THE EFFECTS OF Na-ASCORBATE ON ASCORBATE CONCENTRATION IN THE PLASMA AND TISSUES

*Voja Pavlovic * and Zoran Pavlovic ***

Male guinea pigs of a Hartley-derived strein, a mean starting weight of 325 g + 70 g (s.d.), immunized with bovine serum albumine, were studied in animals maintained on various amount of ascorbic acid for 28 days. Animals were paid-fed on ascorbate-free diet (standard "dry" meal (Wagner Guinea Pig Diet). The animals were immunized with bovine serum albumine (BSA, Miles) 0.2 mg being administered in complete Freund's adjuvans on day 0 (0.025 mg/foot pad and 0.1 mg in the nuchal skin) and again on day 14 in 1% saline into the nuchal skin. The animals were separated into five categories of five animals each and put on a daily schedule of intraperitoneal injections of 0, 10,25, l00 i 250 mg Na-ascorbate (Bronson). Blood was taken by cardiac puncture from each animal on experimental days 0, 14 and 28. At the end of experiment all animals were anesthetized and tissue samples were taken for evaluation of vitamin C with Zannoni methods. The immunized guinea pigs receiving no supplementation showed a reduction in ascorbic acid concentration of 59% at 14 days to 67% at 28 days. In the meantime, no difference between the 10 and 25 mg doses in relative change. Guinea pigs receiving the mega doses (100 mg and 250 mg/day) exhibited increased plasma levels, the former showing a greater increase than latter. At the end of the experiment the gain in the 100 mg group had dropped to 12%, but in the 250 mg group it continued to raise.

Of greater significance than the plasma levels are the ascorbate concentrations measured in various tissues of selected animals that had been maintained for 28 days on these ascorbate regimens. Intraperitoneal administration of Na-ascorbate a rapid increase of ascorbic acid plasma concentration. The greatest the ascorbate concentrations was obtained in the case of the adrenal (95.3), pituitary (89.1) and spleen (29.7) per 100 g in the respective cases. Under the same circumstances the ascorbate concentrations in the brain and in the eye dropped only to 20% of the control value. *Acta Medica Medianae 2003; 42 (4):23-27.*

Key words: ascorbic acid, plasma, tissue

Institute of Physiology, Medical faculty, Nis*
Clinic of Hemalology, Medical center, Nis**

Corespoiulence to: Voja Pavlovic
Institute of Physiology, Medical faculty
Brace Taskovic 81
18000 Nis, Serbia and Montenegro
Tel.: 018/334 221, e-mail: vojapav@yahoo.com

Introduction

On the basis of in vitro examination of tissue homogenate extracts Burns is (1) concluded that, man, monkey and guinea pig are unable to convert L-gulonolactone to L-ascorbic acid, and that this is the "missing step" in the biosynthesis in the livers of these species which makes them dependent on exogenous ascorbic acid for their vitamin C requirements. Chatterjee et al. (2) concluded that there exists an additional "missing step", namely that performed in other species by the enzyme glucuronolactonase in converting D-clucuronolactone to L-gulonolactone. More specifically Burns (1), Stone (3) and Chatterjee et al. (2) considered that the " missing step" is due to gene deletion.

In his seminal paper "Evolution and the Need for Ascorbic Acid" Linus Pauling (4) concluded that the loss of the ability to synthesize ascorbic acid probably occurred in the common ancestor of the primates. A rought estimate of the time at which this mutational change occurred is twenty-five million years ago (5).

Weighty experimental and theoretical considerations will be advanced in favour of the thesis that vitamin C deficiency in a number of species including humans is not due to rotal inability to biosynthesis ascorbate, but rather to a very limited biosynthetic ability which normally cannot be stopped up to meet the minimum metabolic/physiological requirements (6, 7, 8, 9).

In the present paper we report the effects of supplemental doses of Na-ascorbate on blood and tissue ascorbic levels from immunized, pair-fed guinea pigs maintained on an ascorbate free diet but supplemented with 0, 10, 25, 100 or 250 mg vitamin C, given in daily intraperitoneal doses.

