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Comparative Bioavailability of Synthetic and Natural Vitamin C in Guinea Pigs

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Abstract
This study was undertaken to compare the bioavailabilities of synthetic ascorbic acid and a natural vitamin C which contains bioflavonoids. Adult male guinea pigs were orally dosed with 50 mg of ascorbate and the serum levels measured using a fluorometric method. The two forms of ascorbic acid gave similar peak concentrations of serum ascorbate but the natural vitamin C peaked later and remained in the serum for a longer time period. The bioavailability of the natural vitamin C was significantly greater (148%, $p < 0.001$) than that of the synthetic ascorbic acid.

Introduction

The requirement of ascorbic acid (vitamin C) is a common property of living organisms. Practically all animals except the guinea pig, monkey and man can synthesise this vitamin. Vitamin C plays an important role in many metabolic reactions including synthesis of collagen [1] and synthesis of proteins of the immune system [2]. There is now considerable interest in ascorbate supplementation in amounts that are relatively large when compared to the recommended dietary intake. This interest is due to recent books by Linus Pauling on the efficacy of ascorbic acid in the treatment of colds [3] and treatment of cancer [4].

Although natural and synthetic ascorbic acids are chemically identical, citrus fruits and other natural sources of vitamin C contain other compounds including bioflavonoids which could affect the bioavailability of ascorbic acid. In fact, bioflavonoids have been shown to improve the utilisation of ascorbic acid and increase its storage in guinea pigs [5, 6, 7]. However, human studies have shown conflicting results. The comparison of serum and urine levels after oral intake of comparable doses of natural and synthetic vitamin C have led to the conclusion that natural vitamin C shows greater [8], equal [9], or less [10] availability than synthetic ascorbic acid. Most recently, Nelson [11] by an intraluminal perfusion technique found the two forms to be similarly absorbed.

Since the absorption of ascorbate into the plasma determines its metabolic availability to the tissues [11], the plasma concentrations of the vitamin following an oral dose may be used to determine the bioavailability of a supplement. Guinea pigs are an appropriate animal model for vitamin C since the guinea pig, like man, has an active transport system [12]. Also, the guinea pig minimises the large intersubject variation in bioavailability which are often seen in human studies. The present study describes the relative bioavailability of a synthetic and natural vitamin C after a single dosing of guinea pigs.

Materials and Methods

Subjects: Ten adult male Hartley guinea pigs were assigned to one of two groups on the basis of weight so that the average weight of the two groups was statistically identical. They were fed a standard guinea pig chow for several weeks until the time of the experiment.

Formulations: The two formulations were synthetic L-ascorbic acid (Fisher Scientific Company, Pittsburgh, PA.) and Renatured vitamin C in Citrus Fruit Media. The vitamin C in the latter was synthetic ascorbic acid added to natural proteins, carbohydrates and bioflavonoids 18%, proteins 15% and carbohydrates 30%. The product was a light brown, water soluble powder.

Dosage Schedule: The guinea pigs were fasted overnight preceding dosing. Each guinea pig received by means of an analytical pipette 1 ml of a 50 mg/ml ascorbic acid solution freshly prepared in distilled water. Blood samples (0.2 ml) were taken from the heart during light ether anaesthesia before dosing and periodically after dosing. Blood samples were collected with EDTA as an anticoagulant and centrifuged at 4000 rpm for 10 minutes. Plasma (0.1 ml) was taken for ascorbic acid analysis and assayed the same day or alternatively the proteins were precipitated with metaphosphoric acid and the sample frozen at -20°C until analysis within 2 days.

Measurement of Ascorbate: Ascorbic acid was measured in plasma by fluorescence [13] following precipitation of proteins with metaphosphoric acid and reaction with 1,2-naphthoquinone-4-sulphonic acid. A standard curve was determined using freshly prepared aqueous standards. Quercetin, a representative bioflavonoid, gave zero fluorescence at a concentration of 5 mg/100 ml. Thus, bioflavonoids which are present in the natural vitamin C are not an interference in the assay procedure.

