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THE EFFECT OF ASCORBIC ACID ON BLOOD HISTAMINE LEVEL AND DELAYED-TYPE HYPER-SENSITIVITY IN GUINEA PIGS

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ABSTRACT

The effect of ascorbic acid on blood histamine level and delayed-type hypersensitivity was studied in thirty male guinea pigs. Animals were sensitized to B.C.G., trichophytin and mallein during the first four weeks. The diet was *ad libitum* during this period. After this "sensitization period" animals were tested intradermally with tuberculin, trichophytin and mallein. The number and diameter of positive delayed type hypersensitivity responses (antigenic and cumulative scores) were evaluated as an effector state of cell mediated immunity. Animals were fed low, adequate or high ascorbate diets (0.5, 2.0 or 50 mg ascorbate×100g body $wt^{-1}\times d^{-1}$) for the next four weeks. Antigenic and cumulative scores were evaluated after this period once again.

Mean liver ascorbate paralleled dietary intake and the values obtained were significantly different in the three groups. Blood histamine was significantly depressed in the high ascorbate group compared to the adequate and low ascorbate groups, and liver ascorbate was inversely correlated to blood histamine levels ($r= -0.97$, $p<0.001$). Although mean antigenic and cumulative scores in the low ascorbate group were lower than those of adequate and high ascorbate groups, the differences were not significant.

It was concluded that ascorbate may enhance immune function through detoxifying histamine, an immunodepressor compound. However, the immunostimulatory effect of ascorbate on cell mediated immunity was not confirmed in this study. In chronic ascorbate deficiency, there may be a predisposition to infectious diseases due to depressed immune function, at least because of disturbances in metabolism of other nutrients influencing immune responsiveness, especially iron and folate.

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INTRODUCTION

Histamine (Hm), which is formed during decarboxylation of histidine by the pyridoxal phosphate dependent enzyme L-histidine decarboxylase,[1] has immunosuppressive effects on various aspects of the immune system.[2-4] This immunosuppression may involve the activation of suppressor T-cells that have Hm type 2 receptors.[2] Although some experimental and clinical studies have demonstrated the Hm lowering effect of ascorbate,[5-9] there are some controversies about its immunostimulatory effect. Vitamin C supplementation gave rise to increasing T-cell/B- cell ratios in guinea pigs.[10] This was not, however, confirmed in man.[11] Although the immunostimulatory effect of ascorbate on humoral immunity has not been demonstrated,[12] evidence indicates that delayed-type hypersensitivity to tuberculin[11] and leukocyte chemotaxis[9] are enhanced in man by ascorbate.

This study was undertaken to examine the possibility that ascorbic acid lowers blood Hm level and promotes cell-mediated immunity (CMI) *in vivo*.

MATERIAL AND METHODS

Experimental design

Thirty male Dunkin Hartley guinea pigs (w~460g, Razi Institute, Hesarak, Karaj) were housed in wire-meshed cages (5 in each cage) in a temperature regulated (22-25°C), light-controlled room (lighted from 0700 to 1900 h). After the initial acclimatization period, all animals were sensitized to three antigens, B.C.G., trichophytin and mallein, according to Table I. After two weeks, a booster dose of 0.05 mL of trichophytin and mallein was injected subcutaneously to all animals. After another two weeks all animals were tested intradermally with tuberculin (P.P.D.), trichophytin and mallein. In this study an induration diameter > 3mm was considered positive, according to the antigen manufacturers recommendation. The number and diameter of positive responses, which were considered as antigenic and cumulative scores, respectively,[13] were evaluated after 48 hrs. Animals were then randomly assigned to one of the three vitamin dosage groups: low ascorbate (0.5 mg ascorbate/100g body wt/d), control (2.0 mg ascorbate /100 g body wt/d) and high ascorbate (50 mg ascorbate /100 g body wt/d). These dosage levels have been used successfully in previous studies on ascorbic acid and immune function.[7,8] All vitamin mixtures were prepared immediately prior to use in distilled water and administered orally through rubber tubing attached to a 1mL syringe. Throughout the study all animals were fed with a convenient scorbutogenic diet designed for rabbits and water *ad libitum*. After a four-week experimental period, all animals were tested intradermally with 0.1 mL of tuberculin, trichophytin and mallein. Antigenic and cumulative scores were evaluated after 48 hrs. All animals were then killed by heart puncture under diethyl ether anesthesia.

Liver ascorbate analysis

The liver was excised immediately and 1g of its right lobe was homogenized in 9 mL ice-cold 5% trichloroacetic acid. Following centrifugation (3500 rpm, 0°C, 5 min), the supernatant was stored (-20°C) less than 5 days and analyzed colorimetrically for ascorbate content using 2,4 dinitrophenyl hydrazine reagent.[7,8,14]

Blood histamine assay

All blood samples were tested in triplicate. One mL aliquot of freshly drawn blood anticoagulated with oxalate was mixed with 0.9 mL distilled water to lyse red cells and 0.1 mL 60% perchloric acid to precipitate all the proteins in the sample, then centrifuged at 3500 rpm for 10 min. The Hm from the supernatant was extracted by n-butanol and re-extracted into an aqueous phase prior to condensation with o-phthaldehyde. Fluorescence at 440 nm was measured after excitation at 380 nm (Hitachi 204 A fluorometer).[15]

Statistical analysis

Data are reported as means±SEM. One way analysis of variance was used to examine the differences between group means. The Pearson product-moment correlation test was used for correlation. All statistical analyses were done using SPSS-PC package.

