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IN VIVO EFFECT OF ASCORBIC ACID ON ENHANCEMENT OF HUMAN NATURAL KILLER CELL ACTIVITY

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ABSTRACT

The in vivo effect of ascorbic acid on human natural killer (NK) cell activity was determined. Twenty control healthy subjects were given ascorbic acid at a concentration of 60 mg/kg, and blood was drawn at 0,1,2,4,8, 24 and 48 hours after treatment with ascorbic acid. Peripheral Blood Lymphocyte-NK activity was measured by a 4-hr. -^{51}Cr-release assay using K562 tumor cells as targets. Treatment with ascorbic acid was shown to have a biphasic effect on NK activity: a transient slight suppression between 1 to 2 hrs. (20% of control) was followed by a significant enhancement (an over-shoot) at 8 hrs. that was further increased at 24 hrs., then the activity returned to the normal level by 48 hrs. Changes in the activity of ascorbate treated NK cells were inversely related to the E:T ratio; namely 231%, 189%, 141% and 127% at 6:1, 12:1, 25:1 and 50:1 E:T ratio respectively. Flow cytometry analysis indicated no quantitative changes in the NK cell sub-populations post treatment with ascorbic acid in the experimental subjects as compared with control untreated subjects. Simultaneous to measurement of NK count and activity, ascorbic acid and its uptake by PBL was measured in the plasma. The uptake of the vitamin was maximized at 2-4 hours and maintained at a high level up to 24 hours. We conclude that ascorbic acid is a potent immunomodulator and its effect in enhancement of NK cytotoxicity may explain one mechanism by which ascorbic acid exerts its probable anti cancer activity.

KEY WORDS: Ascorbic Acid, Natural Killer Cell, Lymphocyte, Activity

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INTRODUCTION

Several studies suggest anti-cancer activity for ascorbic acid (1-3). A recent symposium sponsored by the National Institute of Health in September 1990 extensively evaluated ascorbic acid's role in cancer prevention and possibly therapy. The overall conclusion was that consistent epidemiologic evidence support ascorbic acid's having a protective effect on a variety of human tumors. A summary of this symposium goes into great detail on the many different mechanisms of how ascorbic acid may have its protective and beneficial effect (3). In studies that may have therapeutic implications, observations on hypovitaminosis C in patients treated with interleukin-2 (IL-2) and lymphokine activated killer (LAK) cells were reported (3). During the first phase of treatment with IL-2 alone, plasma levels of ascorbic acid dropped by more than 80%. These levels of ascorbic acid were lower than the first phase in the majority of patients after the third treatment phase with IL-2 plus LAK cells. The finding that in some patients the cytotoxic activity of lymphocytes cultured in the presence of IL-2 was stimulated by ascorbic acid may indicate that IL-2 placed an increased demand on ascorbate requirements (3).

An increasing body of evidence implicates the Natural Killer phenomenon as a means of host defense against the growth and dissemination of tumor cells (4-6). NK cells have the ability to mediate natural resistance against tumors, and may play an important role in immune surveillance (7,8). Therefore, factors enhancing or influencing NK activity could be relevant for resistance to malignant disease. Based on this rationale, we examined the effect of ascorbic acid on human NK function in vivo. The results show that ascorbic acid augments NK activity as early as 8 hrs. after oral administration and that this effect maximized at 24 hrs., returning to normal 48 hrs. after treatment.

METHODS

Human Subjects:
Twenty healthy control subjects (5 females and 15 males) who were members of a college swimming team in Southern California were selected for participation in this study. The subjects ranged in age from 20 to 24 years, with a mean of 22 years. They had not ingested any medications or vitamins for at least 2 weeks before participation in this study. In addition, the subjects were not having a history of chronic diseases and they were in regular training of 2-4 hours daily. Informed consents were obtained from the participants and the study was approved by our institutional review board.

Treatment with Ascorbic Acid:
All the subjects were given ascorbic acid orally in the form of powder provided by Metagenics, Inc. San Clemente, CA. The vitamin was given at a concentration of 60 mg/kg body wt. by disolving between 3.5 - 5.6 gm of ascorbic acid in water, and 10-15 ml blood were drawn from each individual at different intervals; 0,1,2,4,8 hrs. and 1, and 2 days post treatment.