Results

Concentrations of ascorbate in plasma: The results for the determination of ascorbate in plasma after an oral dosing of 50 mg of ascorbate in the form of synthetic ascorbate and natural vitamin C are shown in Table 1. The two groups were statistically compared by means of a student's *t*-test. The pre-dose levels were not significantly different for the two groups. Also, the maximum concentration of ascorbate were not significantly different (0.221 mg/dl for the synthetic group and 0.214 mg/dl for the natural group). The peak concentration was reached sooner for the synthetic group, approximately 1.5 hours after dosing as compared with 2 hours for the natural vitamin C. The natural vitamin C stayed in the plasma longer than the synthetic ascorbic acid as it took more than 4 hours for the natural group to return to pre-dose level and less than 3 hours for those receiving the synthetic material.

Table 1: Mean Plasma Concentrations of Ascorbate (mg/dl). After Oral Administration of Synthetic and Natural Vitamin C to guinea pigs.

Time (hours)	Plasma Ascorbate Concentration (± SD)	
	Synthetic Group (n = 5)	Natural Group (n = 5)
0	0.080 + 0.014	0.078 + 0.22
0.5	0.140 + 0.034	0.132 + 0.006
1.0	0.204 + 0.029	0.186 + 0.013*
1.5	0.221 + 0.013	0.199 + 0.047**
2.0	0.123 + 0.011	0.214 + 0.017**
3.0	0.076 + 0.018	0.140 + 0.013**
4.0	-----	0.092 + 0.016

* $p < 0.1$, Natural vs. Synthetic
** $p < 0.001$, Natural vs. Synthetic.

Apparent biological half-lives were calculated by a least squares regression analysis of the plot of $\log_e$ vs. time over the last three sampling times where the concentration of ascorbate was decreasing. The half-life of synthetic vitamin C was 1.0 hours and of the natural material was 1.6 hours. These half-lives are not elimination half-lives since they also reflect absorptive and distributive phases in ascorbate pharmacokinetics.

Bioavailability of Ascorbate: The relative bioavailability of the two forms was calculated by comparison of the area under the plasma concentration-time curve after administration of each formulation. The areas were determined by means of a planimeter and the results are shown in Table 2. The bioavailability of the natural vitamin C was 148% that of the synthetic ascorbic acid and the difference was highly significant ($p < 0.001$).

Table 2: Area (Arbitrary units) under the Plasma Ascorbate Concentration-time Curve after oral administration of Synthetic and Natural Vitamin C to Guinea Pigs.

Synthetic Group		Natural Group	
Subject Weight (g)	Plasma Area	Subject Weight (g)	Plasma Area
530	426	540	627
552	463	545	541
554	419	550	726
570	449	560	647
570	452	560	729
Mean ± S.D.			
555 ± 16	442 ± 18	551 ± 9	654 ±78**

** $p < 0.001$, Natural vs. Synthetic.

Discussion

Previous guinea pig studies have been long term feeding experiments which have compared the effects of synthetic ascorbic acid alone or mixed with bioflavonoids. Parrot [5] in 1948 found that catechin, a bioflavonoid, when given with ascorbic acid increased the ascorbate levels in the liver, spleen, kidney and adrenals of guinea pigs and also prevented scourbutic lesion which were present when ascorbic acid was given alone at a low dose. Blanc and von der Mühll [7] also found a synergistic action between ascorbic acid and bioflavonoids with respect to the concentration of ascorbate in the internal organs.

The best quantitative study was by Crampton and Lloyd [6] in 1950 who fed guinea pigs daily sub-optimal doses of vitamin C (0.5-2.0 mg) in the form of synthetic ascorbate or orange-grapefruit juice. This vitamin C was given alone or with 100 mg of rutin, a bioflavonoid. After 42 days, the biological potencies were determined by measuring the height of the odontoblast cells of the incisor teeth. There was no difference between the biopotency of natural or synthetic vitamin C. However, rutin increased the biopotency by an average of 56% and was most effective at low doses of Vitamin C. However, the large excess of rutin/ascorbic acid of 200/1 to 50/1 is not realistic for animal of human supplementation because isolated bioflavonoids are much more expensive than ascorbic acid.

