REVIEW

Dietary antioxidants in the prevention of hepatocarcinogenesis: A review

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In this review, the role of dietary antioxidants in the prevention of hepatocarcinogenesis is examined. Both human and animal models are discussed. Vitamin C, vitamin E, and selenium are antioxidants that are essential in the human diet. A number of non-essential chemicals also contain antioxidant activity and are consumed in the human diet, mainly as plants or as supplements, including β-carotene, ellagic acid, curcumin, lycopene, coenzyme Q_{10}, epigallocatechin gallate, *N*-acetyl cysteine, and resveratrol. Although some human and animal studies show protection against carcinogenesis with the consumption of higher amounts of antioxidants, many studies show no effect or an enhancement of carcinogenesis. Because of the conflicting results from these studies, it is difficult to make dietary recommendations as to whether consuming higher amounts of specific antioxidants will decrease the risk of developing hepatocellular carcinoma.

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1 Introduction

In the United States, liver cancer is the fifth leading cause of death from cancer in males and the eighth leading cause in females [1]. Worldwide, hepatocellular carcinoma (HCC) is the third leading cause of death from cancer [2]. The incidence of and mortality from HCC are variable worldwide, with the Far East and sub-Saharan Africa having the highest incidences. The primary risk factors for HCC are infection with hepatitis B and hepatitis C viruses, and long-term exposure to aflatoxin [3]. In the United States, chronic alcoholism leading to chronic liver disease is a significant risk factor [4]. The prognosis for HCC is poor, with the 5-year survival rate at diagnosis being only 11% [1]. A number of molecular changes have been identified with hepatocarcinogenesis. These include mutations in the p53, Rb, β-catenin, and insulin-like growth factor receptor 1 genes [3, 5]. Other genes are overexpressed, including c-myc, c-jun, and cyclin D_1 [3, 5].

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Abbreviations: AAF, 2-acetylaminofluorene; **AFB_1,** aflatoxin B_1; **ATBC,** α-Tocopherol, β-Carotene$_1$; **Caret,** β-Carotene and Retinol Efficacy Trial; **CV-3611,** 2-*O*-octadecylascorbic acid; **DEN,** diethylnitrosamine; **EGCG,** epigallocatechin gallate; **GGT,** γ-glutamyl transpeptidase; **GPx,** glutathione peroxidase; **HCC,** hepatocellular carcinoma; **3′-Me-DAB,** 3′-methyl-4-dimethylaminoazobenzene; **NAC,** *N*-acetyl cysteine; **OHdG,** 8-hydroxydeoxyguanine; **PB,** phenobarbital; **PCBs,** polychorinated biphenyls; **PCB-77,** 3,3′,4,4′-tetrachlorobiphenyl; **PGST,** placental glutathione-*S*-transferase; **PH,** partial hepatectomy; **Se,** selenium; **SELECT,** Selenium and Vitamin E Cancer Prevention Trial; **TBARS,** thiobarbituric acid-reactive substance; **TGF-α,** transforming growth factor-α; **TrxR,** thioredoxin reductase

Liver cancer can be induced experimentally by a number of agents, including aromatic amines, azo dyes, nitrosamines, and aflatoxin [6]. In initiation–promotion protocols, the administration of a carcinogen (such as diethylnitrosamine (DEN), 2-acetylaminofluorene (AAF), or aflatoxin B_1 (AFB_1)) along with a proliferative stimulus (see next paragraph) followed by the long-term feeding of chemicals, such as phenobarbital (PB), 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, or polyhalogenated biphenyls (PCBs), leads to a high incidence of hepatocellular adenomas and carcinomas [6, 7]. Transgenic mouse models of liver carcinogenesis have also been developed [8].

There are several different methods for inducing cell proliferation in the liver. Partial hepatectomy (PH) in adult animals was examined initially by Higgins and Anderson and later in other studies [9–14]. Peraino *et al.* [15] used neonatal animals, when hepatic cell proliferation is higher [16, 17], during the initiation phase. Another method is to use a necrogenic dose of an initiator [18–20]; many of the studies reviewed below have used necrogenic doses of hepatocarcinogenic agents, such as DEN or AFB_1 in the absence of other proliferative stimuli. A different approach was described by Pani and colleagues, who reported that liver initiation could be brought about by fasting animals for 4 days, refeeding, which induces hepatic cell proliferation, and then administrating a subnecrogenic dose of an initiator 24 h after refeeding [21–23]. However, Espandiari *et al.* [24] observed that the fasting/refeeding method was not as effective as the PH or neonatal methods. All of these different methods of inducing cell proliferation have been used in hepatocarcinogenesis studies.

