Effect of ascorbic acid on incidence of spontaneous mammary tumors and UV-light–induced skin tumors in mice[1,2]

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ABSTRACT Two large-scale studies of the effect of different amounts of L-ascorbic acid in the food on tumor-free survival have been conducted. One involved the incidence of spontaneous mammary tumors in RIII mice, with seven ascorbic acid and three control groups, 50 mice per group. With increasing ascorbic acid in the diet, there was a highly significant delay before appearance of the first tumor. Median age at first tumor was 82.5 wk in ad libitum controls, 124.9 wk in the highest-dose ascorbate group. The proportion of mice with tumors was also reduced. The other study involved dermal neoplasms in mice irradiated with ultraviolet light. A pronounced effect of vitamin C in decreasing the incidence and delaying the onset of malignant lesions was observed with high statistical significance. By 20 wk approximately five times as many mice had developed serious lesions in the zero-ascorbate as in the high-ascorbate group. *Am J Clin Nutr* 1991;54:1252S–5S.

KEY WORDS Ascorbic acid, vitamin C, cancer, neoplasms, therapy, prevention

Introduction

Ascorbic acid is a molecule synthesized by most nonhuman animal species, with an array of functions, including antioxidation and free radical scavenging (1, 2), synthesis of hormones and neurotransmitters, synthesis of collagen and carnitine (3–6), immune-system functions (7, 8), and reconstituting vitamin E (9, 10). Animal studies (11–22), some human studies (23, 24), and epidemiologic studies (25) have suggested a role for high doses of ascorbic acid in delaying the incidence or reducing the severity of cancers. These observations indicated the need for large, carefully conducted, and well-controlled animal studies on the role of vitamin C in cancer.

Here we report the results of two large studies of the effects of increasing dietary amounts of ascorbic acid on tumor-free survival in mice, first in a spontaneous mammary-tumor model and then in ultraviolet (UV) light–induced dermal tumors.

Methods and results

Incidence of spontaneous mammary tumors in RIII mice

RIII/Imr is an inbred strain of mouse characterized by transmission of mammary-tumor virus through maternal milk and by a high spontaneous mammary-tumor incidence. In this strain the mean age of tumor onset is 12 mo. Five hundred virgin RIII mice were obtained at age 7 wk and housed in a certified biologically clean (by Centers for Disease Control) facility, isolated from direct exposure to humans and noise to reduce the stress on test animals. These methods and results were described in detail (26).

The animals were randomly assigned to 10 groups of 50 mice each. Six test diets, two control diets, and two crossover diets were administered. The test diets contained ascorbic acid in the following amounts (in g/100 g): 0.076, 1.86, 2.9, 4.2, 8.0, and 8.3. The control diets consisted of an ad libitum group and a restricted group that received 90% of the average food consumed by the test groups. The ascorbic acid content of all diets was confirmed by high-performance liquid chromatography. All diets were equicaloric. Ingredients were steam-pelleted to control *Salmonella.* Assays of the nutrient content of the diets revealed an almost total loss of thiamin, presumably as a result of the steam-pelleting; as a result, thiamin was added in the drinking water. Nutrient analyses showed other nutrients to be present in adequate amounts. Diets were identified by color code so that investigators were masked with respect to the test diets.

Mammary tumors were first observed when the mice were 31 ± 1 wk old, and each test mouse was palpated weekly thereafter. All mice, including those surviving at the conclusion of the study, were necropsied. A section of each mammary tumor was examined histologically. Most were adenomatous mammary neoplasms. Approximately one-third were well-differentiated noninvasive adenomata; the bulk were definitely malignant, were moderately invasive, and tended to invade the adjacent tissues.

The outcome variable in this analysis was age at detection of the first mammary tumor. Tarone's test for trend (27), essentially the same as the method recommended by the International Agency for Research on Cancer (IARC) (28), was applied to the ages of the mice when the first mammary tumor was detected. Semilog Kaplan-Meier survival plots were also examined (29). The linearity of such plots corresponds to a first-order process and indicates that a single additional oncogenic factor is required

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as the last step in the progression to cancer, which may be affected by the amount of vitamin C in the diet.

Increasing the amount of ascorbic acid in the diet was associated with an older age at appearance of first tumor (**Table 1**). The statistical significance of the trend test over all the test dosages was $P = 0.0000005$. The median age at appearance of first tumor was 82.5 wk in the ad libitum controls and 124.9 wk in the highest-dose ascorbate group. The proportion of mice with tumors was also reduced by high-dose ascorbate, from 56% in the ad libitum controls to 30% in the highest-dose ascorbate group.