Materials and methods

Animals

Male guinea pigs of a Hartley-derived strain, having a mean starting weight of 325 g + 47 g (s.d.), were obtained from a commercial supplier. These animals were obtained as young adults (5 - 6 weeks), ear-tagged on receipt, and distributed into experimental groups by weight stratification to ensure comparable weights within each group. The animals were usually housed in small groups of 5 per cage. The animals were exposed to equal 12 hour periods of light and dark and pair-fed only a standard "dry" meal (Wayne® Guinea Pig Diet) with no supplement. An ample supply of fresh water was made available to all groups.

Materials

All inorganic chemicals used in this study were of analytical grade. The following specialized organic reagents were used: sodium ascorbate (USP, Bronson), ascorbic acid (Mitheson, Coleman and Bell), bovine serum albumin (Miles Laboratories), alfa'alfa'dipyridyl (Aldrich Chemical Co.), heparin (Invenex). Freund's adjuvans for immunization was obtained from Sigma.

Immunization

All animals were kept on an ascorbate-free diet for 28 days. The animals were immunized with bovine serum albumin (BSA, Miles) 0.2 mg being administered in complete Freund's adjuvant on day O (0.025 mg/foot pad and 0.1 mg in the nuchal skin) and again on day 14 (in 1% saline) intracutaneoulsy and subcutaneously into the nuchal skin. Two studies were made, each involving 15 animals. Group A received 0, 10 or 100 mg/day ascorbate while group B received 0, 25 or 250 mg/day ascorbate.

Within each group, animals were separated into three categories of five animals each and put on a daily schedule of intraperitoneal injections of 0, 10, 25, 100, or 250 mg Na-ascorbate (Bronson). Taking into account that the sodium content of crystalline Na-ascorbate is 11,8% these were prepared in 2 ml aliquots of varying NaCl solution such that the daily sodium load was 29.5 mg/animal.

Plasma and tissue analysis

Blood was taken by cardiac puncture from each animal on experimental days 0, 14, and 28, into a syringe containing 200 units of Na-heparin (Invenex) in 0.2 ml. At the end of the experiment all animals were anesthetized with sodium pentobarbital, and tissue samples were taken for evaluation of vitamin C. In all cases blood sample (day 14 and 28) and tissue samples (day 28) were removed from animals 20 hr after the ascorbate injection made on the previous day.

L-ascorbic acid in the plasma and tissues of experimental animals was estimated by a micromethod described by Zannoni et al. (10). Blood was taken by cardiac puncture from each animal on experimental days 0, 14 and 28, into a syringe containing 200 units of Na-heparin (Ivenex) in 0.2 ml. Proteins are precipitated by adding 0.24 ml of 40% trichloracetatic acid to 2.0 ml of plasma on ice. The samples are centrifuged at 10.000 x g for 15 min. at 4"C and the following reagents then added to 1.0 ml of supernatant: 0.05 ml of 85% orthophosphoric acid, 0.05 ml of 8% alfa'alfa'dipyridyl in 95% ethanol, and 0.05 ml of 3% aqueous ferric chloride. The ferrous-dipyridyl chromophore is allowed to develop for 20 minutes at room temperature and then read to 525 nm. Standards for each set of experimental samples are determined in duplicate.

At the end of the experiment all animals were anesthetized with sodium pentobarbital, and tissue samples were taken for evaluation of vitamin C. In all cases blood samples (day 0, 14 and 28) and tissue samples (28 day) were removed from animals 20 hr after the ascorbate injection made on the previous day.

Results

In this experiment we present the results of two studies made 4 months aparat of different groups of animals receiving the equivalent of 0, 10, 25, 100, or 250 mg ascorbic acid per day. In bouth experiments, immunized animals receiving no supplementation showed a reduction in ascorbic acid concentration of 55 to 60% at 14 days and 67% to 74% at 28 days. The decreases in the maintenance level were 25% and 39% for the two periods, there being no difference between the 10% and 25 mg doses in relative change (table 1).

