Effect of Ascorbic Acid Nutriture on Blood Histamine and Neutrophil Chemotaxis in Guinea Pigs¹,²

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ABSTRACT Histamine suppresses certain immune responses, including neutrophil chemotaxis. The present study examined whether the histamine-lowering effect of ascorbate was accompanied by enhanced chemotaxis in guinea pigs. Animals were fed low ascorbate, adequate or high ascorbate diets (0.5, 2.0 or 50 mg ascorbate·100 g body wt⁻¹·d⁻¹) for 4 wk. Mean liver ascorbate paralleled dietary intake, and these values differed significantly. 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 = −0.64, P < 0.001). The random migration of neutrophils was not significantly affected by vitamin dosage. Leukocyte chemotaxis was significantly impaired in low ascorbate animals compared to that of animals with adequate ascorbate nutriture. Leukocyte chemotaxis in high ascorbate animals did not differ significantly from that in the adequate or low ascorbate groups. Furthermore, chemotaxis was significantly lower when cells extracted from animals with adequate ascorbate nutriture were incubated in low ascorbate or high ascorbate serum rather than in autologous serum. These data suggest that the histamine-lowering effect of supplemental ascorbate does not appear to enhance leukocyte chemotaxis and that serum from guinea pigs fed low or high levels of ascorbate appears to contain factors that depress chemotaxis. J. Nutr. 121:126-130, 1991.

INDEXING KEY WORDS:
- ascorbic acid
- chemotaxis
- histamine
- guinea pigs

The autooxidation of ascorbic acid to dehydroascorbic acid ruptures the imidazole ring of histamine, producing the products of histamine degradation (1, 2). Adding ascorbic acid to tissue cultures significantly reduces both endogenous histamine levels (3, 4) and histidine decarboxylase activity, a measure of the histamine-forming capacity of cells (4). In humans, plasma ascorbate levels > 45.4 μmol/L are associated with low blood histamine levels (5), and the therapeutic use of the vitamin protects against histamine-induced anaphylactic shock (6).

Oh and Nakano (4) recently demonstrated that a parameter of the immune response, lymphocyte blastogenesis, was enhanced by ascorbic acid administration because the vitamin lowered cell culture histamine levels that were immunosuppressive. The present study further examined the possibility that ascorbic acid promotes immune function by attenuating the effects of immunosuppressive histamine. Chemotaxis was the immune response chosen for investigation because it has been established that chemotaxis is enhanced by ascorbate (7, 8) and depressed by histamine (9).

Leukocyte chemotaxis in 50% serum was measured using cells and serum extracted from guinea pigs fed graded doses of ascorbic acid for 4 wk. Our objective was to determine whether the serum histamine-lowering effect of supplemental ascorbate was accompanied by enhanced chemotaxis.

METHODS

Experimental design. Thirty male Hartley guinea pigs (~150 g, Charles River Laboratories, Wilmington, MA) were housed individually in wire-meshed cages in a temperature-regulated [22°C], light-controlled (lighted from 0700 to 1900 h) room. Throughout the study, all animals were fed a convenient scorbuto-genic diet, a nonpurified diet designed for rabbits (Purina rabbit chow, Ralston Purina, St. Louis, MO), and water ad libitum.


²Supported by donations to the Arizona State University Foundation.
During the initial 2 wk acclimation period, all animals received a single, oral dose of L-ascorbic acid [2.0 mg/100 g body wt-d], a level adequate for both growth and incisor development (10). Animals were then randomly assigned to one of three vitamin dosage groups: low ascorbate, 0.5 mg ascorbate/100 g body wt-d; control, 2.0 mg ascorbate/100 g body wt-d; and high ascorbate, 50 mg/100 g body wt-d). These dosage levels have been used successfully in previous work examining ascorbic acid and immune function (11, 12). All vitamin mixtures were prepared immediately prior to use in distilled water and administered orally through rubber tubing (0.125-inch i.d.) attached to a 1-mL syringe. After a 4-wk experimental period, animals were killed by heart puncture under halothane anesthesia.

**Blood histamine and liver ascorbate analysis.** All tissue samples were tested in duplicate. A 5-mL aliquot of freshly drawn blood anticoagulated with oxalate (gray top Vacutainer, Becton Dickenson, Rutherford, NY) was mixed with 4.5 mL deionized water, to lyse red cells, and 0.5 mL concentrated perchloric acid, then centrifuged for 10 min at 500 × g (13). The histamine from the supernatant was extracted by n-butanol and reextracted into an aqueous phase prior to condensation with o-phthalaldehyde as originally described by Shore et al. (14). Fluorescence at 450 nm (filter no. 7-60) was measured after activation at 360 nm (filter no. 7-60).

The liver was excised immediately and homogenized in 9 mL ice-cold chloroform-acetic acid per gram of tissue. Following centrifugation [3500 × g, 0°C], the supernatant was stored [−20°C] less than 5 d and analyzed colorimetrically for vitamin content using the dinitrophenylhydrazine reagent (15).

