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Antioxidants and Cancer

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As a corollary of the free radical theory of carcinogenesis, antioxidants have been postulated to exert an anticarcinogenic effect by inhibiting the activation of carcinogens via oxidative processes in vivo, stabilizing potential precursors of carcinogens or cocarcinogens such as polyunsaturated fatty acids, and acting as scavengers of genotoxic free radicals. High concentrations of the food antioxidants BHA and BHT protect against chemically induced neoplasia in animals by a mechanism that appears to involve their capacity to accelerate the catabolism and excretion of carcinogens via the mixed function oxidase complex rather than their antioxidant properties. Also, the protective effect of diets high in selenium against chemically induced carcinogenesis appears to be unrelated to the normal physological role of this element as a constituent of the enzyme glutathione peroxidase. The natural antioxidants vitamin E and vitamin C are less active as inhibitors of chemical carcinogenesis, but they inhibit the formation of potentially carcinogenic products of oxidative rancidity in foods and their action in quenching genotoxic free radicals is suggestive of an anticarcinogenic effect in vivo.

Interest in the possible anticarcinogenic effect of antioxidants stems from the free radical theory of carcinogenesis, according to which tumor formation is a result of the genotoxic action of oxy and peroxy radicals formed by one-electron oxidation of carcinogens, or of reduced oxygen species such as hydroxyl and superoxide radicals, hydrogen peroxide, and organic peroxides formed as secondary products (Ts'o et al., 1977). Evidence in support of this hypothesis includes the demonstration that ionizing radiation produces reduced oxygen compounds in biological materials (Bielski and Gebicki, 1977), that some organic peroxides have mutagenic and tumorigenic activity (Kotin and Falk, 1963), that diets high in polyunsaturated fatty acids predispose to chemically induced carcinogenesis (King et al., 1979; Hopkins et al., 1981; Ip, 1982), and that malonaldehyde, a decomposition product of fatty acid peroxides formed in vivo, is carcinogenic (Shamberger et al., 1974; Bird et al., 1982). The demonstration that under certain conditions vitamin E and certain synthetic antioxidants as well as selenium, a constituent of a peroxide-decomposing enzyme, have anticarcinogenic activity in animals has provided further support for this hypothesis.

As a corollary of this theory, antioxidants have been postulated to exert their anticarcinogenic effect by inhibiting the activation of carcinogens via oxidative processes in vivo, by stabilizing potential precursors of carcinogens or cocarcinogens such as polyunsaturated fatty acids, and by acting as scavengers of genotoxic free radical intermediates. However, there is no single mechanism that explains the anticarcinogenic action of all antioxidants, and recent research indicates that the inhibitory effect of some such compounds is due in part to their ability to alter the action of enzymes involved in the metabolism of carcinogens rather than to their antioxidant properties per se.

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Table I. Inhibition of Chemically Induced Carcinogens in Rodents by BHA[a]

carcinogen	site(s)
benzo[*a*]pyrene	lung, forestomach
benzo[*a*]pyrene-7,8-dihydrodiol	forestomach, lung, lymphoid
7,12-dimethylbenz[*a*]-anthracene	lung, forestomach, skin, breast
7-(hydroxymethyl)-12-methylbenz[*a*]anthracene	lung
dibenz[*ah*]anthracene	lung
diethylnitrosamine	lung
4-nitroquinoline *N*-oxide	lung
uracil mustand	lung
urethane	lung
methylazoxymethanol acetate	large intestine
trans-5-amino-3-[2-(5-nitro-2-furyl)vinyl]-1,2,4-oxadiazole	forestomach, lung, lymphoid

[a] Wattenberg (1982).

Synthetic Antioxidants. Protection against chemically induced neoplasia has been most clearly demonstrated in the case of the food antioxidants butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). Numerous experiments, notably those of Wattenberg and co-workers, have shown that BHA has anticarcinogenic activity against a wide range of chemical carcinogens with several different target organs. A summary of these findings, taken from a recent review by Wattenberg (1982), is given in Table I. It is noteworthy that BHA apparently is effective against carcinogens that require metabolic activation as well as those, such as uracil mustard, that do not.

BHT has been found to have anticarcinogenic activity comparable to that of BHA in the prevention of benzo[*a*]pyrene (BP)-induced tumors in the forestomach of A/HeJ mice when fed at a concentration in the diet of 5 mg/g (Wattenberg, 1972). It also reduces 1,2-dimethylhydrazine (DMH)-induced colon tumors in rats (Jacobs and Griffin, 1979) and BALB/c mice (Clapp et al., 1979), 7,12-dimethylbenz[*a*]anthracine (DMBA)-induced mammary adenocarcinomas in rats (King and McCay, 1983), and neoplasms of the forestomach, skin, breast, liver, and large intestine produced by various chemical carcinogens in mice and rats (Wattenberg, 1978).

