Effect of mega doses of vitamin C on bactericidal ativity of leukocytes[1]

Prakash G. Shilotri,[2] Ph.D. and K. Seetharam Bhat,[3] Ph.D.

ABSTRACT Effect of ingesting mega doses of ascorbic acid was studied on the leukocyte function in five normal human subjects. During the first 15 days the subjects received daily supplements of 200 mg of ascorbic acid, and during the next 2 weeks they were given 2 g of vitamin C per day. Supplementation of 200 mg as well as 2 g of ascorbic acid stimulated hexose monophosphate shunt activity of resting leukocytes indicating an increase in resting metabolism. Intakes of 200 mg of ascorbic acid per day did not affect bacterial killing by leukocytes. On the other hand, daily intakes of 2 g of ascorbic acid for 2 weeks significantly impaired bactericidal activity. Four weeks after withdrawal of the viatmin supplementation, bactericidal activity returned to normal. *Am. J. Clin. Nutr.* 30: 1077–1081, 1977.

Numerous clinical reports claim that large doses of ascorbic acid supplements are beneficial in a variety of stress conditions such as burns, injuries, surgical operations, and infections (1, 2). On the basis of results of clinical trials and the nontoxic nature of ascorbic acid, Pauling (3) has strongly recommended a daily intake of 2 g or more of vitamin C for an adult with an energy requirement of 2500 Kcal/day. However, the beneficial and prophylactic effects of mega doses of ascorbic acid supplements have been questioned by other workers (4–6). The use of massive doses of ascorbic acid is thus a controversial issue. There is, however, a lack of definite information on the effects of large doses of ascorbic acid on the functional parameters of biological systems under normal conditions.

Immune mechanism is one of the functions believed to be affected by ascorbic acid. Phagocytosis and microbicidal activity of leukocytes are among the major defence mechanisms of the host against infection. The process of phagocytosis by human polymorphonuclear leukocytes (PMN) is accompanied by a major change in oxidative metabolism that includes an increase in hexose monophosphate shunt (HMS) activity (7). It has been shown that HMS activity of leukocytes plays an important role in the bactericidal activity of PMN (8). We report here, the effects of mega doses of ascorbic acid on the bactericidal and HMS activities of the leukocytes obtained from normal adult volunteers.

Materials and methods

Five apparently healthy male volunteers, ages between 23 and 28 years were investigated. A sample of venous blood was obtained at the beginning of the study. To each of them, an oral supplement of 200 mg of ascorbic acid was given daily in two divided doses for a period of 15 days to ensure that they were saturated with respect to the vitamin as indicated by their leukocyte ascorbic acid levels. Samples of blood were again obtained for biochemical investigations. All the subjects were then supplemented daily with 2 g of ascorbic acid in four equally divided doses for a further period of 15 days. Twelve hours following the last dose of the vitamin supplement, a sample of blood was obtained. The treatment was withdrawn thereafter and a repeat blood sample was obtained 4 weeks after the withdrawal of the vitamin treatment. Blood was collected under heparin and PMN were isolated by a modified dextran flotation technique as described earlier (9) and suspended in Krebs Ringer phosphate buffer, pH 7.4. Phagocytic and bactericidal activities were determined in the leukocytes of all samples of blood.

Bactericidal activity of leukocytes was measured using *Escherichia coli* as the test organism, and HMS activity of resting and phagocytizing PMN was assayed by the conversion of glucose-1-^{14}C to $^{14}CO_2$ as described by Selvaraj and Bhat (9). Total ascorbic acid in leukocytes was estimated by the method of Denson and Bowers (10) and plasma cortisol by the competitive protein binding technique (11). In parallel experiments, the effect of in vitro additions of ascorbic acid

[1]From the National Institute of Nutrition, Indian Council of Medical Research, Hyderabad-500007, India.

[2] Assistant Research Officer. [3] Research Officer.

and dehyroascorbic acid (DHA) in final concentrations of 1 mM on the bactericidal system of leukocytes was investigated. Similarly the effect of preincubation of cells with DHA on the bactericidal activity was also studied.

Glucose-1-^{14}C (specific activity 50 mCi/mmole) was obtained from BARC, Bombay, India. [$^1/_3$, $^2/_3$ (n)-^{3}H] Corticosterone (specific activity 34 Ci/mmole) was purchased from the Radio Chemical Centre, Amersham. Hydrocortisone was obtained from Sigma, U.S.A. DHA was prepared freshly whenever needed, by adding bromine to a solution of ascorbic acid and removing excess bromine under nitrogen. All other chemicals were of analytical grade, and used without further purification.

