LETTER TO THE EDITOR

Long-term Cancer Bioassays of Ascorbic Acid

James Huff1

National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709

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Kuroiwa et al. (2008) report on the lack of carcinogenesis of coadministration of ascorbic acid and sodium nitrite in male F344 rats, despite causing oxidative DNA damage–associated mutagenicity in vitro and increased levels of 8-hydroxydeoxyguanosine in rat forestomach epithelium. Earlier studies showed that forestomach carcinogenesis was enhanced by this combination after initiation with N-methyl-N’-nitro-N-nitrosoguanidine (Okazaki et al., 2006), and forestomach papillomas were induced without prior initiation, using sodium nitrite and sodium ascorbate (Yoshida et al., 1994). Ascorbic acid enhanced butylated hydroxyanisole–induced forestomach lesions (Shibata et al., 1993), sodium L-ascorbate following initiation with several nitrosamines inhibited liver but promoted renal pelvis and bladder carcinogenesis (Thamavut et al., 1989), and sodium ascorbate but not ascorbic acid induced neoplastic and preneoplastic lesions of the urinary bladder (Fukushima et al., 1986), implicating the influence of the sodium ion and increased pH on bladder carcinogenesis. Following initiation with 1,2-dimethylhydrazine, sodium L-ascorbate increased the incidence of adenomas and the number of tumors per rat of the colon (especially of the distal colon) (Shirai et al., 1985). Ito et al. (1983, 1986) posed that antioxidants have different effects (promoting or inhibitory influences) depending on the organ studied and suggest the importance of a whole-body approach to their investigation.

Not mentioned by Kuroiwa et al. (2008) were long-term carcinogenesis bioassays of L-ascorbic acid done by the National Toxicology Program (NTP) (Douglas et al., 1984; NTP, 1983). Hence, the intent of this letter. Our studies were conducted by offering diets containing 0, 25,000, or 50,000 ppm L-ascorbic acid (>97% pure) to groups of 50 F344/N rats and 50 B6C3F1 mice of each sex for 103 weeks. Overall, there were no observed increases in toxicity or neoplasia between control and exposed groups that were considered causally related to L-ascorbic acid.

However for one lesion, undifferentiated (mononuclear cell) leukemia, the incidence of low-dose female rats (control, 6/50; low dose, 17/50; high dose, 12/50) was increased (p < 0.02) compared to controls, and the high-dose group was double the control rate. Adding the few lymphomas observed gave rates of 9/50 versus 19/50 and 12/50. Using the Poly-3 statistical tests indicates a marginal trend (p = 0.07), a low-dose increase (p = 0.001), and a high-dose no effect (p = 0.07). Altogether, NTP considered these malignant tumor increases not related to administration of L-ascorbic acid because they were not significantly increased in the high-dose group, the dose-response trend test was not significant, and no increases were observed for male rats (17/50 vs. 16/50, 14/50). For male mice, there was a negative trend (p < 0.03) for lymphoma/leukemia (9/50 vs. 8/50, 3/50) with the high-dose group being significantly lower (p = 0.035) than controls. No differences were seen for female mice: 14/50 versus 13/50, 17/50.

Compared to the initiation-promotion studies (Okazaki et al., 2006; Yoshida et al., 1994), there were no indications of forestomach or esophageal tumors in any of the sex-species groups given L-ascorbic acid alone for 2 years (Douglas et al., 1984; NTP, 1983). Likewise tumors enhanced in the other two-stage carcinogenesis models cited above were not increased or considered target organs in the NTP carcinogenesis studies.

There were some decreasing tumor trends the NTP studies in rats. For preputial or clitoral glands, negative trends (p < 0.05) were observed for males with adenocarcinomas of the preputial gland and for females with adenocarcinomas of the clitoral gland. Interstitial-cell tumors of the testis occurred with a negative age-adjusted trend (p < 0.03), but none of the control/dose group comparisons were significant. Adenomas/carcinomas of the pituitary gland showed a decreased trend (p < 0.05) in female rats.

In female rats, several nonneoplastic lesions usually seen in aged animals showed significant dose-related declines: myocardial degeneration, nephropathy, liver inflammation, hyperplasias of the thyroid and adrenal glands, and osteopetrosis of the femur. While it seems reasonable to relate decreases of degenerative changes to ascorbic acid exposure, similar changes were not found in the male rats. And no pattern of degenerative lesions was seen in mice of either sex. Thus, the significance of these findings in female rats is uncertain.

Thus, under the conditions of these NTP studies, L-ascorbic acid given in the diet at 2.5 or 5.0% for 103 weeks was not

1 For correspondence via E-mail: huff1@niehs.nih.gov.

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toxic or carcinogenic for F344/N rats or for B6C3F1 mice. While the decreased effects in rats and mice given ascorbic acid seem logical and reasonable with respect to an antioxidant mechanism of ascorbic acid, NTP decided “These borderline increases and decreases in neoplastic lesions, as well as decreases in nonneoplastic effects in female rats, were considered to be insufficient evidence for a compound related effect.”

REFERENCES