Preparation of Mononuclear Cells:
Lymphocytes were prepared from fresh heparinized peripheral venous blood by Ficoll-Hypaque density gradient centrifugation. Cells were washed three times with Hanks' balanced salt solution (HBSS) and re-suspended to a concentration of 10 X 10^6 cells/ml in a complete medium (CM), that consisted of RPMI-1640 supplemented with 10% fetal calf serum and 1% antibiotics (100 U penicillin and 100 ug/ml streptomycin) within an hour of isolation cells used for different assays.

^{51}Cr-release Assay for Measuring NK Activity:
A standard 4-hour ^{51}Cr-release assay was employed. Briefly, 1 X 10^4 ^{51}Cr-labelled K562 tumor target cells in 0.1 ml CM were added to different wells of microtiter plate. Effector cells were then pipetted into quadruplicate wells to give effector:target (E:T) ratios of 6:1, 12:1, 25:1 and 50:1. After a 4-hour incubation at 37°C, the plates were centrifuged at 1400 rpm for 5 minutes and 0.1 ml of supernatant from each well was collected and counted in a gamma counter. The percentages of isotope released were calculated by the following formula:

$$\% \text{ Lysis} = \frac{\text{Exp. Rel - Sp. Rel}}{\text{Total Rel - Sp. Rel}} \times 100$$

Spontaneous release (SP) from the target cells was always no more than 8-10% of total release. Total release was measured my adding 0.1 ml Trinton X-100 to designated wells. Lytic units (LU) were calculated from effector titration curves. One LU was defined as the number of effector cells required to achieve 20% lysis. This serial performance of NK cytotoxic assay was based on criteria for a reproducible NK assay and intended to help minimize errors associated with NK cytotoxicity assays (9).

Measurement of Ascorbic Acid in Plasma and its Uptake by PBL:
Ascorbic acid in plasma or its uptake by peripheral blood lymphocytes was determined according to the spectrophotometric method previously described (10). In brief, 5 X 10^6 cells were suspended in 0.2 ml of PBS, sonicated for 30 sec., and added to 1 ml of 5% trichloroacetic (TCA). The mixture was centrifuged to remove the precipitates and the supernatant was used to measure the level of ascorbic acid. 1.2 ml of this supernatant or 0.5 ml plasma was mixed with 0.4 ml of dinitrophenyl-hydrazine-thiourea-copper (DTC) reagent, incubated at 37°C for 3 hr., then transferred to an ice-water bath for 10 min. Then 2 ml of cold 12M H2S04 was added and the mixture was kept at room temperature for 20 min. Ascorbic acid was measured in controls (both serum and PBL from the individuals prior to ascorbic acid usage), and ascorbic acid-treated specimens at 520 nm and results were calculated from a calibration graph.

NK Sub-populations:
NK cell subset enumeration was carried out in ascorbate-treated and non-treated cell populations. A single laser flow cytometer (Epics Profile: Coulter Epics, Inc., Hialeah, FL), which discriminates forward and right-angle light scatter, as well as two colors, was used with a software package (Quad Stat: Coulter). Mononuclear cell populations were determined by two-color direct immunofluorescence, by using a whole-blood staining technique with the appropriate monoclonal antibody and flow cytometry (11). The fluorescein isothiocyanate (FITC), or phycoerythrin (PE)-conjugated monoclonal antibodies (Coulter Immunology) CD56-PE and CD3-FITC were selected for determination of total NK cells and its

subpopulations ($NKHT3^+$/$NKHT3^-$cells). To monitor lymphocyte markers, bit maps were set on the lymphocyte population of the forward-angle light scatter versus a 90° light scatter histogram. The percentage of positively stained cells for each marker pair, as well as the percentage of doubly stained cells, was determined. Estimates of the absolute numbers of lymphocyte positive for the respective surface markers were determined by multiplying peripheral lymphocyte cell count by the percentage of cells positive for each surface marker.