Other studies have used non-genotoxic carcinogens. These are chemicals that induce cancer, but which do not covalently bind to DNA. Examples of non-genotoxic carcinogens in the liver include peroxisome proliferators, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, and choline deficiency [25–28]. Possible mechanisms of carcinogenesis include the induction of oxidative stress, increased cell proliferation, and the inhibition of apoptosis.

Several endpoints have been examined in studies examining the effects of antioxidants on carcinogenesis. Gross tumors that are observed include hepatocellular adenomas and HCCs [29]. In addition, foci of putative preneoplastic hepatocytes appear before the development of gross tumors. These foci, known as altered hepatic foci or enzyme-altered foci, contain cells that exhibit qualitatively altered enzyme activities or alterations in one or more cell functions, such as iron or glycogen accumulation [6, 30]. The enzymes most frequently studied include γ-glutamyl transpeptidase (GGT) and placental glutathione-*S*-transferase (PGST), which are normally not present in adult liver but are often present in foci; and ATPase and glucose-6-phosphatase, which are normally present but are frequently missing from foci [31, 32]. Altered hepatic foci can also be identified on hematoxylin- and eosin-stained tissue [33, 34].

The Solt–Farber-resistant hepatocyte model [18, 35, 36] has been a valuable tool for the study of hepatocarcinogenesis. In this protocol, visible nodules or altered hepatic foci rapidly appear and their expansion is dose dependent [18, 35, 36]. In this model, initiation consists of either a necrogenic dose of a hepatocarcinogen or a non-necrogenic dose in combination with PH. Initiation is then followed by a selection phase, consisting of AAF feeding or injection with a proliferative stimulus, normally PH or carbon tetrachloride injection, midway through AAF administration. Visible nodules and/or altered hepatic foci appear at the end of the selection phase. If the animals are maintained for a longer period of time, with either no further treatment or treatment with a promoting agent, such as PB, the nodules regress but hepatocellular adenomas and carcinomas appear several months later.

In this review, we will examine the role that dietary antioxidants may play in the possible prevention of liver cancer. We will discuss epidemiological studies, clinical trials in humans, and experimental hepatocarcinogenesis studies in which antioxidants were studied. The induction of oxidative stress has been proposed as one mechanism by which chemicals exert their initiating and/or promoting activities in the liver [25, 37, 38]. Therefore, studies that have examined dietary antioxidants provide clues to the mechanisms of hepatocarcinogenesis as well as providing a possible mechanism for the prevention of the human disease.

2 Vitamin E

Vitamin E is a generic term for a group of compounds, tocopherols and tocotrienols. Each class includes four isoforms (α, β, δ, and γ), which differ in the number and position of methyl groups on their chromanol ring [39]. The Dietary Reference Intake recommendation for vitamin E is currently 15 mg/day for males and females; this recommendation is for α-tocopherol alone, as this is the vitamin E form maintained in plasma and the form recognized by α-tocopherol transfer protein in the liver [40]. Recent data for vitamin E intake in the United States indicate that only 5% of males and 4% of females consume the Recommended Dietary Allowance for vitamin E daily [41].

Based on limited prospective studies, higher serum vitamin E levels do not seem to correlate with liver cancer prevention. For example, in a population-based 11.7-year follow-up study on mortality rates from cancer in a Japanese population, higher serum tocopherol levels did not correlate with reduced risk of mortality from cancer, including liver cancer [42]. In a 15-year follow-up prospective study in males, high serum levels of α-, γ-, and δ-tocopherols did not reduce the risk of developing HCC [43].