Table 1. Effect of Na-ascorbate on plasma ascorbate levels in immunized male guinea pigs maintained on an ascorbate free diet

Group	Na-ascorbate (mg)	Plasma ascorbate (means µg/ml±SD)				
		Day 0	Day 14	dl%	Day 28	d2%
A	0	3.28±0.41	1.49±0.27	-55	0.85±0.35	-74
	10	2.76±0.24	2.14±0.36	-22	1.66±0.56	-39
	100	3.40±0.17	5.87±1.95	+73	3.81±0.66	+12
B	0	4.94±0.46	2.03±0.45	-59	4.65±0.23	-67
	25	3.57±0.68	2.61±0.81	-27	2.19±0.25	-39
	250	3.51±4.97	4.97±0.94	+42	5.61±0.99	+60

Guinea pigs receiving the megadoses, 100 mg and 250 mg/day, exhibited increased plasma levels, the former showing a greater increase than the latter (73% occuring at 14 days). At the end of experiment the gain in the 100 mg group had dropped to 12%, but in the 250 mg group it continued to rise, reaching a concentration of 5.6 micro g/ul, or an increase of 60% above control. The eventual accommodation of the plasma level to high doses of ascorbate is confirmed by the results of the terminal plasma levels obtained on a separate group of animals (table 2). The values for the maintenance and megadose categories are identical.

Of greater significance than the plasma levels are the ascorbate concentration measured in various tissues of selected animals than had been maintained for 28 days on these ascorbate regimens. The greatest absolute and relative reductions in tissue ascorbate in animals completely deprived of vitamin C, occurred in the case of the pituitary, adrenal and spleen: 74 to 3.5; 68 to 1.0; and 32 to 0.3 mg ascorbate per 100 g in the respective cases. Under the same circumstances the ascorbate concentrations in the brain (cerebrum or hypothalamus) and in the eye dropped only to 20% of the control value. At a 10-fold increase of the maintenance dose, to the 250 mg, there was no further increment in the tissue ascorbate levels except in the case of the pituitary and adrenal, but these were below the acceptable level of statistical significance.

Discussion

Hartley strain male guinea pigs with a mean weight of 325 g maintained ad libitum on a complete Reid-Briggs diet without vitamin C, but supplemented intraperitoneally with 10,25, 100 and 250 mg ascorbate per day, for a period of 28 days.

It is known that the concentration of ascorbate in leucocytes is one of the highest known and is considered to be second only to that in the adrenals. The leucocytes are actively engaged in transporting large quantities of ascorbate to demaged tissues to an extern greater than that possible using blood or lymph in the absence of leucocytes because of the threshold limit of c. 1.4 to 2.4 mg ascorbate per 100 ml plasma enforced by the kidney (6).

Polymorphonucler leucocytes and mast cells (11) of various mammalian species contain high concentrations of ascorbate. During the course of viral infections there is a rapid depletion of cellular ascorbate (4), the level returning on normal after recovery. The active accumulation of ascorbate by leucocytes increases in phase with chemotaxis of polymorphonucler leucocytes to infected areas (11) and reduced in response to external agents such as steroids (12) which, according to various authors (13), act to suppress the hexose monophosphate shunt, eventually, depleting the polymorphonucler leucocytes of ascorbate.

Several body tissues contain higher concentrations of ascorbic acid than plasma. Such a situation must involve active transport. The experimentally determined values for a given tissue can vary significantly with the individual's age, with different individuals of the same age and with investigation - since strict comparisons of individuals are not possible in post-mortems with different time intervals between death and the post-mortem - and with different procedures adopted by different investigators (14, 15, 16, 17).

In our experiment, imunized guinea pigs receiving no supplementation showed a reduction in ascorbic acid concentration of 59% at 14 days to 67% at 28 days. In the meantime, no difference between the 10 and 25 mg doses in relative change. However, guinea pigs receiving the megadoses (100 mg and 250 mg/day) exhibited increased plasma levels, the former showing a greater increase than latter. At the end of the experiment the gain in the 100 mg group had dropped to 12%, but in the 250 mg group it continued to rise.

Of greater significance than the plasma levels are the ascorbate concentrations measured in varios tissues of selected animals that had been maintained for 28 days on these ascorbate regimens. Intraperitoneal administration of Na-ascorbate a rapid increase of ascorbic acid plasma concentration. The greatest the ascorbate concentrations was obtained in the case of the adrenal (95,3), pituitary (89,1) and spleen (29,7) per 100 g in the respective cases. Under the same circumstances the ascorbate concentrations in the brain and in the eye dropped only to 20% of the control value (18,19,20).