Statistical Analysis:
The SAS procedure of analysis of variance (ANOVA) was used to examine: 1) The effects before and after treatment with ascorbic acid in two different determinations, 2) the effect of changing the ratios between E:T cells, and 3) the interaction of the two effects.

RESULTS

Effects of Ascorbic Acid on NK Activity:
The time-course of the changes in NK activity in the healthy subjects treated with ascorbic acid is shown in Fig. 1. Because of the complexity of the study and the number of intervals, only five individual subjects were tested for NK activity before (o time) and after (1,2,4,8,24,48 hours) ascorbic acid usage. The additional fifteen subjects were tested before and 24 hours after treatment. A slight decrease in NK activity took place at 1-2 hrs post treatment in all subjects, which was followed by a sharp increase (an over-shoot) in its value at 8 hrs. The activity was further increased at 24 hr, then returned to an almost normal level at 48 hrs. This increase in NK activity at 8 and 24 hours post ascorbic acid statistically was highly significant $P<0.001$. In order to test the reproducability of the results, the present study was repeated with the same individuals after a two week interval, and by assaying NK function at different E:T ratios (6,12,25 and 50:1). The results of both experiments were similar regarding the enhancement of NK function by ascorbic acid. Therefore, the second set of data is not shown.

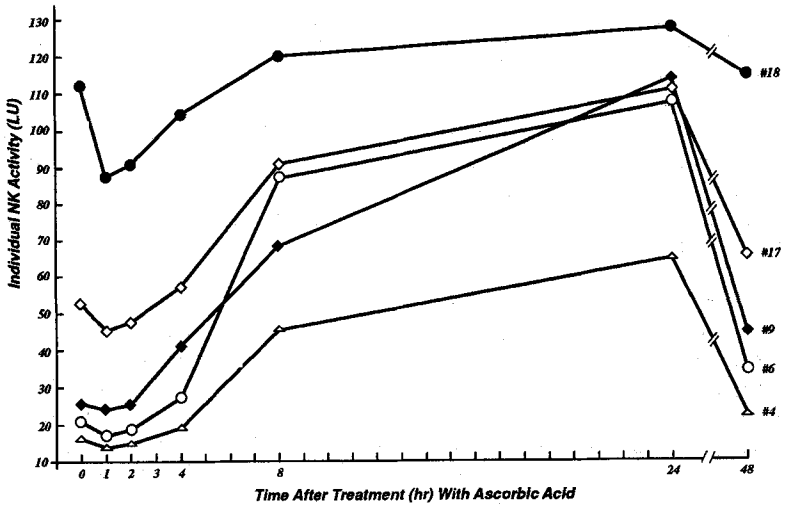

Fig. 1 NK activity of five different individuals expressed as number of lytic units at 0,1,2,4,8,24 and 48 hr post treatment with ascorbic acid. Activity in NK cells was measured by 4-hr ^{51}Cr-release assay.

Activity at Different E:T Ratios:

The relationship between NK activity and the ratios of effector:target (E:T) cells was examined at 24 hr. post-treatment with ascorbic acid (Table 1). Treatment with ascorbic acid resulted in a significant induction of NK activity at 24 hrs. $P<0.001$. The change in enhancement of activity was found to be inversely related to the E:T ratio, i.e. higher enhancement of ascorbic-treated NK activity was achieved at lower ratio. A maximum induction of NK activity (231%) was observed at the E:T ratio 6:1, and decreased with increasing E:T ratio; namely 189% at E:T 12:1, 141% at E:T 25:1, and 127% at E:T 50:1.