Although serum vitamin E may not correlate with liver cancer prevention in humans, some *ex vivo* studies have observed lower serum vitamin E levels with some liver

diseases. Chronic hepatitis can be a precursor to hepatic cancer in humans [44], and the serum levels of α-tocopherol in hepatitis patients are typically decreased. The diminished serum α-tocopherol may be due to decreased β-lipoproteins, which can occur as a result of liver disease [45]. One study observed reduced plasma α-tocopherol in patients with HCC with cirrhosis; those patients also had decreased hepatic α-tocopherol in cirrhotic areas in comparison with controls who had undergone perlaparoscopic cholecystectomy [46]. However, other studies have not correlated low vitamin E with liver diseases. For example, one study did not see a difference in hepatic vitamin E levels between carcinoma tumors and adjacent liver tissue, and no correlations were observed between blood levels of vitamin E and HCC or normal tissue [47]. One comparative study did not see a significant decrease in plasma α-tocopherol in patients with hepatitis, cirrhosis, or HCC, but the levels in hepatoma patients approached significance ($p = 0.0561$) [44]. Another comparative study also did not find plasma vitamin E levels to be different between controls and patients with HCC or those with a carcinoma that had metastasized to the liver [48]. One case–control study with newly diagnosed HCC patients found vitamin E status, as assessed by dietary vitamin E and serum levels, to be no different from cases *versus* control subjects [49].

Based on various clinical trials, vitamin E supplementation seems to provide no overall benefit for total cancer prevention in males [50, 51] or females [52]. One study examined the incidence of and mortality from several cancers, including liver cancer, after supplementation with several vitamin–mineral combinations for 5.25 years. One arm of the study investigated a β-carotene (15 mg), α-tocopherol (30 mg), and selenium (Se) supplement (50 μg); however, this supplement combination did not reduce the incidence or death rates of liver cancer. One of the limitations of this randomized factorial intervention trial was that it was impossible to evaluate the independent effect of α-tocopherol alone [53].

As chronic hepatitis C virus patients frequently develop liver cirrhosis and HCC, one study investigated the effect of α-tocopherol supplementation on hepatocarcinogenesis in patients with cirrhosis and a history of chronic hepatitis C viral infection. After 5 years of supplementation, there was no statistical improvement in liver function, hepatocarcinogenesis suppression, or cumulative survival of the treatment group *versus* the control group, although the tendency was for improved cumulative tumor-free survival, and overall survival was higher in the vitamin-E-supplemented group [54]. However, short-term vitamin E supplementation (3 months or less) has been shown to significantly improve liver function in patients infected with viral hepatitis [55, 56]. In patients with diagnosed HCC, vitamin E improves the survival rate as a supportive treatment to tamoxifen and all-trans-retinoic acid [57].

Data from animal studies, which have been used extensively to study hepatic injury and hepatocarcinogenesis, more consistently show anticancer activity for vitamin E (Table 1). The level of vitamin E found in the AIN-93 diet is 75 IU/kg diet (which is equal to 150 mg all-rac-α-tocopheryl acetate *per* kg diet) [58]. In several of the studies, the level of vitamin E far exceeds the AIN-93 recommendation. For example, 15 g vitamin E/kg diet is 100-fold higher than the recommended level. In addition, for some of the studies, the exact dose of vitamin E cannot be determined because the form fed was not listed. Some of the animal models support the hypothesis that hepatic cancer stems from the complications associated with oxidative stress. The earliest study found that vitamin E supplementation decreased the incidence of liver tumors induced by 3′-methyl-4-dimethylaminoazobenzene (3′-Me-DAB) when fed after its administration but had little effect when fed during 3′-Me-DAB administration [59]. One transgenic mouse model overexpresses the proto-oncogene c-myc and transforming growth factor-α (TGF-α) in the liver to produce a line of mice with increased susceptibility to spontaneous liver tumors [60]. At 3 wk of age, the double transgenic mice were fed a control diet or a diet supplemented with 2000 units DL-α-tocopherol acetate/kg of diet. After supplementation for 10 wk, the tocopherol-supplemented mice had decreased intracellular peroxides, reduced chromosomal aberrations, decreased dysplasia, and a 65% reduction in hepatic adenoma incidence. Other studies using male Wistar rats supplemented the diets with 1 mg/kg vitamin E (tocopherol) 1 wk before a single dose of 500 μg AFB_1. The rats stayed on the supplemented diets for either 3 more weeks [61], 3 more months [62], or the remainder of the study (24 months) [63]. The rats that were fed the vitamin E supplemented diets for 3 wk or 3 months were placed on a control diet until the end of the studies (23 or 20 months, respectively). All three studies found reduced incidences of hepatic tumors and increased activity in cytochrome P-450 (isozyme not specified) with supplemented dietary vitamin E. The authors hypothesized that the increased induction of cytochrome P-450 in rats fed the high vitamin E diets may have led to more rapid detoxification of AFB_1. One study in trout, in which *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine was used as the initiating agent and hydrogen peroxide was used as the promoting agent, found the incidence of hepatic tumors to be unaffected by dietary vitamin E [64]. Kakizaki *et al.* [65] examined whether either deficient or high levels of dietary vitamin E could influence hepatocarcinogenesis in transgenic mice that overexpress TGF-α. The mice receiving the control level of vitamin E (20 mg α-tocopheryl acetate/kg diet) developed the most tumors, the mice receiving the highest level (500 mg/kg diet) had a significantly lower incidence, and the mice receiving the deficient diet had a tumor incidence between that of the other two groups.