RESULTS

Weight gains were similar for all guinea pigs during the experimental period and no significant difference was noted

Table I. Antigens, dosages and route of injection to sensitize the guinea pigs.

Antigen	Dosage (mL)	Incomplete Freund's Adjuvant (mL)	Route of Injection
B.C.G.	0.1	___	I.D.
Trichophytin	0.1	0.1	S.C.
Mallein	0.1	0.1	S.C.

I.D.: intradermal; S.C.: subcutaneous.

Table II. Effect of ascorbic acid nutriture on guinea pig body weight, liver ascorbate, blood histamine, antigenic and cumulative scores.[1]

Measure	Low Ascorbate[2]	Control[2]	High Ascorbate[2]
Initial body weight (g)	456±10.1[a]	471±12.6[a]	450±10.7[a]
Final body weight (g)	505±6.9[a]	511±12.9[a]	511±8.5[a]
Liver ascorbate (μmol/g)	0.28±0.003[a]	0.46±0.006[b]	0.92±0.02[c]
Blood histamine (ng/mL)	157±0.6[a]	135±2.2[b]	87±1.2[c]
Initial antigenic score[3]	1.5±0.28[a]	1.5±0.15[a]	1.4±0.15[a]
Final antigenic score[3]	1.2±0.28[a]	1.6±0.15[a]	1.5±0.15[a]
Initial cumulative score (mm)[3]	7.0±1.1[a]	7.0±0.6[a]	6.0±0.8[a]
Final cumulative score (mm)[3]	5.3±1.2[a]	8.0±0.9[a]	7.5±0.9[a]

1. Male guinea pigs (n= 10/group) were sensitized to three antigens (B.C.G., Trichophytin, and Mallein) during the first four weeks. Animals were then fed a non-purified, scorbutogenic diet supplemented with a single, daily dose of ascorbic acid for another 4 weeks. Values are mean±SEM. Means in a row not sharing a common superscript letter are significantly different ($p<0.001$).

2. Ascorbic acid dose was 0.5, 2.0, and 50 mg/100g body weight in the low, control and high ascorbate groups, respectively.

3. Antigenic score: the total number of positive DTH responses (induration diameter ⩾ 3mm); cumulative score: the total diameter of positive DTH responses.

in initial and final body weight between the three groups (Table II). Mean liver ascorbate paralleled dietary intake and differed significantly between the three groups (0.283+0.003, 0.462±0.006 and 0.919±0.02 μmol/g for the low, control and high ascorbate groups, respectively (Table II).

Mean antigenic and cumulative scores (the number and diameter of positive responses) were lower in the low ascorbate group than in control and high ascorbate groups. Also, in the low ascorbate group final antigenic and cumulative scores were lower than in the initial ones. However, none of these differences was significant (Table II).

DISCUSSION

Our findings demonstrate the antihistaminic effect of ascorbic acid and confirm the findings of other authors.[5-7,9,10] It was thought that the auto-oxidation of ascorbic acid to dehydroascorbic acid ruptures the imidazole ring of Hm causing Hm degradation products, e.g. hydantoin acetic acid, to be produced.[5] It was previously thought that ascorbic acid deficiency caused only one problem, scurvy, which can begin to develop when the plasma reduced ascorbic acid level falls below 0.2 mg/dL, but only becomes fully developed when all reduced ascorbic acid has disappeared from the blood. However, it is now evident that a metabolite, histamine, begins to accumulate in the blood long before the plasma reduced ascorbic acid levels below 0.7 mg/dL are associated with significantly elevated blood Hm levels.[5]

Histamine is an important neurotransmitter, which is in high concentrations in the lateral cerebral ventricles and hypothalamus and has an inhibitory effect on feeding behavior in rats.[16] However, similar weight gain was noted for all guinea pigs during the experimental period (Table II). Considering that loss of appetite is one of the symptoms of scurvy,[17] it is probable that Hm may play a role in inducing the anorexia seen in this disease. Also, it is likely that if the experimental period was longer, significant loss of weight would be observed in the low ascorbate group.

There is disagreement as to the effect of vitamin C on immune function and *in vivo* resistance to disease. This may be due to inadequate knowledge of what components of the immune system are affected by the vitamin and the dosages required to achieve stimulation.[12]

Although stimulation of H_2 receptors activates suppressor T-cells,[2-4,8] no significant difference in antigenic or cumulative scores was noted between the three groups. Although the immunostimulatory effect of ascorbate on CMI, as judged by DTH, was not confirmed in this study, it is probable that in ascorbate deficiency, there may be a predisposition to infectious diseases due to depressed immune function, at least because of disturbances in

metabolism of other nutrients influencing immune responsiveness, especially iron and folate.[19-22] On the other hand, some recent papers claim that ascorbate selectively influences the proliferation of B-cells and negatively acts on interleukin-2 production by T-cells when a threshold of saturation is exceeded[23] and that sustained levels of ascorbate may be toxic and immunosuppressive for human T-cells.[24] Considering these controversies, there still seems to be a long way to clarification of the exact mechanism(s) of ascorbate immune modulation.

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