TABLE 1
INDIVIDUAL NK CELL ACTIVITY BEFORE (B) AND AFTER (A) TREATMENT WITH ASCORBIC ACID AT DIFFERENT E:T RATIOS

			LYMPHOCYTE TO TARGET CELL RATIO							
		BODY WEIGHT	50:1		25:1		12:1		6:1	
SUBJECT	SEX	KG	B	A	B	A	B	A	B	A
1	M	88.6	13.3	55.5	15.4	36.0	8.3	21.5	6.2	18.2
2	M	88.5	14.2	20.0	11.9	11.8	10.5	11.2	3.0	7.0
3	F	73.6	18.8	71.4	18.1	31.8	11.3	25.0	8.6	21.4
4	M	93.2	19.2	55.5	19.4	41.6	12.0	31.3	5.2	14.0
5	M	77.3	21.0	20.4	20.0	19.4	15.6	10.4	9.4	5.2
6	M	77.5	21.7	>100	20.5	41.0	11.2	39.0	5.6	27.0
7	M	79.5	22.7	57.1	20.1	47.8	14.5	24.3	10.5	16.8
8	F	75.0	24.2	66.6	23.9	43.7	15.4	30.9	6.4	20.7
9	M	77.3	27.7	>100	24.9	68.2	13.9	48.3	7.3	39.1
10	F	72.7	31.0	27.7	26.2	19.2	18.1	13.0	6.9	7.0
11	M	88.6	33.3	52.6	31.7	35.6	19.8	29.3	6.6	12.2
12	M	79.5	38.4	>100	30.6	51.4	21.7	42.2	13.6	32.9
13	M	67.3	41.6	52.6	31.8	24.6	25.5	21.4	10.6	20.0
14	M	72.7	47.6	>100	42.4	47.2	22.6	51.0	11.9	45.0
15	M	81.8	50.0	66.5	43.8	54.6	23.9	35.2	15.3	14.3
16	M	90.9	55.5	83.3	38.3	32	29.8	24.0	14.7	23.6
17	M	69.5	55.5	>100	37.5	43.3	29.1	41.0	18.1	36.8
18	M	73.6	>100	>100	49.2	52.0	37.4	47.0	27.5	29.0
19	F	59.1	>100	>100	50.0	41.1	46.8	37.3	34.6	33.6
20	F	59.3	>100	>100	64.0	56.3	55.9	46.8	36.1	41.3

Differential Subject Responses to Ascorbic Acid:
Among the subjects studied, basal NK cytotoxicity expressed as number of LU, could be divided into three categories; low (10-20 LU) 4 out of 20, medium (21-60 LU) 13 out of 20 and high (>60 LU) 3 out of 20 (Fig. 2). A majority of the subjects (18/20) responded to ascorbic acid with increased NK activity, but there was a differential response toward the immune augmentory effect of the vitamin. In comparison to low and high basal NK cytotoxicity, the group with medium NK activity (except for one subject) demonstrated a higher response to ascorbic acid. (Fig. 2). This enhancement of NK activity by Ascorbic Acid was not different in male or female subjects (Table 1).

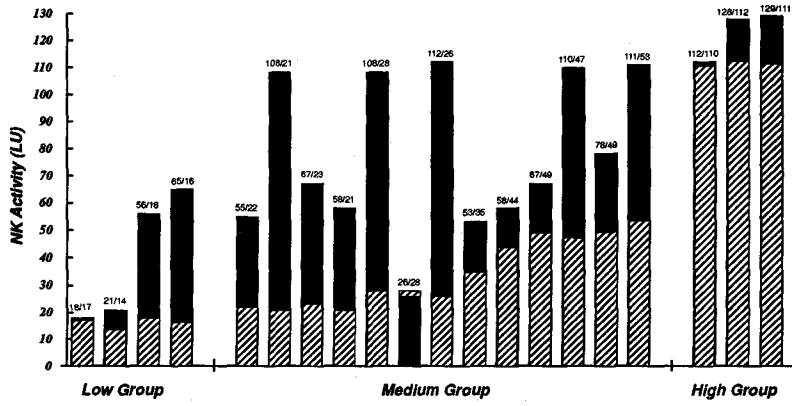

Fig. 2 NK cell activity expressed as number of LU for each individual examined separately at 24 hr. NK activity before ▨, and after ■ treatment with ascorbic acid.