Many animal studies have used altered hepatic foci as an endpoint in studying hepatocarcinogenesis, because these foci can be detected early after initiation [66]. Several studies have supplemented with vitamin E and treated with various hepatic carcinogens to show that increased dietary vitamin E

Table 1. Effect of vitamin E on experimental liver carcinogenesis

Reference	Form and dose of vitamin E	Species, strain, and sex	Induction/initiation regimen	Promotion regimen	Endpoint	Results	
						Initiation (or complete carcinogenesis)	Promotion
Swick and Bauman [59]	α-Tocopherol (1–1.6 g/kg diet)	Male Sprague-Dawley or Holtzman rats	3′-Methyl-4-dimethyl-aminoazobenzene (0.64 g/kg in diet) for 4 wk	None	Liver tumors	Vitamin E supplementation decreased the incidence of liver tumors when fed after the administration of 3′-Me-DAB, but had little effect when fed during 3′-Me-DAB administration	Not studied
Moore *et al.* [67]	α-Tocopherol (10 g/kg in diet)	Syrian golden hamsters	DOPN (20 mg/kg, s.c.)	None	PGST-positive foci and tumors	Inhibition of foci	Not studied
Ura *et al.* (two studies) [72]	DL-α-tocopherol acetate (3.6-15 g/kg in diet)	Male Fisher 344 rats	DEN (200 mg/kg, i.p.)	PH 1 wk after starting the vitamin E diets	GGT-positive foci	Not studied	Dietary DL-α-tocopherol acetate decreased the number and size of GGT-positive foci
	DL-α-tocopherol acetate (3.6, 7.2, and 15 g/kg in diet)	Male Fisher 344 rats	PH/DEN (20 mg/kg, i.p.)	AAF/CCl_4 selection followed by 14 wk with no further chemical treatment	GGT-positive foci	Not studied	Rats fed either 3.6 or 7.2 g/kg DL-α-tocopherol acetate after DEN-initiation and selection had increased number and size of GGT-positive foci
Nyandieka *et al.* [62]	Tocopherol (1 mg/kg diet)	Male Wistar rats	AFB_1 (500 μg/kg)	None	Tumors	Inhibition of tumor development	Not studied
Glauert *et al.* [80]	α-Tocopherol acetate (10, 50, or 500 mg/kg diet)	Female Sprague-Dawley rats	Ciprofibrate (0.25 g/kg in diet)	None	Tumors and GGT-positive, ATPase-negative, and G6Pase-negative foci	Increased incidence of tumors and number and volume of foci in rats fed higher levels of vitamin E	Not studied
Nyandieka *et al.* [61]	Tocopherol (1 mg/kg diet)	Male Wistar rats	AFB_1 (500 μg/kg)	None	Tumors	Inhibition of tumor development	Not studied
Hendrich *et al.* [70]	α-Tocopherol (50 or 500 mg/kg diet)	Female Fischer-344 rats	DEN (10 mg/kg i.p. at 4 days of age)	PB (500 ppm in diet) or butylated hydroxyanisole (5000 ppm in diet)	GGT- and PGST-positive foci	Not studied	Supplemental α-tocopherol (500 ppm) did decrease the mean focal volume after initiation with and promotion with PB and