Results

The mean ascorbic acid concentration in leukocytes at the beginning of the study was 9.3 ± 0.61 μg/10^8 cells. At the end of 15 days supplementation with 200 mg of the vitamin per day and 2 g of ascorbic acid per day the levels increased significantly ($P < 0.005$) to 14.1 ± 0.39 μg/10^8 cells and 16.1 ± 1.02 μg/10^8 cells, respectively. Administration of large doses of ascorbic acid was not associated with alterations in circulating levels of cortisol (12.4 ± 1.59 μg/dl and 11.1 ± 0.29 μg/dl before and after dosing).

Data on bactericidal activity are presented in Table 1. Initially, the mean number of viable bacteria was 0.28 ± 0.081 × 10^5 at the end of 30 min incubation period. Supplementation of 200 mg of ascorbic acid/day did not bring about any significant changes in this number (2.17 ± 1.28 × 10^5). However, the number of viable *E. coli* markedly increased to 28 ± 9.2 × 10^5 ($P < 0.01$) when the dose was raised to 2 g/day, indicating reduced bactericidal capacity of the leukocytes. The impaired bactericidal activity was restored to initial levels after withdrawal of ascorbic acid (Fig. 1).

In Figure 2A are shown the effects of ascorbic acid and DHA, on the bactericidal activity of intact leukocytes, when added to the medium at 1 mM concentration. Neither ascorbic acid nor DHA had any effect on microbicidal activity under the conditions used. Similarly no effect was seen when the cells were pre-incubated with DHA (Fig. 2B).

Results of oxidation of glucose-1-^{14}C to $^{14}CO_2$ by leukocytes during resting and phagocytizing conditions are presented in Table 2. Although there was an increased production of $^{14}CO_2$ during phagocytosis following the intake of 200 mg as well as 2

TABLE 1
Effect of mega doses of vitamin C administration on bactericidal activity of human PMN

Subjects	Number of viable bacteria × 10^5			
	Plasma control	Initial	200 mg vitamin C[a]	2 g vitamin C[a]
			30 min	
N	100	0.37	0.11	56.0
P	100	0.07	6.50	43.0
R	100	0.50	3.70	15.0
S	100	0.32	0.20	19.0
SP	100	0.12	0.32	7.0
Mean ± SE	100	0.28 ± 0.081	2.17 ± 1.28[b]	28 ± 9.2[c]

[a] Every subject received 200 mg of vitamin C per day for 15 days followed by 2 g of vitamin C per day for 15 days. [b] Not significant. [c] $P < 0.01$ using paired t test.

TABLE 2
Effect of mega doses of ascorbic acid on HMS activity of PMN in five human subjects

Description	Glucose-1-^{14}C-$^{14}CO_2$ (mμmoles)/10^6 cells		
	Resting	Phagocytizing	P.E.[a]
Initial	1.77 ± 0.56	2.85 ± 0.44	261 (115–595)[c]
Vitamin C (200 mg)[b]	2.77 ± 0.33	4.00 ± 0.33	149 (113–184)[c]
Vitamin C (2 g)[b]	2.99 ± 0.81	5.76 ± 1.33	208 (130–296)[c]

[a] P.E. = phagocytic effect in % (100%−no effect). [b] Each subject received 200 mg per day for 15 days followed by 2 g of vitamin C/day for 15 days. [c] Range.