Ascorbic Acid in the plasma and its uptake by PBL:
Data on ascorbic acid in the plasma is shown in Fig. 3. The mean level of the vitamin before treatment was 1.5 mg/dl, which was markedly increased (4.2mg/dl) at 1 hr, and maximized (6.5 mg/dl) at 2-4 hr. The level then declined but was still high (3.8 mg/dl) up to 24 hr, as compared with control untreated subjects. Uptake of the vitamin by PBL showed a similar pattern of increase, up to 24 hr. post-treatment (Fig. 4).

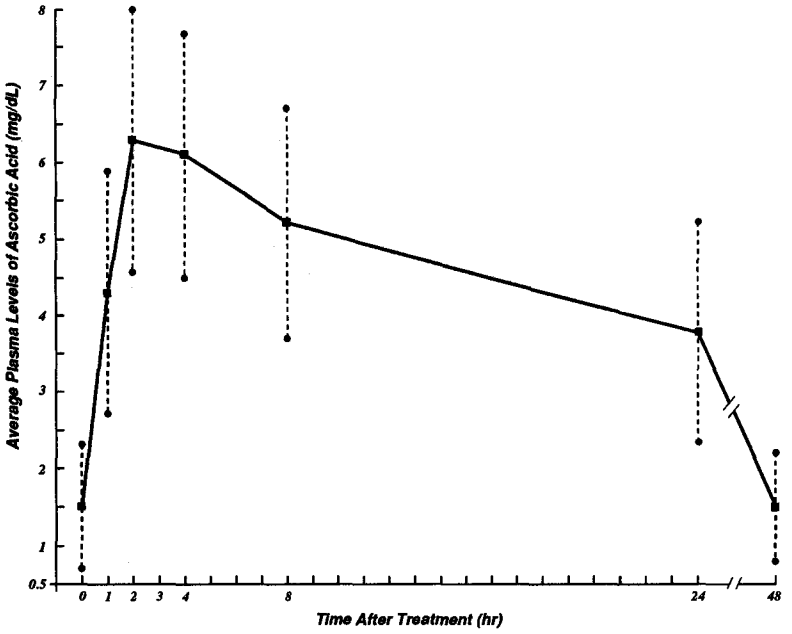

Fig. 3 Ascorbic acid level in the plasma. Level of the vitamin was determined spectrophotometrically at different intervals; 0-48 hr. and the average of twenty individuals with standard deviation is presented.

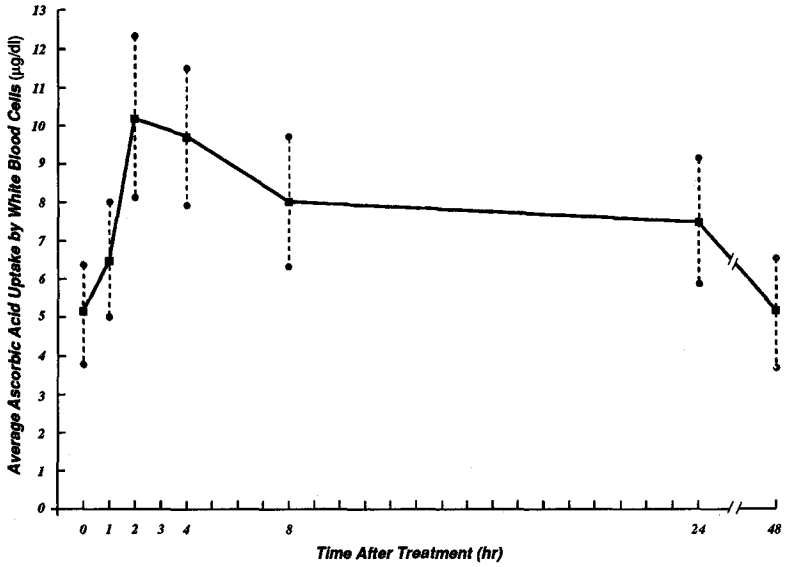

Fig. 4 Uptake of ascorbic acid by PBL. The uptake of ascorbic acid was determined at different intervals up to 48 hr. post treatment and the average of twenty individuals with standard deviation is presented.