Table 1. Continued

Reference	Form and dose of vitamin E	Species, strain, and sex	Induction/initiation regimen	Promotion regimen	Endpoint	Results	
						Initiation (or complete carcinogenesis)	Promotion
							butylated hydroxyanisole after 3 months; however, the effect was not observed after 11 months
Kelly *et al.* [64]	Vitamin E, 20 or 1000 mg/kg in diet	Shasta rainbow trout	23-day-old trout embryos exposed to *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (25 mg/kg in water) for 30 min	Hydrogen peroxide (0, 600, or 3000 ppm in diet)	Liver tumors	Not studied	The incidence of hepatic tumors was unaffected by dietary vitamin E
Nyandieka and Wakhisi [63]	Vitamin E in diet (1 mg/kg body weight)	Male Wistar rats	AFB_1 (500 μg/kg, p.o.)	None	Tumors	Inhibition of carcinogenesis	Not studied
Rahmat *et al.* [75]	Tocotrienols (30 mg/kg diet)	Male rats	DEN (200 mg/kg i.p.) followed by AAF (0.2 g/kg in diet) for 2 months	None	Liver nodules	Inhibition	Not studied
Tsuda *et al.* [71]	α-Tocopherol (15 g/kg in diet)	Male Fisher rats	PH/2-amino-3-methylimidazo [4,5-f]quinoline (100 mg/kg p.o.)	PB (0.05% in diet) + a single dose of D-galactosamine (100 mg/kg i.p.)	PGST-positive foci	Inhibition	Not studied
Mizumoto *et al.* [69]	DL-α-tocopherol (100, 200, 600, and 1100 mg/kg diet)	Male Fischer-344 rats	Choline-deficient, amino acid-defined diet	None	GGT-positive foci	Vitamin E produced a dose-dependent response in decreasing the number and size of GGT-positive liver foci (complete carcinogenesis)	Not studied
Kolaja and Klaunig [79]	Vitamin E (0, 50, 250, and 450 mg/kg diet)	Male B6C3F1 mice	35 mg DEN/kg body weight twice a week until focal lesions were formed (8 wk). The mice were then started on a diet with varying levels of supplementary DL-α-tocopherol acetate for 60 days.	None	Altered hepatic foci identified by H&E staining	Not studied	The highest dose of dietary vitamin E (450 mg/kg diet) resulted in increased number and volume of the hepatic focal lesions
Lii *et al.* [77]	α-Tocopherol acetate (0, 5, or 10 g/kg diet)	Female Sprague-Dawley rats	DEN (15 mg/kg i.p. at 24 h of age)	PB (500 ppm in diet)	GGT-positive foci	Not studied	No effect

Table 1. Continued

Reference	Form and dose of vitamin E	Species, strain, and sex	Induction/initiation regimen	Promotion regimen	Endpoint	Results	
						Initiation (or complete carcinogenesis)	Promotion
	added to purified diet containing AIN-76 vitamin mix						
Makpol *et al.* [73]	Supplemental α-tocopherol or γ-tocotrienol (30 and 300 mg/kg diet, respectively)	Male rats	DEN (200 mg/kg, i.p.)	0.02% AAF for 2 wk with PH at 1 wk; rats then maintained for 12 or 16 wk	PGST- and GGT-positive foci	Foci formation decreased when tocopherol or tocotrienol supplemented at the same time as DEN and AAF	Supplementation begun after DEN and AAF treatments had no effect
Klaunig and Kamendulis [68]	Vitamin E (400 mg DL-α-tocopherol acetate/kg diet)	Male B6C3F1 mice	DEN (25 mg/kg i.p., 2 × /wk for 8 wk)	Dieldrin (10 mg/kg diet)	Neoplastic and preneoplastic lesions using H&E staining	Not studied	Decreased hepatic focal lesion volume
Lii *et al.* [78]	α-Tocopherol (0, 100, or 5000 mg/kg diet)	Female Sprague-Dawley rats	DEN (15 mg/kg i.p.) at 24 h of age	PB (500 ppm in diet)	GGT- and PGST-positive foci	Not studied	No effect on altered hepatic foci
Factor *et al.* [60]	DL-α-tocopherol acetate (2000 units/kg of diet)	Male TGF-α/c-myc double transgenic mice	TGF-α/c-myc overexpression	None	Tumors	Reduction in hepatic adenoma incidence	Not studied
Kakizaki *et al.* [65]	α-Tocopherol acetate (<1, 20, and 500 mg/kg diet)	Male CD-1 mice overexpressing TGF-α in the liver	DEN (5 mg/kg i.p.)	TGF-α overexpression	Tumors	Not studied	The mice receiving the control level of vitamin E (20 mg α-tocopheryl acetate/kg diet) developed the most tumors, the mice receiving the highest level (500 mg/kg diet) had a significantly lower incidence, and the mice receiving the deficient diet had a tumor incidence between that of the other two groups.
Glauert *et al.* [76]	α-Tocopherol acetate (10, 50, or 250 mg/kg diet)	Female Sprague-Dawley rats	DEN (150 mg/kg p.o.)	PCB-77 or PCB-153 (300 μmol/kg i.p. every 14 days for 4 injections)	PGST-positive foci	Not studied	No effect

