfort to the subject. The blood was collected in EDTA tubes and the plasma was separated immediately after a sampling. In a random crossover experimental design, each subject drank either 500mg AA, 2g placebo CE or 2g CE dissolved in 50ml of a solution of 278mmol glucose/l. The placebo CE provided 4mg ascorbate and the CE, 502mg. Finger-prick samples were taken 1, 2, 3, 4, 6 and 8 hours after dosing. Subjects were not allowed to eat until after the last sampling but were allowed to drink water as desired. A 0.1ml aliquot of blood plasma was immediately mixed with an equal volume of 1.56 mol metaphosphoric acid/l and frozen at -20°C. The next day the samples were analysed by a fluorometric method (12) that measures unoxidised ascorbate. Bioflavonoids did not give a fluorescence with this procedure and therefore did not interfere. At weekly intervals the subjects ingested the other formulations and the sampling procedure was repeated.

Six males aged 21 - 41 years with an average age of 26 ± 8 years volunteered for the first urine study. The subjects were saturated with AA in a protocol similar to that of Robinson (13). After 2 weeks of consuming 1g AA to saturate the body stores, the subjects avoided ascorbic acid for 2 days before the experimental day. During the second day of this 2 day period, a 24 hour urine collection was made for use as a blank. After fasting overnight the subjects ingested in the morning 50mg of Ascorbate in one of two forms, AA or CE (dissolved in 50ml of a solution of 278 mmol glucose/l), in a random crossover experimental design. The subjects were then allowed to eat lunch and dinner while still avoiding foods high in ascorbic acid. A 24 hour

urine collection was made. The following day the subjects resumed taking 1g AA for 5 days and then stopped for 2 days before ingesting the other form. Twenty-four hour blank and post-dose urine was collected as before. Urine was stored in plastic specimen bottles over 50ml of 3.12 mol metaphosphoric acid/l, kept refrigerated between collections and frozen at -20°C until analysis within 1 week. The 24 hour volume was recorded and an aliquot was taken for fluorometric analysis of total ascorbate after reduction of dehydroascorbic acid with mercaptoethanol.

Twelve subjects (six males and six females) volunteered for the second urine study. They ranged in age from 18-41 years with an average age of 22 ± 6 years. A blank 24 hour urine collection was made the day before dosing. after an overnight fast subjects consumed either AA, placebo CE or CE as in the plasma study. Twenty four hour urine was collected as described previously. One week later another 24 hour urine blank was collected. The other forms were given and the procedure was repeated. The subjects avoided foods high in ascorbic acid and in their diet 1 week before the experiment and during the week of the experiment.

Data Analysis

Areas under the plasma ascorbate concentration-time curves were measured for each individual by manual planimetry with a reproducibility of 2.5%. The points for determining the time for maximal ascorbate concentration were plotted by a cubic spline curve-fitting programme on an IBM PC (model XT) computer (Armonk, NY).

Statistical comparisons of the results were made by a paired or two sample Student's *t*-test.

Results

The initial mean ± SD fasting plasma ascorbate was 35.2 ± 13.7 μmol/l (range 14.8-69.8 μmol/l). These are typical values of a normal population (14). There was no significant difference between male and female subjects, with levels of 35.1 ± 14.2 and 35.2 ± 11.1 μmol/l, respectively. The extent of absorption of ascorbate was determined by comparing the areas under the plasma ascorbate concentration-time curves after administration of each formulation. Because absorption of AA is dose-dependent (15) comparison of AA and CE was made at the same dose of ascorbic acid. The areas were measured for each subject and the data are shown in Table 1. There was no significant difference between males and females for either AA or CE; therefore, the results were combined. All subjects showed a greater area under the curve for CE than for AA. The ascorbate in the CE was found to be significantly more absorbed than the AA ($p < 0.001$).

Table 1: Areas under the plasma ascorbate time-concentration curves after oral administration of 500mg ascorbate in the form of synthetic ascorbic acid (AA) alone or in a citrus extract (arbitrary units).

			Plasma ascorbate area	
Subject	Age (years)	Sex	AA	Citrus Extract
1	18	F	571	824
2	18	F	748	842
3	19	F	389	629
4	41	M	672	816
5	21	M	470	873
6	22	M	565	772
7	21	M	656	879
8	18	M	651	748
$\bar{x} \pm$ SD			590 ± 117	797 ± 82*

* Significantly different by a paired *t*-test ($p < 0.001$).

The average plasma ascorbate curves for the three formulations are shown in Figure 1. The maximal ascorbate concentrations were 197 ± 20.2 μmol/l for AA, 209 ± 32.9 μmol/l for CE and 56.8 ± 11.9 μmol/l for placebo CE. The time for maximal plasma ascorbate was 2.9 ± 0.3h for AA and 4.1 ± 0.5h for CE ($p < 0.001$). A statistical evaluation of the AA and CE curves in Figure 1 indicates that there was no significant difference between the two dose forms except at 4 hours after ingestion when the CE produced a 68% greater plasma ascorbate than AA ($p < 0.005$). The placebo CE produced a small but significant increase in plasma ascorbate after 3 hours compared with the 0 hour value ($p < 0.05$).

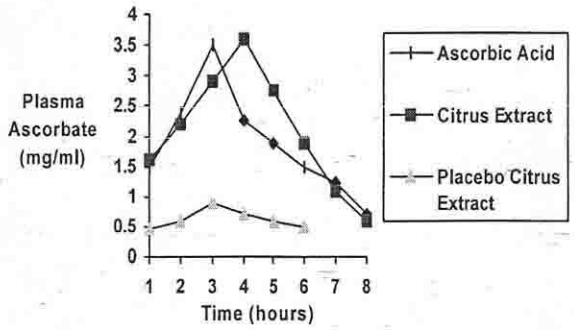

Fig. 1. Plasma time-concentration curves for eight fasting subjects ingesting 500 mg of ascorbate as synthetic ascorbic acid (AA) alone or as 2g of a citrus extract, or 2g placebo citrus extract, or 2g placebo citrus extract ($\bar{x} \pm$ SD). (To convert mg/dl to μmol/l, multiply by 56.78).