The mean body weights over the entire course of the study (9–114 wk) were not different among the study groups, averaging 24.5 g in the ad libitum controls and 23.7 in the 8% ascorbate group. Ad libitum controls consumed somewhat more food (36 g/wk) than did any of the other study groups. One group of control mice, the restricted controls, received only 90% of the average caloric intake of the other study groups. That group had a somewhat greater delay before appearance of the first tumor than did the ad libitum controls but less than the animals receiving ascorbic acid in their diets.

One group of animals on a test diet experienced a poorer pattern of tumor appearance than did either of the control groups. This was the group receiving the lowest concentration of ascorbic acid in their diets, 0.076%. The median age at appearance of first tumors in this group was 69.5 wk compared with 82.5 wk in the ad libitum controls. This observation, also seen in the second study reported below, prompted two further studies on the effect of exogenous ascorbic acid intake on tissue ascorbate and biosynthesis of ascorbic acid by mice (30, 31). The results indicated that in this ascorbic acid–synthesizing species, consumption of a small amount of exogenous ascorbic acid produces a decrease in the rate of biosynthesis and a decreased concentration of tissue ascorbic acid. It is likely that this is due to a feedback control mechanism that regulates ascorbic acid biosynthesis. This phenomenon occurs in mice and possibly other animals that synthesize ascorbic acid but has no significance for species that cannot synthesize the vitamin, such as humans, other primates, and guinea pigs. It does suggest the need for great caution in the design and interpretation of ascorbate-cancer studies in laboratory animals that can synthesize the vitamin.

Incidence of dermal neoplasms induced in mice by UV light

The hairless mouse is a mutant that loses its hair at about 3 wk of age thus affording easy visibility of skin lesions. It does not have the thymus defect of the nude mouse. Female SK hhr strain hairless mice were obtained at age 6–9 wk and stabilized for 2 wk before test diets were begun. Irradiation was begun 10 d later and continued 5 d/wk for ~15 wk, for a total exposure of 135 J/cm^2. The exposure was begun at 1.13 J/cm^2 and increased gradually to compensate for epidermal thickening. Mice were observed during irradiation to confirm that each mouse received a full dorsal exposure. These methods and results were described in detail (32).

Diet supplements were mixed with Purina certified rodent chow No. 5002, pelleted, and kept frozen until needed. There were four test diets, containing 0%, 0.3%, 5% and 10% ascorbic acid. The control group consisted of 45 mice and each of the other groups consisted of 38–40. In addition to these four irradiated groups, there were an additional four groups of unirradiated mice, 20 per group, receiving the four test diets but no irradiation. A total of 720 observations were made of the unirradiated mice and no lesions were observed. Thus, the lesions in the irradiated mice can be attributed to the radiation and not to the diets. The intake of food by the different groups was the same to within 6% over a 3-wk period.

TABLE 1
Effect of varying ascorbic acid intake on incidence of spontaneous mammary tumors in RIII mice

Ascorbate in diet*	Time to first tumor (weeks)	Mice with tumors
%	wk	%
0 (ad libitum)	82.5	56
0 (restricted)	87.3	44
1.86	92.4	44
4.2	109.9	38
8.1	124.9	30

* $n = 50$ per group.

The irradiated mice were inspected and weighed every 14 d beginning 4 wk before the end of the 15-wk irradiation period and continuing for nine inspections. The gross readings of the lesions were performed by the same person. Lesions classified as papillomas or carcinomas were counted and recorded. Other lesions, such as small keratoses, wounds, or areas of thickened skin, were noted but not included in this analysis. After the final reading, representative lesions on the control and 10% ascorbic acid groups were assigned code numbers and examined histologically. Most tumors ≥ 6 mm in diameter were found to be squamous-cell carcinomas. A histopathologic study demonstrated good agreement among four pathologists examining 60 of the lesions (33).

The outcome, length of time to appearance of the first persistent lesion in the four test groups, was tested by using the method formulated for the IARC (28). As the study progresses, only animals that have not yet experienced an endpoint are in the at-risk denominator for the subsequent time. Data were right censored when the animals died from causes unrelated to the experiment. (Only two animals of the 162 were censored in this way, one in the control group and one in the 5% ascorbic acid group.) The appropriate test for trend over the four dosage groups for censored survival data of this type is Tarone's (27) procedure based on the framework developed by Peto and Peto (34), Mantel (35), and Breslow (36). Data were analyzed and reported separately for lesions > 2, 4, 6, and 10 mm in diameter.