The nomenclature for identifying the form of vitamin E is the same as in the original papers. Abbreviations: ATPase, adenosine triphosphatase; CCl_4, carbon tetrachloride; DOPN, 2,2′-dioxo-*N*-nitrosodipropylamine; G6Pase, glucose-6-phosphatase; GGT, gamma-glutamyl transpeptidase; H&E, Hematoxylin and Eosin; PCB-77, 3,3′,4,4′-tetrachlorobiphenyl; PCB-153, 2,2′,4,4′,5,5′-hexachlorobipenyl.

decreased the number of foci or the focal volume [67–70]. In the study by Moore *et al.* [67], altered hepatic foci were induced in Syrian golden hamsters with 2,2′-dioxo-*N*-nitrosodipropylamine. The hamsters receiving 10 g/kg α-tocopherol in their diets had a decreased number and size of PGST-positive foci [67]. Klaunig and Kamendulis [68] saw decreased hepatic focal lesion volume with increased dietary vitamin E (400 mg DL-α-tocopherol acetate/kg diet) in male B6C3F1 mice, which received DEN as the initiating agent and dieldrin as the promoting agent. Mizumoto *et al.* [69] found DL-α-tocopherol to exert a dose-dependent response in decreasing the number and size of GGT-positive liver foci and in decreasing the oxidative stress indicators, 8-hydroxydeoxyguanine (OHdG) and thiobarbituric-acid-reactive substance (TBARS). Supplemental α-tocopherol (500 mg/kg diet) did decrease the mean focal volume after initiation with DEN and promotion with PB and butylated hydroxyanisole after 3 months; however, the effect was not observed after 11 months [70].

Some studies saw the most protection against hepatic-altered foci during the initiation and early promotion stages of hepatocarcinogenesis. Tsuda *et al.* [71] observed that α-tocopherol inhibited the initiation of altered hepatic foci by 2-amino-3-methylimidazo[4,5-f]quinoline. Dietary DL-α-tocopherol acetate decreased the number and size of GGT-positive foci when fed for 6 wk after DEN initiation with the administration of a PH 1 wk after beginning the experimental diets [72]. However, supplemental α-tocopherol had no protective effect once GGT-positive foci were formed. In fact, rats fed either 3.6 or 7.2 g DL-α-tocopherol acetate/kg diet after finishing the Solt–Farber protocol (DEN initiation and selection by 2 wk of AAF feeding with carbon tetrachloride (CCl_4) administration 1 wk after starting AAF feeding) had increased number and size of GGT-positive foci, compared with rats fed an unsupplemented unrefined diet. A 16-wk study found GGT- and PGST-positive foci as well as plasma GGT to be significantly reduced after initiation by DEN, selection by AAF feeding and PH, followed by a maintenance period when rats were fed a diet with either supplemental α-tocopherol or γ-tocotrienol (30 and 300 mg/kg diet) [73]. The two antioxidants were only effective against altered hepatic foci formation when administered simultaneously with DEN and not 4 or 8 wk later. Other studies found γ-tocotrienol to be effective in decreasing preneoplastic marker enzymes when given simultaneously with the tumor initiator, AAF, as detected with liver microsomal uridine diphosphate glucuronyltransferase and plasma GGT activities [74] or liver and plasma activities of GGT and alkaline phosphatase [75]. Rahmat *et al.* [75] also observed that vitamin E inhibited the induction of liver nodules.

Some animal studies have shown no effect or increased altered hepatic foci with vitamin E supplementation. One study found vitamin E (250 ppm α-tocopheryl acetate) treatment for almost 2 months to be ineffective in protecting against the induction of altered hepatic focal lesions by polychlorinated biphenyls (PCBs) in female Sprague-Dawley rats [76]. Others have also found supplementary α-tocopherol to have no effect on altered hepatic foci [77, 78]. In one study, male B6C3F1 mice were treated with 35 mg DEN/kg body weight twice a week until focal lesions were formed (8 wk). The mice were then started on a diet with varying levels of supplementary DL-α-tocopherol acetate for 60 days. The highest dose of dietary vitamin E (450 mg/kg diet) resulted in increased number and volume of the hepatic focal lesions, especially those of basophilic variety; DNA synthesis was also enhanced [79]. The mice treated with no dietary vitamin E also had enhanced focal lesion growth, but less than what was observed for the highest level of dietary vitamin E. Another study investigating higher levels of dietary vitamin E (500 mg α-tocopheryl acetate/kg diet) found an increased induction of altered hepatic foci and tumors in female Sprague-Dawley rats fed 0.25 g/kg ciprofibrate for 21 months [80].