Quantities of NK Sub-populations:
Total NK cell population and its sub-populations post-treatment with ascorbic acid were determined by Flow Cytometry, using Anti-CD56 and Anti-CD3 monoclonal antibodies respectively. Results in Table 2 demonstrated that treatment with the vitamin had no significant effect on the percentages of total NK cell population, or its sub-populations, examined at 0 and 24 hours.

TABLE 2

CHANGES IN NK CELL POPULATIONS BEFORE AND AFTER TREATMENT WITH ASCORBIC ACID

	% TOTAL		% NKH T3+		% NKH T3-	
SUBJECT	Before	After	Before	After	Before	After
1	14.6	13.5	3.8	4.2	11.1	9.8
2	18.2	17.6	1.9	2.1	16.1	15.8
3	8.5	8.6	3.6	3.4	4.8	5.2
4	10.8	11.2	3.5	3.4	2.2	2.6
5	6.3	6.1	2.3	2.1	4.1	4.4
6	16.9	17.1	5.8	6.3	10.7	11.2
7	8.2	7.4	2.1	2.1	6.1	5.3
8	6.3	5.4	1.9	1.6	4.4	3.8
9	12.7	13.2	3.4	3.5	9.3	7.7
10	8.6	7.9	2.5	2.3	6.1	5.6
11	13.4	12.3	4.9	5.1	8.5	7.2
12	12.6	12.1	3.8	4.0	8.8	8.1
13	12.3	12.9	2.7	2.5	9.6	10.0
14	11.4	10.3	4.2	3.9	7.2	6.4
15	8.8	9.7	1.7	2.1	7.1	7.6
16	21.8	19.5	8.3	7.4	13.5	12.1
17	10.3	10.9	2.6	2.8	7.7	8.1
18	11.7	10.6	3.1	3.5	8.6	7.1
19	9.8	10.4	1.7	2.1	8.1	8.3
20	13.2	14.5	4.1	4.6	9.1	9.9

Differences in results from before and after treatment with Ascorbic Acid are not statistically significant.

DISCUSSION:

During the last decades, only a few studies have been done on ascorbic acid effects on NK cells (3,12,1,2,8). All these studies so far have been done using an animal model or human PBL in vitro. It has been postulated that the NK cell may function as immunosurveillance mechanisms against tumor cells in the early stage of tumor development (6). In this connection, it has been further noted that different lymphokines, such as interleukin-2 and interferon, increases the natural cytotoxicity of lymphocytes as target tumor cells (2,13).

The present study developed, for the first time, an overall picture of the in vivo changes in human NK activity post-treatment with ascorbic acid, correlated with the level of the vitamin in plasma and its uptake by PBL. The results showed a clear biphasic pattern of NK function: an early, slight transient inhibition in NK activity that lasts for about 2 hours, followed by a significant enhancement of activity at between 8-24 hours after treatment (Fig. 1).

The mechanism of the apparent early transient inhibition of NK activity is not yet understood, but could be attributed to increased acidity of the blood by ascorbic acid, since the level of ascorbic acid in the plasma and PBL was maximized at 1-2 hours. However, this speculation was not checked by measuring the plasma Ph. The rise in NK activity between 8-24 hours was correlated with a decline in the level of ascorbic acid in the plasma and PBL, suggesting that metabolites of ascorbic acid could contribute to the observed enhancement of NK cytotoxicity. An alternative explanation could be that ascorbic acid enhances NK function only indirectly through the production of lymphokines such as interferon (14), which is known to activate NK cytotoxic activity (15). As shown in Fig. 2, based on their baseline NK cytotoxicity the individuals we studied could be classified into low, medium and high basal NK cell activity. In fact, there were some subjects who had high NK activity that exceeded above the level of calculation and their LU were expressed as >100. This distribution of NK cytotoxicity in our subjects was in agreement with a previous report (13), who demonstrated that 90% of their control donors had medium or high NK activity and 10% with low NK activity. A majority of our subjects responded significantly to the augmentary effect of ascorbic acid; however, we noticed that three individuals - one from the group of medium basal NK activity and two from the group of low basal NK activity, did not react to the ascorbic acid (Fig. 2). The reason for this is not yet understood, but could be attributed to the genetic variation of the subjects or to the loss of granzymes which are necessary for cell-mediated cytotoxicity. (Fig. 3).