A biological action of bioflavonoids in animals and man was first suggested in 1936 by Szent-Gyorgi [14] who reported that these compounds prevent capillary fragility and bleeding in scorbutic animals. A dietary role for bioflavonoids is suggested by evidence of a widespread low-level blood cell aggregation in apparently healthy human subjects which is inhibited *in vitro* by bioflavonoids [15].

Somogyi [16] first presented a mechanism for the effect of bioflavonoids on vitamin C in physiological fluids. He hypothesised that flavonoids act as sparing factors in slowing down the oxidation of Vitamin C. This antioxidant effect was shown by *in vitro* studies with oxidants such as ascorbic acid oxidase, copper and peroxidase.

In the present study, a natural vitamin C product containing bioflavonoids was found to be more readily absorbed by Guinea pigs than synthetic ascorbate. These results indicate that human supplementation with natural vitamin C might prove efficacious.

References

1. Alfano, M.C., Drummond, J.F. and Muller, S.A. Effect of Ascorbic acid on the Biosynthesis of Basement Membrane Collagen. *J. Dent. Res.*, **54**, L107, 1975.
2. Vallance, S. Relationship between Ascorbic Acid and Serum Proteins of the Immune system. *Brit. Med. J.*, **2**, 437, 1977.
3. Pauling, L. Vitamin C and the Common Cold. Freeman Publishing Company, San Francisco, 1970.
4. Cameron, E. and Pauling, L. Cancer and Vitamin C. Warner Books, Inc., New York, 1979.
5. Cotereau, E.W., Gabe, M., Gero, E. and Parrot, J-L. Influence of Vitamin C upon the amount of Ascorbic Acid in the organs of the Guinea Pig. *Nature*, **161**, 557, 1948.
6. Crampton, E.W. and Lloyd, L.E. A Quantitative Estimation of the effect of rutin on the biological potency of Vitamin. *J. Nutr.*, **41**, 487, 1950.
7. Blanc, B. and von der Mühll, M. Interaction of a Flavonoid and Vitamin C: Its influence on the weight of the guinea pig and the vitamin C content of its organs. *Int. Z. Vitaminforsch,* **37**, 156, 1967.
8. Elmby, A. and Warburg, E. The inadequacy of synthetic ascorbic acid as an antiscorbutic agent. *Lancet*, **11**, 1363, 1937.
9. Todhunter, E.N. and Fatzes, A.S. The comparison of the utilisation by college women of equivalent amounts of ascorbic acid in red raspberries and in crystalline form. *J. Nutr.*, **19**, 113, 1940.
10. Pelletier, O. and Keith, M. Bioavailability of synthetic and natural ascorbic acid. *J. Am. Dietet. Assoc.*, **64**, 271, 1974.
11. Nelson, E.W., Streiff, R.R. and Cerda, J.J. Comparative bioavailability of Folate and Vitamin C from a Synthetic and a Natural source. *Am. J. Clin. Nutr.*, **28**, 1014, 1975.
12. Stevenson, N. and Brush, M.K. The existence and characterisation of Na^+-Dependent active transport of Ascorbic Acid in the Guinea pig. *Am. J. Clin. Nutr.*, **22**, 318, 1969.
13. Hubman, B., Monnier, D. and Roth, M. Rapid, accurate determination of Ascorbic Acid in blood plasma. *Clin. Chim. Acta.*, **25**, 161, 1969.

14. Bentsath, A., Rusznyak, I. and Szent-Gyorgi, A. Vitamin P: Flavonoids as vitamins. *Nature*, **138**, 798, 1936.
15. Robbin, R.C. Effect of methoxylated flavones on Erythrocyte aggregation and sedimentation in blood of normal subjects: Evidence of a dietary role for flavonoids. *Int. J. Vit. Nutr. Res.*, **43**, 494, 1973.
16. Somogyi, J.C. An investigation of substances which inhibit vitamin C degradation. *Z. Vitaminforsch*, **16**, 134, 1945.