The minimum effective concentration of BHT in the diet is influenced by diet composition, by the level of the carcinogenic challenge, and by species. There are indications that BHA is more effective against BP-induced stomach cancer in mice at a concentration of 10 than at 0.5 mg/g and more effective against DMBA-induced neoplasia at 10 than at 2 mg/g (Wattenberg, 1972). King and McCay (1983) found BHT to be superior to BHA as an inhibitor of mammary tumors in Sprague-Dawley rats challenged with DMBA but to be ineffective against mammary tumors induced by nitrosomethylurea (NMU). Cohen et al. (1982) observed dose-dependent inhibition of carcinogensis in three model systems (DMBA-induced mammary carcinoma, azoxymethane-induced colon carcinoma, and 2-(acetylamino)fluorine-induced hepatocarcinoma) over a range of 0.3–6 mg of BHT/g of diet. All levels of BHT exhibited protection against a low dose (5 mg) of DMBA.

There is less information available concerning the anticarcinogenic properties of other antioxidants, but it is apparent that activity is not restricted to compounds with the structural or physical characteristics of BHA and BHT. The commercial lipid antioxidant Ethoxyquin has inhibitory activity against DMBA-induced carcinogenesis comparable to that of BHA at a concentration of 10 mg/g of diet (Wattenberg, 1972) (Table II). Ethoxyquin is also effective against lung cancer induced by BP, breast cancer caused by DMBA, and lung cancer produced by diethylnitrosamine or 4-nitroquinoline *N*-oxide (Wattenberg, 1978).

Table II. Comparison of the Inhibitory Effects of BHA, Ethoxyquin, and Vitamin E on Neoplasia of the Forestomach Induced in Ha/ICR Mice by DMBA[a]

carcinogen, mg/g of diet	antioxidant, mg/g of diet	tumor incidence, %	tumors/animal (*N*)
DMBA, 0.05	none	100	6.9
	BHA 10	58[b]	1.3[b]
	ethoxyquin 10	41[b]	0.7[b]
	DL-α-tocopherol	95	4.1

[a] Wattenberg (1972). [b] $P < 0.01$.

Vitamin E. In view of the low structural specificity required for anticarcinogenic activity among these lipid-soluble antioxidants, it is noteworthy that the natural lipid antioxidant vitamin E is less effective against chemically induced carcinogenesis than the major commercial synthetic food antioxidants. The relative ineffectiveness of DL-α-tocopherol in preventing stomach cancer in DMBA-treated animals (Table II) is illustrative. King and McCay (1983) found that vitamin E is ineffective against DMBA-induced mammary tumorigenesis, and Kozumbo et al. (1983) observed that α-tocopherol had little potency as an inhibitor of ornithine decarboxylase induction, an early indicator of promotor activity. Notwithstanding, this vitamin has been reported to provide some protection against carcinogenesis induced by methylcholanthrene in C57 mice (Haber and Wissler, 1962), by DMBA in Syrian golden hamsters (Shklar, 1982), and by dimethylhydrazine in LACA mice (Cook and McNamara, 1980) and to inhibit the chromosomal damage to mammalian cells grown in culture caused by BP (Smalls and Patterson, 1982). Ip (1982) observed that a low vitamin E diet had little effect on the incidence of DMBA-induced mammary tumors in rats when the diet contained 5% stripped corn oil but had a significant protective effect when it contained 20% stripped oil. No additional protection was afforded by feeding a high level of vitamin E in the diet (1000 mg/kg) over that provided by a moderate level (30 mg/kg). In a further study, vitamin E was found to potentiate the protective effect of a high-selenium diet on DMBA-induced tumorigenesis, whereas it was ineffective alone (Horvath and Ip, 1983). The authors concluded that vitamin E acts only in the proliferative phase of tumorigenesis and that the inhibitory action of selenium is not due to its role in glutathione peroxidase.

Mechanisms. The fact that vitamin E is less effective than some synthetic antioxidants as an inhibitor of chemically induced carcinogenesis raises an important consideration regarding the relationship between antioxidant activity and anticarcinogenic activity. Vitamin E (and probably some water-soluble reducing substances such as vitamin C) appears to act by preventing the formation of carcinogens by oxidative reactions in the diet and in vivo. Inhibition by vitamin E of the formation of fatty acyl endoperoxides, which are the precursors of malonaldehyde, is an example. Malonaldehyde excretion in the urine is increased in vitamin E deficient rats (Polensek and Draper, 1982).