**Leukocyte chemotaxis.** Red cells and mononuclear leukocytes from freshly heparinized blood (~8 mL, green top Vacutainer) were sedimented by the addition of 0.75 mL methyl cellulose (2% in normal saline) (16). Leukocytes from the neutrophil-rich plasma were counted in a hemocytometer and washed twice in Hanks’ balanced salt solution (HBSS), pH 7.2 (17). Cell suspensions [2.5 × 10⁷ cells/mL] in 50% autologous plasma, 50% heterologous plasma or HBSS were used for studies on agarose gel.

The agarose assay is a simple, rapid and inexpensive method for measuring leukocyte locomotion (18). Clean microscope slides were dipped in 0.5% gelatin, rinsed in deionized water and air-dried. A 2% agarose-0.5% gelatin mixture in deionized water was dissolved over boiling water, diluted with an equal volume of HBSS and carefully layered onto microscope slides (19, 20). After the gel hardened (4°C for 30 min, two sets of a series of three holes, 2.5 mm apart, were punched in the agarose of each slide using a metal template. Agarose plugs were removed with a hypodermic needle. A 10-μL aliquot of the cell suspension was placed in the center well. Ten microliters of a chemotactic solution (f-Met-Leu-Phe, 10⁻⁴ mol/L in dimethyl sulfoxide in the presence of 2 × 10⁻⁴ mol/L 2-mercaptoethanol, diluted to 10⁻⁵ mol/L prior to use) (18) was added to one of the outer wells, and 10 μL of HBSS with gelatin (0.5%) was placed in the remaining outer well. Slides were incubated 3 h at 37°C, fixed in absolute ethanol for 30 min and left overnight in fresh absolute ethanol (20). The agarose was gently removed after immersion (30 min) in water, and the slide was stained (Wright stain, Diagnostic Systems, Gibbstown, NJ) and air-dried. Random migration (mm) and the chemotactic index (movement toward chemotactic solution divided by movement toward buffer) were measured microscopically using an eyepiece micrometer disc and a 40× objective. Each cell suspension was tested in duplicate.

**Statistical analysis.** Data are reported as means ± SEM. One-way analysis of variance and least significant difference tests were used to examine the differences between group means (21). The Pearson product-moment correlation test was used for correlation analysis.
RESULTS

Similar weight gains were noted for all guinea pigs during the experimental period. The mean initial and final body weights for the three dietary groups did not differ significantly (Table 1). Mean liver ascorbate paralleled dietary intake and differed significantly among the three groups (0.70 ± 0.05, 1.12 ± 0.05 and 1.80 ± 0.05 μmol/g for the low ascorbate, control, and high ascorbate groups, respectively, Table 1). Blood histamine was inversely related to ascorbate nutriture (r = -0.64, p < 0.001, Fig. 1); mean blood histamine values fell from 151 ± 15 and 142 ± 11 ng/mL in the low ascorbate and control groups, respectively, to 80 ± 4 ng/mL in the high ascorbate group. The mean blood histamine levels observed for the low ascorbate and control groups did not differ significantly, but both of these values were significantly higher than that observed for the high ascorbate group (Table 1).

The random migration of leukocytes in 50% autologous serum did not differ significantly among the dietary treatment groups; however, cells extracted from the low ascorbate and high ascorbate animals migrated only 40-50% of the distance traveled by control cells (Table 1). Leukocyte chemotaxis in 50% autologous serum was significantly greater for cells extracted from control sera than for cells extracted from low ascorbate animals (p < 0.05, Table 1). Although the mean chemotactic response in autologous serum of leukocytes from the high ascorbate animals was 41% lower than that noted for control cells, this difference was not significant.

Neither random migration in autologous serum nor chemotaxis in autologous serum were related to blood histamine levels. Furthermore, these parameters were not related to liver ascorbate.

Figure 2 depicts the chemotaxis and random migration data for extracted leukocytes incubated in the various test sera and buffer. Chemotaxis of cells from guinea pigs fed a low ascorbate diet was not altered significantly by the various media, but locomotion was 57–87% less when these cells were incubated in autologous or high ascorbate sera relative to that in buffer or control sera (Fig. 2a). Chemotaxis of cells from control guinea pigs was significantly depressed when cells were incubated in low ascorbate serum or high ascorbate sera compared to autologous sera (p < 0.05, Fig. 2a). Chemotaxis of cells from control animals was 87% greater in autologous sera than in buffer, but the difference was not significant. The directed migration of cells from animals fed the high levels of ascorbate was about 50% less in low ascorbate serum than in buffer, control sera or autologous sera.

The random migration of leukocytes from guinea pigs fed low ascorbate diet was 71% greater when cells were incubated in control sera compared to autologous sera, and it was 168% greater when cells were incubated in high ascorbate sera (Fig. 2b). A similar, but less pronounced, trend was noted for cells from control animals incubated in low ascorbate,
control and high ascorbate sera. The cells from guinea pigs fed the high ascorbate diet, however, exhibited the lowest random migration in autologous sera; it was 62% less than the migration in buffer (Fig. 2b). None of the serum-dependent changes in random migration were significant.