Table 2. Effects of ascorbate supplementation on tissue ascorbate levels in immunized male guinea pigs

Daily ascorbate supplementation	0 mg	25 mg	250 mg
	Tissue ascorbate (mg/100 g)		
Cerebral hemisphere	3.7±0.1	18.0±1.9	19.9±1.1
Hypothalamus	3.1±0.1	13.6±0.8	14.4±0.8
Pituitary	3.5±1.2	73.6±13.3	89.1±9.8
Adrenal	1.0±0.5	68.0±10.4	95.3±14.1
Eye	2.1±1.3	10.2±1.6	12.6±1.3
Spleen	0.3±0.0	32.2±9.6	29.7±5.8
Plasma	0.1±0.0	0.3±0.1	0.3±0.1

There appears to be some indication that the plasma concentrations can be permanently elevated by "excessive" amounts of vitamin C (21, 22, 23). However, if there exist secondary reservoirs, such as the tissue receptors,the plasma concentration is not so significant a parametar as is tissue storage (21, 14). In this connection we see that the ascorbate levels in et last two key metabolic tissues, the adrenal and pituitary, can be greatly increased by excessive amounts of vitamin C.

Conclussion

Peritoneal aplication of diferent dosages of ascorbic acid increases the level of ascorbic acid in the plasma as well as in the tissues. The largest quantity of ascorbic acid is concentrated in adrenal, pituitary and spleen and less in the cerebral hemisphere, hypothalamus and eye lence.

Ascorbic acid is concentrated in the tissues wich have greater metabolic processes.

References

1. Burns JJ, Evans C. The synthesis of L-ascorbic acid in the rats from D-glucuronolactone and L-gulonolactone. J Biol Chem 1956;223:905-11.
2. Chatterjec IB, Majumder AK, Nandi BK, Subramanian N. Synthesis and Some Mayor Functions of Vitamin C in Animals. In Kingand Burns, eds., Second Conference on Vitamin C. New York Academy of Sciences: New York; 1975.
3. Stone I. The Genetic Disease Hypoascorbemia. Acta Geneticae Medicae et Genellologiae 1967; 16: 52-8.
4. Pauling L. Evolution and the need for ascorbic acid. Proc Nat Acad Sci 1970; 67: 1643-50.
5. Zuckerkandi E, Pauling L. Molecular Disease, Evolution, and Genie Heterogeneity, in Horizons in Biochemistry. Academic Press: New York 1962; 189: 225-32.
6. Cameron E, Pauling L. The Orthomolecular Treatment of Cancer. I. The Role Of Ascorbic Acid in Host Resistence. Chem Biol Inter 1974; 9: 273-83.
7. Kalden JR, Guthy EA. Prolonged skin allograft survival in vitamin C-deficient guinea pigs. European Surg Res 1972; 4: 114-9.
8. Yonemoto RH, Chretien PB, Fehniger TF. Enhanced lymphocyte blastogenezis by oral ascorbic acid. Proc Am Assoc Caner Res 1976; 17: 288-93.
9. Yonemoto RH. Vitamin C and immunological response in normal controls and in cancer patients. Medico Dialogo 1979; 2: 23-30.
10. Zannoni V, Lynch M, Gokdstein S, Sato P. A rapid micromethod for the determination of ascorbic acid in plasma and tissues. Biochem Med 1974; 11: 41-7.
11. De Chatelet LR, McCall CE, Cooper MR, Shirley PS. Ascorbic acid levels in phagocytic cells. Proc Soc Exptl Biol Med 1974; 145: 1170 -79.
12. Goetzi EJ, Wasserman SI, Gigli I, Austen KF. Enhancement of randon migration and chemotactic response of human leukocytes by ascorbic acid. J Clin Invest 1974; 54: 823-7.
13. Kumar M, Axerod AE. Circulating antibody function in scorbutic guinea pigs. J Nutr 1969; 98: 43-8.
14. Martin A, Prior R, Shuckitt-Hale B, Cao G, Josef JA. Effect of fruits, vegetables, or vitamin E and C distribution in peripheral and brin tissues: implications for brain function. J Gerintol Biol Sci Med 2000; 55(3): 1144-9.
15. De la Fonte M, Ferrandez MD, Burgos MS, Soler A, Prieto A, Miquel J. Immune function in aged women is improved by ingestion of vitamin C and E. Can J Physiol Pharmacol 1998; 76(4): 373-8.
16. Sauberlich HE. Pharmacology of vitamin C. Annu Rev Nutr 1994; 14:71-91.
17. Van de Pols JC. A possible role for vitamin C in age-related cataract. Proc Nutr Soc 1999; 58(2): 295-301.
18. Berger J, Shepard D, Morrow F, Taylor A. Relationship between dietary intake and tissue levels of reduced and total vitamin C in non scorbutic pig. J Nutr 1989; 119(5): 734-40.
19. Grunewald RA. Ascorbic acid in the brain. Brain Res Rev 1993; 18(1): 123-33.
20. Racek J, Holecek V, Trefil L. Antioxidative properties of ascorbic acid. Cas Lek Cesk 2000; 139 (19): 583-7.
21. Jacques PF. The potential preventive effects of vitamine C for cataract and age-related macular degeneration. Int Vitam Nutr Res 1999; 69(3): 198-205.
22. Jacques PF, Taylor A, Hankinson SE, Willett WC, Mahuken B, Lee Y. et al. Long-term vitamin C supplement use and prevalence of early age-related lens opacities. Am J Clin Nutr 1997; 66(4): 911-6.
23. Simon JA, Hudes ES. Serum ascorbic acid and other correlates of self-reported cataract older Americans. J Clin Epidemiol 1999; 52(12): 1207-11.