Although BHA and BHT are effective inhibitors of lipid peroxidation in foods, in contrast to certain nonphenolic antioxidants such as *N*,*N*-diphenyl-*p*-phenylenediamine, they are ineffective as in vivo substitutes for vitamin E in

the prevention and treatment of vitamin E deficiency diseases (Draper, 1980). Their effectiveness against chemical carcinogenesis appears to be due primarily to their ability to act as blocking agents, i.e., to modify the metabolism of carcinogens so as to reduce their carcinogenic activity (Miller, 1978; Wattenberg, 1982). Recently, Kozumbo et al. (1983) observed that BHA and BHT inhibited the induction of ornithine carboxylase activity by the tumor promoter 12-*O*-tetradecanoylphorbol. Vitamin E was less potent in this regard and vitamin C was inactive. The protection afforded by propyl gallate against carcinogenesis induced by DMBA (King and McCay, 1983) suggests an analogous mechanism. However, the observation that the effectiveness of BHT as in inhibitor of DMBA-induced mammary tumorigenesis is affected by the degree of unsaturation of dietary fat (King et al., 1979) indicates that the anticarcinogenic activity of this compound may be in part due to its antioxidant properties.

Recent research into the mode of action of blocking agents such as BHA and BHT has revealed that they induce changes in the metabolism of various xenobiotic compounds including several carcinogens. BHA administration alters the metabolism of some carcinogens by the mixed function oxidase system so as to accelerate their conversion to hydroxylated derivatives. There is an associated increase in the activity of several conjugase enzymes including UDP-glucuronyl transferase, epoxide hydratase, and glutathione *S*-transferase, which convert these derivatives to noncarcinogenic forms. Hence BHA activates a coordinated enzyme system that enhances the detoxification of a number of xenobiotic compounds. Incubation of BP with microsomes from BHA-treated mice reduces its binding to DNA to half that observed in control mice (Speier and Wattenberg, 1975). Reduced BP binding is associated with a decrease in epoxidation (an activation process) and an increase in hydroxylation (a detoxification process). Research in this field has been reviewed by Wattenberg (1982).

These observations indicate that the inhibition of chemical carcinogenesis by BHA and BHT is a manifestation of a general enzymatic defense system against xenobiotic compounds that are capable of being hydroxylated by the mixed function oxidase complex. As food antioxidants, BHA and BHT also inhibit the formation of potentially carcinogenic products of lipid peroxidation in the diet. However, their lack of vitamin E activity indicates that they are less effective than the tocopherols as inhibitors of lipid peroxidation in vivo. Thus while BHA and BHT appear to be superior to vitamin E as inhibitors of chemical carcinogenesis (and then only at relatively high concentrations), vitamin E may be a superior inhibitor of carcinogenesis caused by lipid free radicals in vivo.

Human Cancer. The relevance to human nutrition of the demonstration that synthetic antioxidants protect animals against some forms of chemical carcinogenesis is debatable. Under typical conditions used in animal experiments, more than 1 mg of BHA of BHT per g of diet was required for effective inhibition of carcinogenesis. The concentration of these antioxidants used in the major fats and oils in the human diet is 0.01–0.02%. Since the diet contains about 20% fat, the average concentration of BHA or BHT in the total diet appears to be in the range of 0.002–0.004% (0.02–0.04 mg/g). This concentration is well below that required for protection against the levels of chemical carcinogens used in acute experiments on animals. However, it can be presumed that the levels of human exposure to carcinogens are also well below those used to produce short-term effects in animals. It appears unlikely that the concentrations of BHA and BHT in the human diet are sufficient to provide the stimulation of the mixed function oxidase system that is necessary for their anticarcinogenic effect. Also, not all carcinogens in the human environment are metabolized by the mixed function oxidase system. While there is evidence that the feeding of antioxidants extends the life span of experimental animals, the evidence is inconsistent and it is not clear that any such extension is due to a reduced incidence of spontaneous tumors.

The introduction of BHA and BHT as food antioxidants has been cited as a possible factor in the decline in gastric carcinoma in the United States (Shamberger et al., 1972). Any cause and effect relationship between these events is speculative, but there is clear evidence that BHA and BHT inhibit nitrosamine formation in the stomach and that they inhibit the formation of potentially carcinogenic products of lipid peroxidation in foods. In the absence of convincing evidence of adverse effects, these considerations justify their continued use as food additives.

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