Our study suggests that the increase in NK activity from ingestion of ascorbic acid was not due to an increase in the actual quantity of effector cells, but rather to an induction in the activity per cell. This was confirmed through two observations. First, the analysis of percentages of NK cells showed no significant changes in NK sub-populations before and after treatment with the ascorbic acid. Second, a maximal induction in NK activity was achieved at lower E:T ratios (6:1 and 12:1), as compared with 25:1 and 50:1 ratios.

Results are controversial concerning the action of ascorbic acid and NK function. Earlier studies reported an inhibition of human NK activity by ascorbic acid in vitro (12), but in vivo studies using an animal model were mixed - they showed either no change in activity (2), or inhibition (16). The inhibition observed in animal studies is probably due to differences in the dose of ascorbic acid, the route of administration, or the time of treatment. Siegel and Morton (2) placed mice on an ascorbic acid regimen of 250% mg in the drinking water, and tested NK activity 4-5 weeks later. The controversy in human NK studies between our data and that of earlier report (12) could be attributed to differences in the experimental approach, in vivo vs in vitro. The estimated ascorbate requirements for humans vary widely from one authority to another. Irwin and Hutchins (16) reported that the ascorbic acid intake necessary to prevent scurvy is very small. The WHO/FAO commission and a whole series of national commissions recommend a daily intake of 30mg of ascorbic acid per subject, whereas the recommended dietary allowance in USA is 45mg/day. On the other hand, a high "mega-dose" (10-30gm/subject) has been recommended by other investigators (17-19,20). In the present study, we chose an intermediate dose (60mg/kg/body weight), which resulted in a significant enhancement of NK activity. However, whether or not this represents and optimal dose, and whether similar immune augmentation will be achieved in patients with immune disorders such as cancer and AIDS, needs to be further investigated.

Regarding the beneficial effect of ascorbic acid in cancer prevention available information is also contradictory. One in vitro study suggested the vitamin could kill malignant human T-cell line (PFI-285) within hours (21), while in vivo studies suggested that skin tumors (22) and oestrogen-induced renal tumors (3) were reduced in ascorbate-treated animals. Further studies have demonstrated the anti-cancer activity of ascorbic acid against breast and gastric cancers (23,24). Howe et al (23) showed that ascorbic acid had the most consistent, statistically-significant inverse association with breast cancer in 12 case-controlled studies of diet and breast cancer, while others (24) reported that the risk of gastric cancer among 1016 patients and 1159 controls decreased in proportion to the intake of ascorbic acid, vitamin B and vegetables. These apparent immunomodulatory effects of ascorbic acid on NK function and T-cell mediated immunity may be one mechanism for anti tumor activity of ascorbic acid. Additional studies with cancer patients and patients with immune disorders are needed in order to ascertain the beneficial effect of ascorbic acid on NK cytotoxic activity.

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REFERENCES:

1. Pauling, L. Vitamin C therapy of advanced cancer. New Engl. J. Med, 1980 302:694-698.

2. Siegel BV, Morton J.I.: Vitamin C and Immunity: Natural Killer (NK) cell factor. Int. J. Vitam Nutr. Res, 1983; 53:179-183.

3. Henson DE, Block G and Levine M. Ascorbic acid: Biologic functions and relation to cancer. J. National Cancer Institute, 1991; 83:547-550.

4. Ghoneum M, Gill G, Wojdani A, Payne C, Alfred LJ. Suppression of basal and corynebacterium parvum-augmented NK activity during chemically induced tumor development. International Journal Immunophar, 1987; 9:71-78.

5. Ghoneum M, Salem F, Gill G, Jackson K, Betru Y, Allen H. NK cell activity and tumor development of age-related variation to 3-methylcholanthrene. In: Ades EW, Lopes G, eds. natural Killer Cells and Host Defense. Basel: Karger 1989; PP, 278-84.