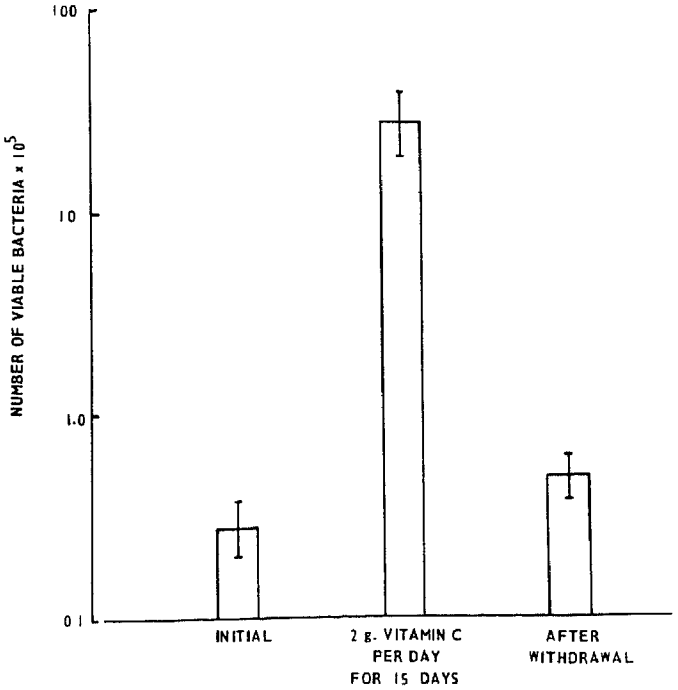

FIG. 1. A profile of bactericidal activity of leukocytes after withdrawal of supplementation with mega doses of ascorbic acid. The data are mean ± SEM for the five human subjects.

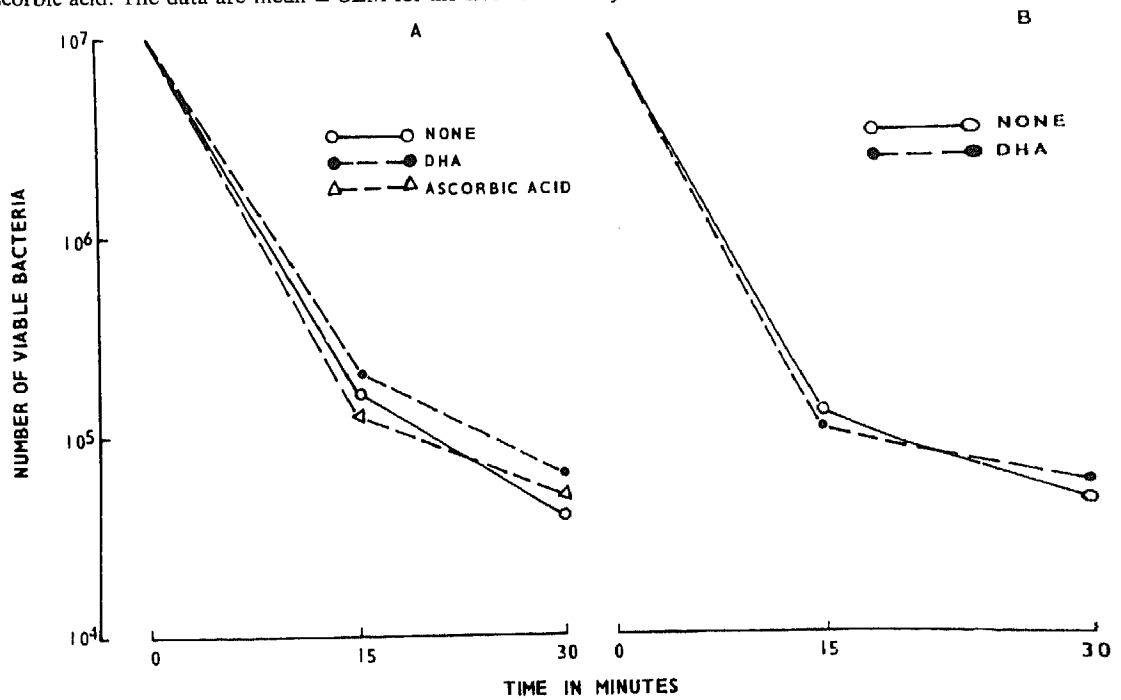

FIG. 2. A, the effect of ascorbic acid and DHA on the bactericidal activity of human leukocytes. The assay system consisted of leukocytes and bacteria (*E. coli*) in the ratio 1:2, 1 μmole of ascorbic acid or DHA and 20% heat inactivated (56°C, 30 min.) autologous plasma in the final volume of 1 ml Krebs Ringer phosphate medium, pH 7.4. B, the effect of preincubation of leukocytes with DHA on the bactericidal activity. PMN were incubated with DHA 1 μ mole in 1 ml Krebs Ringer phosphate medium, pH 7.4 for 30 min at 37 C. The cells were washed and their bactericidal activity was determined.

g/day of ascorbic acid, the phagocytic effect expressed as percentage of resting activity was not different from that of the initial because of increased CO_2 production in the resting cells.