The results of the first urine excretion study after a previous saturation of the body stores with ascorbate are shown in Table 2. All six subjects showed a greater excretion of ascorbate after ingestion of CE compared with AA ($p < 0.05$).

Table 2: Net urinary excretion of ascorbate after oral administration of 500 mg synthetic ascorbic acid (AA) alone or in a citrus extract to AA saturated subjects (mg).

	Urinary Ascorbate	
Subject	AA	Citrus Extract
1	8.0	26.4
2	73.4	157.7
3	49.6	189.9
4	102.1	172.1
5	36.8	43.2
6	108.4	262.1
$\bar{x}$ ± SD	63 ± 38.9	141.9 ± 90.5*

* Significantly different by a paired *t*-test ($p < 0.05$).

Results of the second urine study of subjects who were not saturated with ascorbate are displayed in Table 3. The placebo CE produced no net excretion of ascorbate. There was no significant difference between the male and female excretion data for AA or CE although males tended to excrete more ascorbate after AA ingestion than the females. Five of the six males and four of the six females excreted more ascorbate after ingestion of AA than after ingestion of CE and the difference between the two forms was significant ($p < 0.05$).

Table 3: Net 24 hour urinary excretion of ascorbate after oral administration of a placebo citrus extract or 500 mg synthetic ascorbic acid (AA) alone or a citrus extract (mg)*

	Urinary ascorbate			
Subjects	AA	Placebo citrus extract	Citrus extract	p**
Males (n = 6)	181 ± 89	-2.3 ± 11.4	72 ± 35	< 0.05
Females (n = 6)	93 ± 32	-26.9 ± 38.7	71 ± 29	NS
All Subjects	135 ±79	-10.5 ± 23.2	72 ± 31	< 0.05

* $\bar{x}$ ± SD.

** Significance of difference between AA and citrus extract by a paired *t*-test.

Discussion

A comparison of the areas under the plasma ascorbate concentration-time curves (Table 1) allows the determination of the relative bioavailability of the two forms. The ascorbate in the CE was found to be 35% more bioavailable than AA alone ($p < 0.001$). This result is similar to the 48% greater bioavailability of CE found in our guinea pig study (8). These findings seem to contradict an earlier human study by Pelletier and Keim (6) who found that AA plus the bioflavonoid rutin produced a slightly lower serum ascorbate increase than did AA alone. However, that study measured ascorbate 2 hours post-dose, at which time our data showed no significant difference between AA alone and the CE that contained bioflavonoids (Fig. 1). The area under the placebo CE curve was 9.4% of that under the CE curve. This percentage is more than would be expected form the ascorbate in the placebo CE, which is only 0.8% of the CE ascorbate. This result from a human study corroborates previous data reviewed by Hughes and Wilson (5) that showed elevated tissue ascorbate in guinea pigs after administration of bioflavonoids.

As seen in Figure 1 the CE produced a peak plasma ascorbate concentration at a longer time interval after ingestion than did AA. This was not a dosage form difference because both CE and AA were given in solution. The result indicates that the CE was more slowly absorbed than the AA. The time for maximum concentration

for AA was 2.9 hours which is nearly identical to the 2.8 hours found by Zeitler et al (15). The time for the CE was 4.1 hours, similar to the 4-5 hours found by other workers after ingestion of timed release formulations of AA (13, 16).

To use urinary excretion data to compare the absorption of the two forms of ascorbic acid it is necessary to saturate the body stores because the retention of ascorbate in the body is influenced by the nutritional status of the individual (13). The fact that the subjects were saturated is corroborated by the significantly greater blank 24 hour urinary ascorbate in the saturated group than in the unsaturated group in the second urine study (158.0 ± 59.5 mg and 77.5 ± 35.2 mg, respectively [$p < 0.05$ by a two sample *t*-test]). The excretion from the saturated group is similar to that reported by Jones (16), 237 ± 25.4 mg. The data on presaturated individuals in Table 2 confirm the plasma results that indicated that the CE was absorbed more than AA alone. The urine results are similar to a previous study (6) which showed that AA plus rutin or fresh orange juice alone produced a greater ascorbate excretion than AA alone in pre-saturated subjects. Another report that confirmed these results (16) showed that black currant juice, high in bioflavonoids, produced a greater urinary excretion of ascorbate than did AA alone.

The second urine study (using non-saturated subjects) produced different results from the first study. The results in table 3 demonstrate that the CE produces significantly less ascorbate excretion than AA alone in non-saturated subjects. In light of the plasma study and the first urine study, which showed increased absorption for the CE compared with AA alone, the second urine study results may be interpreted to indicate that the ascorbate in the CE is sequestered to a greater extent than AA alone in the tissues of non-saturated subjects.

The finding that the ascorbate in CE is more bioavailable and more slowly absorbed than AA alone, i.e., that it acts as a timed release formulation, is advantageous for supplementation. The water soluble CE does not suffer from the disadvantages of previous times release forms which were found to be less bioavailable than conventional forms of AA (17). Although the mechanism of the greater bioavailability of the ascorbate in CE is not known, previous studies with bioflavonoids produced two possible mechanisms: enhanced absorption and stabilisation of ascorbate (5).

In conclusion, the ascorbate in the CE was found to be more slowly absorbed and more bioavailable than AA alone and is thus the preferred form of ascorbate for supplementation.

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