**DISCUSSION**

These data demonstrate that the chemotactic response of leukocytes was altered by ascorbic acid nutriture in guinea pigs. Compared to control dosage levels, suboptimal intake of the vitamin (25% of the control dosage for 4 wk) significantly depressed cell chemotaxis, an effect that may involve cellular defects as well as serum inhibitors. The cellular defects appeared to be specifically related to the chemotactic apparatus of the leukocyte and not to basic locomotion mechanisms because the random migration of low ascorbate cells in buffer was not less than that of control cells (Fig. 2b). The chemotactic index of low ascorbate cells in buffer, however, was only 28% of that noted for the control cells (Fig. 2a).

Leukocyte chemotaxis, cell movement directed by external chemical gradients, is probably initiated by the binding of chemoattractants to membrane receptors, followed by second messenger release and subsequent rearrangement of the cytoskeletal proteins, a phenomenon known as polymerization (22, 23). Low leukocyte ascorbate levels may impair the assembly of these contractile proteins. Boexer et al. (24) demonstrated that ascorbic acid promoted intracellular leukocyte microtubule assembly. In a separate report, these investigators showed that ascorbic acid supplementation significantly improved leukocyte chemotaxis in patients with Chediak-Higashi syndrome, a disease characterized by impaired neutrophil microtubule assembly (25). The mechanism of ascorbate-enhanced microtubule assembly is not known, but the vitamin may alter the redox state of tubulin sulfhydryl groups (24).

Exogenous agents modulate leukocyte chemotaxis by altering second messenger release and cell polymerization. Histamine lowers the chemotactic responsiveness of leukocytes, presumably by elevating intracellular cAMP levels via the H-2 receptor (9, 26, 27) and depressing microtubule assembly (28). Conversely, exogenous factors that increase intracellular cGMP, including ascorbic acid (29, 30), enhance leukocyte chemotaxis (31). In the present study, leukocyte chemotaxis was impaired when cells were incubated in low ascorbate serum relative to control serum, a phenomenon that may be attributed, in part, to relatively higher levels of histamine and lower levels of ascorbate. However, leukotaxis was not further amplified by high ascorbate–low histamine serum.

High vitamin intake (25 times the control dosage for 4 wk) did not enhance leukocyte chemotaxis. Furthermore, serum extracted from animals fed high levels of ascorbate appeared to contain inhibitors of chemotaxis since the directed migration of low ascorbate and control leukocytes was lower in high ascorbate serum than in buffer or control serum. The inhibitors were specific for chemotaxis and did not appear to affect the random migration of leukocytes. Hence, any promotive effect of the low histamine–high ascorbate combination was masked by inhibitory serum factors produced by tissue-saturating levels of the vitamin.

These data conflict with reports from other investigators who have demonstrated that supplemental ascorbate enhances leukocyte chemotaxis. Anderson et al. (32) reported that the chemotaxis of neutrophils extracted from healthy subjects following 1 wk of vitamin supplementation (2 or 3 g ascorbate daily) was elevated two- to four-fold ($p < 0.05$); however, chemotaxis was not affected at the 1 g dosage level. In addition, numerous investigators demonstrated that chemotaxis was significantly elevated when normal leukocytes were incubated in 1 to $5 \times 10^{-3}$ mol/L ascorbate (7, 8, 24, 29). In these reports, neutrophil chemotaxis was examined with cells that had been washed and resuspended in buffer, hence, serum-dependent phenomena were not examined. Our data suggest that although cellular defects were not apparent in leukocytes extracted from high ascorbate animals, serum from these animals may contain factors that inhibit chemotaxis. In the present report, chemotaxis of cells from control animals and chemotaxis of cells from guinea pigs fed high ascorbate diets were similar when the cells were incubated in buffer, but chemotaxis of high ascorbate cells was only 59% of that noted for control cells when comparisons were made in autologous serum.

Investigators previously demonstrated that guinea pigs fed high dietary ascorbate had elevated serum levels of dehydroascorbic acid, the oxidized form of the vitamin (1, 33), and that the rise in blood dehydroascorbic acid was accompanied by a concomitant increase in blood glucose (1). Blood glucose levels in animals fed 50 mg ascorbate/100 g body weight, the dosage used in the present report, rose from the baseline value of 100 to 284 mg/100 mL after 15 d of vitamin treatment (1). The hyperglycemic effect of dehydroascorbic acid seems to be associated with abnormalities of the beta cells of the islets of Langerhans of the pancreas (34). Since insuffi-
cient insulin impairs leukocyte chemotaxis (35), this may represent a possible mechanism for chemotaxis inhibition in the high ascorbate serum.

This study demonstrates that low ascorbate nutriture impairs leukocyte chemotaxis. Both cellular defects and serum inhibitors appear to be responsible for this immunodeficiency. High ascorbate nutriture, however, does not promote leukotaxis, although blood histamine, a negative regulator of cell chemotaxis, is significantly depressed. The data suggest that tissue-saturating levels of the vitamin induce serum conditions that depress leukocyte chemotaxis.

LITERATURE CITED