Discussion

Data presented here indicated that daily supplements of 200 mg of ascorbic acid/day did not affect bactericidal activity of leukocytes significantly. On the other hand, daily intakes of 2 g of ascorbic acid for 15 days, significantly impaired bacterial killing by leukocytes. This effect was reversible as evidenced by the return to normal activity following the withdrawal of the vitamin supplement. The mechanism by which large doses of ascorbic acid administration brought about an inhibition in bactericidal activity of leukocytes is not clear. It has recently been shown that elevated levels of plasma cortisol in response to ACTH administration, inhibit bacterial killing by human PMN (12). Encarnacion et al. (13) have reported that animals fed massive amounts of ascorbic acid exhibited high levels of plasma cortisol. However, in the present study such an increase in plasma cortisol was not observed and the decrease in bactericidal activity of leukocytes following the intake of large doses of ascorbic acid cannot therefore be attributed to elevated plasma cortisol levels.

Chatterjee et al. (14) demonstrated that large doses of ascorbic acid supplements in man lead to high levels of DHA in blood, which can be one of the reasons for altered bactericidal activity, since DHA is known to inhibit the NADPH-oxidase activity of PMN, in vitro (P. G. Shilotri, unpublished observation). Leukocyte NADPH-oxidase is considered to be a primary oxidase for the production of hydrogen peroxide (H_2O_2) which is a bactericidal agent (15). McCall et al. (16) have reported the inhibitory action of ascorbic acid on the H_2O_2-myeloperoxidase-halide reactions that have been implicated in the bactericidal activity of neutrophils. The results of the present investigation however, show that exposure of leukocytes to high levels of either ascorbic acid or DHA did not result in any inhibition of bactericidal activity of leukocytes (Fig. 2A and B). Klebanoff and Hamon (17) have also observed similar effects. However, the possibility of inhibitory action of DHA on bactericidal activity of leukocytes cannot be ruled out in a situation wherein cells are exposed to high levels of DHA for long durations.

Daily intakes of 2 g of ascorbic acid have been found to result in increased concentrations of cyclic adenosine monophosphate in blood (18). Bourne et al. (19) have shown that cyclic adenosine monophosphate inhibits the killing of ingested bacteria. Circulating levels of cyclic nucleotides were not measured in the subjects investigated here and it is not possible to comment on this possibility.

In vitro additions of vitamin C have been shown to stimulate HMS activity of resting human PMN (20). In the present investigation too, increased HMS activity of leukocytes was observed following ingestion of large doses of the vitamin. Hanck (21) also reported that activity of transketolase, a key enzyme of the shunt pathway, of blood cells increased in normal humans receiving mega doses of ascorbic acid. Although HMS activity was higher in resting cells, the phagocytic effect remained unchanged after vitamin C supplementation.

Data presented here indicate that not only is there no beneficial effect of mega doses of ascorbic acid supplements on bactericidal activity of leukocytes, but there is a distinct decrease.

Several undesirable side effects attributable to massive doses of ascorbic acid have been reported. These include oxalate stone formation (22), loss of nutritionally required cations, lowering of blood sugar (18), increased mortality of animals fed cereal based diets (23), and abortion in guinea pigs and rats (24). However, the physiological significance of the findings reported here is not clear. The general state of health of the subjects studied in the present investigation was not altered. It is therefore proposed to undertake studies in animals to determine the functional implications, if any, of this altered bactericidal capacity by imposing infection on the animals fed mega doses of ascorbic acid and studying their physiological response.

The authors are grateful to Dr. S. G. Srikantia, Director and Dr. Kamala S. Jaya Rao, Chief, Endocrinology Unit for their keen interest and helpful discussions.