UTICAJ VITAMINA C NA KONCENTRACIJU ASKORBATA U PLAZMI I TKIVIMA

Voja Pavlović i Zoran Pavlović

Kod mladih, muških Hartley zamoraca, telesne težine 300-400 g, koji su bili na skorbutičnoj dijeti (standard "dry" meal Wagne'' Guinea pig diet), proučavanje uticaj razlicitih doza askorbinske kiseline na nivo vitamina C u plazmi i pojedinim tkivima. Životinje su bile imunizovane sa bovin serum albuminom (BSA Miles), 0,2 mg sa kompletnim adjuvansom dato je na početku (0,025 mg/stopalo i 0,1 mg u predeo ušne školjke) eksperimenta i ista doza data je ponovo 14. dana (u 1% NaCl) ogleda u predeo ušne školjke. Životinje su bile podeljene u 5 grupa, od po 5 životinja u svakoj grupi, i primile su, intraperitonealnom inokulacijom, po 0, 1, 25, 100 i 250 mg Na-askorbata (Bronson). Krv je uzimana kardijalnom punkcijom od svake životinje na početku, 14. i 28. dana eksperimenta. Na kraju eksperimenta sve zivotinje su bile anestezirane, a uzorci tkiva su uzimani neposredno posle žrtvovanja i odredivan je nivo vitamina C po metodi Zannoni i sar. Imunizovane životinje koje nisu dodatno dobijale vitamin C pokazuju redukciju koncentracije askorbata od 59% 14. dana i 67% 28. dana. Međutim, nema razlike u primeni doza od 10 i 25 mg. Zamorci koji su primili mega doze (100 i 250mg/nadan) pokazuju porast nivoa askorbata u plazmi. Na kraju eksperimenta, grupa koja je primila 100 mg imalaje pad od 12%, a u grupi koja je primala 250 mg vitamina C je kontinuirano rastao.

Intraperitonealna aplikacija Na-askorbata, rapidno povećava koncentraciju askorbinske kiseline u plazmi i u tkivima. Najveća koncentracija askorbata zabelezena je u tkivu nadbubrega (95,3), hipofize (89,1) i u slezine (29,7), na lOOg ispitivanoga tkiva. Podistim uslovima koncentracija askorbata u mozgu i u očnom sočivu je pala do 20% odkontrolnih vrednosti. *Acta Medica Medianae 2003; 42(4):23-27.*

Ključne reči: vitamin C, plazma, tkiva