For small lesions, the trend test became significant early, and by the ninth reading (12 wk after irradiation ceased) had reached $P < 0.003$ for lesions < 2 mm and $P < 0.007$ for lesions < 4 mm. At that point, 28.8% of control mice and 12.5% of mice in the 10% ascorbic acid group had developed these small lesions.

The effect was even more pronounced for larger lesions (**Table 2**). By the ninth reading, 15.5% of mice in the control group had developed lesions > 10 mm in diameter whereas only 2.5% of mice in the highest-dose ascorbic acid group had developed such lesions. Tarone's test for tumor-free survival in censored data of this sort is significant at $P < 0.01$ for both these groups despite the small numbers of animals experiencing these large tumors. Increasing dosages of vitamin C in the diet of irradiated mice significantly delayed the onset of UV-light–induced tumors.

TABLE 2
Effect of varying ascorbic acid intake on time to first tumor in UV-light exposed hairless mice

Ascorbate in diet	Cumulative percent of mice with tumors by end of study: Lesions > 6 mm	Lesions > 10 mm
%	%	
0 (n = 45)*	22.2	15.6
0.3 (n = 39)	23.1	15.4
5 (n = 38)	10.5	7.9
10 (n = 40)	7.5	2.5
P (trend)†	0.01	0.01

* Number of mice at risk at beginning of the study.
† Tarone's test for trend.

Discussion

The results of these two studies show, with a high level of statistical significance, that increasing amounts of ascorbic acid in the diets of mice delays the onset of spontaneous mammary tumors in RIII mice and of tumors induced by UV irradiation in hairless mice. The absence of carcinogenicity or toxicity of any sort for large amounts of vitamin C in rodents was clearly seen in these studies and was also confirmed by Douglas et al (37).

Studies by others have indicated that ascorbate reduced the tumor growth rate, delayed the onset of tumors, or prolonged the life span of tumor-bearing mice. The origin of the tumors tested has varied: ovarian tumor transplants beneath the subrenal capsule (11), sarcoma-180 subcutaneous implants (12), Ehrlich tumor cells injected intramuscularly (13, 14), Ehrlich carcinoma and L1210 leukemia (15, 16), melanoma cells injected subcutaneously (17, 18), mammary adenocarcinoma transplanted subcutaneously (19), estrogen- or DES-induced renal tumors (20), phorbol ester–induced skin tumors (21), and human mammary-tumor xenografts (22).

The suggestion of a poorer outcome of mice receiving the lowest dose of exogenous vitamin C suggests the need for caution in studies of the role of added ascorbate in cancer and other chronic diseases. Animals that synthesize ascorbic acid apparently have a feedback mechanism such that small amounts of exogenous ascorbate result in alterations in endogenous synthesis or metabolism, resulting in lower rather than higher tissue concentrations of ascorbic acid. Thus, in such species the dosage amounts must be high enough to overcome this feedback mechanism. Such a mechanism is obviously absent in species that do not synthesize ascorbic acid, namely humans, nonhuman primates, guinea pigs, and a few others. It may therefore be questionable to generalize results from synthesizing to nonsynthesizing species unless it is clear that a high enough dosage has been used.

The predominant species used in cancer research are mice and rats, animals that, unlike humans, synthesize ascorbic acid. Not only might this produce questionable results in studies of the role of ascorbic acid, it also should be viewed with caution in studies of the effects of other potential anticancer agents. In ascorbic acid–synthesizing species, animals that experience a physiologic challenge such as a carcinogen or cancer itself were shown to increase their ascorbic acid synthesis (38, 39). This response is not available to humans and thus the effect of the potential anticancer agent may be different in rats or mice than in humans.

An example of such an apparent contradiction may be seen in the research on tumor necrosis factor (TNF). In rodents TNF is an extremely impressive antitumor agent; when used in humans, the toxicity is high and it is difficult to achieve effective doses. This may be an example in which the beneficial effect of TNF seen in rodents is attenuated or obscured in humans because of humans' inability to synthesize increased amounts of ascorbic acid. This suggests both the need to perform studies of potential antitumor agents in nonsynthesizing species and to investigate the effect of substantially increasing the ascorbic acid intake of humans undergoing cancer therapies.

The dosages used in the data presented here were very high. Direct comparisons with dosages in humans are inappropriate, for reasons such as the need to overcome the feedback mechanism in rodents, the different water contents of the foods consumed by rodents vs humans, and the possibility of different digestive handling of solids.

It is very clear from these data, however, that very high dietary intakes of ascorbic acid are both very well-tolerated and statistically significantly beneficial in delaying or preventing the occurrence of spontaneous mammary tumors and UV-light–induced tumors in mice.

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