References

1. GOLDSMITH, G. A. Human requirements for vitamin C and its use in clinical medicine, Ann. N. Y. Acad. Sci. 92: 230, 1961.
2. CHATTERJEE, G. C. Ascorbic acid requirements of animals. In: The vitamins, (2nd ed.) edited by W. H. Sebrell, Jr. and R. S. Harris. New York: Academic Press, 1967, vol. 1, p. 495.
3. PAULING, L. Evolution and the need for ascorbic acid. Proc. Natl. Acad. Sci. 67: 1643, 1970.
4. DIEHL, H. S. Vitamin C for colds. Am. J. Pub. Health 61: 649, 1971.
5. HERRELL, W. E. Had your vitamin C to-day? Clin. Med. 78: 13, 1971.
6. BRIGGS, M. H. Letter: side effects of vitamin C. Lancet 2: 1439, 1973.
7. KARNOVSKY, M. L. Metabolic basis of phagocytic activity. Physiol. Rev. 42: 143, 1962.
8. SELVARAJ, R. J., AND A. J. SBARRA. Relationship of glycolytic and oxidative metabolism to particle entry and destruction in phagocytosing cells. Nature (Lond) 211: 1272, 1966.
9. SELVARAJ, R. J., AND K. S. BHAT. Metabolic and bactericidal activities of leukocytes in protein-calorie malnutrition. Am. J. Clin. Nutr. 25: 166, 1974.
10. DENSON, K. W., AND E. F. BOWERS. The determination of ascorbic acid in white blood cells. A comparison of W.B.C. ascorbic acid and phenolic acid excretion in elderly patients. Clin. Sci. 21: 157, 1961.
11. MURPHY, B. E. P. Some studies of the protein-binding of steroids and their application to the routine micro and ultramicro measurement of various steroids in body fluids by competitive protein-binding radio assay. J. Clin. Endocrinol. Metabol. 27: 973, 1967.
12. SHILOTRI, P. G., AND J. SIVAPRASAD. The effect of ACTH administration on the phagocytic and bactericidal activities of human polymorphonuclear leukocytes. Horm. Metabol. Res. 8: 67, 1976.
13. ENCARNACION, D., M. M. DEVINE AND J. M. RIVERS. Influence of vitamin C nutriture and inanition on ACTH stimulated release of adrenal corticosteroids in guinea pigs. Internatl. J. Vit. Nutr. Res. 44: 309, 1974.
14. CHATTERJEE, I. B., A. K. MAJUMDER, B. K. NANDI AND N. SUBRAMANIAN. Synthesis and some major function of vitamin C in animals. Ann. N. Y. Acad. Sci. 258: 25, 1975.
15. ROSSI, F., D. ROMEO AND P. PATRIARCA. Mechanism of phagocytosis associated oxidative metabolism in polymorphonuclear leukocytes and macrophages. Res. J. Reticuloendo. Soc. 12: 127, 1972.
16. MCCALL, C. E., L. R. DECHATELET, M. R. COOPER AND P. ASHBURN. The effects of ascorbic acid on bactericidal mechanisms of neutrophils. J. Infect. Disease 124: 194, 1971.
17. KLEBANOFF, S. J., AND C. B. HAMON. Role of myeloperoxidase–mediated antimicrobial systems in intact leukocytes. Res. J. Reticuloendo. Soc. 12: 170, 1972.
18. LEWIN, S. Recent advances in the molecular biology of vitamin C. In: Vitamin C. Represent Aspects of Its Physiological and Technological Importance, edited by G. G. Birch and K. J. Parker. London: Applied Science Publishers Ltd., 1974, p. 221.
19. BOURNE, H. R., R. I. LEHRER, K. L. MELMON AND M. J. CLINE. Cyclic adenosine-3′, 5′ monophosphate and regulation of human granulocyte function. J. Clin. Invest. 49: 11a, 1970.
20. DECHATELET, L. R., M. R. COOPER AND C. E. MCCALL. Stimulation of the hexose monophosphate shunt in human neutrophils by ascorbic acid: mechanism of action. Antimicrob. Agents Chemother. 1: 12, 1972.
21. HANCK, A. B. Der vitaminbedarf und seine beeinflussing durch 4 g L (+) – ascorbinsaure pro tag beim gesunden menschen. Internatl. J. Vit. Nutr. Res. 44: 107, 1974.
22. BRIGGS, M. H., P. GARCIA-WEBB AND P. DAVIES. Urinary oxalate and vitamin C supplements. Lancet 2: 201, 1973.
23. NANDI, B. K., A. K. MAJUMDER, N. SUBRAMANIAN AND I. B. CHATTERJEE. Effects of large doses of vitamin C in guinea pigs and rats. J. Nutr. 103: 1688, 1973.
24. HAYES, K. C., AND D. M. HEGSTED. In Toxicants Occurring Naturally in Foods, (2nd ed.) Washington, D.C.: National Academy of Sciences. 1973, p. 239.