6. Herberman RB. Possible role of natural killer cells in host resistance against tumors and diseases. Clin. Immun Allergy, 1983; 3:479-485.

7. Jondal M.: The human NK cell-a short over-view and an hypothesis on NK recognition. Clin Exp Immunol, 1987; 70:255-262.

8. Roder J.C., Pross H.F.: The biology of human natural killer cell. J. Clin. Immun., 1983; 2:249-263.

9. Whiteside TL, Bryant J, Day R, and Herberman R.B.: Natural Killer cytotoxicity in the diagnosis of immune dysfunction: Criteria for a reproducible assay. J Clin Lab Analysis, 1990; 4:102-114.

10. Nino H.V. and Prasad. Vitamins and trace elements. In: Gradwohl's Clinical Laboratory Methods and Diagnosis (Sonnenwirth AC and Jarett L Eds) Volume One. The C.V. Mosby Comp. Toronto, London, 1980; 370-372.

11. Fletcher MA, Baron GC, Asman M, Fischi MA, Limas NG. Use of whole blood methods in assessment of immune paramenters in immunodeficiency states. Diagn. Clin. Immunol. 1987; 5, 69-81.

12. Huwyler T., Hirt A., Morell A.: Effect of ascorbic acid on human natural killer cells. Immunology Letters, 1985; 10:173-176.

13. Lotzova E, Savary CA, and Herberman RB. Induction of NK cells activity against fresh human leukemia in culture with interleukin 2. J. Immunol, 1987; 138:2718-2727.

14. Siegel BV. Enhancement of interferon production by poly (rI), poly (rC) in mouse cell cultures by ascorbic acid. Nature (Lond), 1975; 234:231-532.

15. Balkwill FR. Antitumor effects of interferons in animals. In: N.B. Finter and R.K. Oldham (eds.), Interferon, Vol. 4, In vivo and clinical studies, Elsevier, Amsterdam. 1985; 23-25.

16. Irwin, M.I. and Hutchins, B.K.: A conspectus of research on vitamin C requirements of man. J. Nutr. 1976; 106:821-879.

17. Cameron E. and Campbell A: The orthomolecular treatment of cancer. II. Clinical trial of high-dose ascorbic supplements in advanced human cancer. Chemical-biological Interactions, 1974; 9:285-315.

18. Cameron E. and Pauling L; Ascorbic acid and the glycosaminoglycans: An orthomolecular approach to cancer and other diseases. Oncology, 1973; 27:181-192.

19. Cameron E. and Pauling L; Supplemental ascorbate in the supportive treatment of cancer; prolongation of survival times in terminal human cancer. Proc. National Academy Sciences USA, 1976; 73:3685-3689.

20. Moertal CG, Fleming TR, Creagan ET, Rubin J, O'Connell MJ and Ames MM: High-dose vitamin C versus placebo in the treatment of patients with advanced cancer who had no prior chemotherapy. New England Journal of Medicine, 1985; 312:137-141.

21. Helgestad J, Pettersen R, Strom-Mathisen I, Schejerven L, et al. Characterization of anew malignant human T-cell line (PFI-285) sensitive to ascorbic acid. Eur J. Haematol, 1990; 44:9-17.

22. Slaga TJ, Bracken WM. The effects of antioxidants on skin tumor initiation and aryl hydrocarbon hydroxylase. Cancer Res, 1977; 37:1631-1635.

23. Howe, J. GR, Hirohata T, Hislop TG, Iscovich JM, Yuan JM,Katsouyanni K, Lubin F, Marubin E, Modan B, Roham T et al. "Dietary factors and the risk of breast cancer: combined analysis of 12 case-controlled studies", JNCI 1990; 82:561-569.

24. Buiatti E. Palli D, Decarli A, Amadori D, Avellini C, Bianchi S. Bonaguri C, cipraini F, Cocco P, Giacosa A et al. "A Case-controlled study of gastric cancer and diet in Italy: II Association with nutrients:, Int. J. Cancer, 1990; 45:896-901.

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