Vitamin-E-deficient diets may alter gene expression, which could partially explain the higher incidence of altered hepatic foci and tumors in vitamin-E-deprived animal models. In a long-term rat study (290 days), rats fed vitamin-E-deficient diets had significantly less hepatic γ-glutamylcysteinyl synthetase, which is the rate-limiting enzyme in the synthesis of glutathione [81]. Two weeks of treatment with 0.02% (w/w) α-tocopherol resulted in higher levels of hepatic glutathione-*S*-transferase α levels, glutathione, and glutathione peroxidase activity. The effects on glutathione and glutathione enzymes may be responsible for the chemoprotective character of α-tocopherol [82].

Overall, neither the human nor animal studies support a clear role for vitamin E in the prevention of liver carcinogenesis. In the human studies, neither the prospective nor the intervention studies showed any protective effect of vitamin E. Some but not all studies observed lower serum levels of vitamin E in patients with liver diseases. In animal studies, results were also mixed: protective effects of feeding higher vitamin E were observed in two transgenic models and in some chemically induced models, but in other chemical carcinogenesis models, no effect or even enhancement was observed.

3 Selenium

Dietary Se is an essential mineral for both humans and animals. It functions as a component of several proteins, termed selenoproteins. These include glutathione peroxidases (GPx, several different isoforms), thioredoxin reductases (three isoforms), iodothyronine deiodinases (three isoforms), selenophosphate synthetase, selenoprotein P, and selenoprotein W [83]. Because GPx and thioredoxin reductase function as antioxidants and because an inverse relationship between Se intake and cancer risk was identified in several studies, increasing Se intake has been proposed as a way to prevent the development of some forms of cancer in humans.

Epidemiological data as well as supplementation trials support the hypothesis that Se is likely to be effective in humans. Epidemiological studies on the relationship between Se and cancer have found that Se status is inversely related to some cancer risks. Shamberger reported this association in human subjects and also found that mortality attributed to lymphomas and cancers of the gastrointestinal tract, peritoneum, lung, and breast were lower in subjects living in areas where Se concentration is high in forage crops compared with those living in areas with low-Se-containing forage crops [84, 85]. Clark and Stafford [86], using the same forage data, indicated that colorectal cancer mortality is indeed associated with high Se. Using the estimated Se intake *per* capita, Schrauzer *et al.* [87] noted an inverse association with total cancer mortality rate and age-corrected mortality rate for leukemia and cancers of the colon, rectum, breast, ovary, and lung.

Using serum Se level, several studies reported that low serum or plasma Se level is associated with increased risk for some cancers, such as gastrointestinal cancers, prostate cancer, thyroid cancer, malignant oral cavity lesions, esophageal and gastric cancers, cervical cancer and colorectal adenomas, and non-melanoma skin cancer [88–95]. On the contrary, some studies have reported no significant association between serum Se concentration and cancer risks [96–99]. A recent case–control study found that individuals with higher toenail Se had a decreased risk of developing HCC [100].

Se supplementation trials have been conducted to determine whether Se is effective in reducing liver cancers in human. Most of the supplementation trials were based in China and the remaining trials were in the USA, Italy, and India. The first China trial investigated the preventive effect of Se on primary liver cancer and found that Se supplementation using table salt fortified with sodium selenite (30–50 μg Se/day) resulted in an almost 50% decrease in the primary liver cancer incidence [101, 102]. Another study showed that selenite-fortified salt supplementation reduced the incidence rate of viral infectious hepatitis, a predisposing factor of primary liver cancer [103, 104]. Yu *et al.* [102] reported a significant decrease in primary liver cancer among those receiving Se yeast compared with controls. However, Qu *et al.* [53] found that supplementation with a combination of β-carotene, α-tocopherol, and Se (as Se yeast) for 5.25 years in Linxian, China, did not affect mortality from liver cancer.

A double-blind, randomized trial (Nutritional Prevention of Cancer trial) of Se-enriched yeast involving 1312 patients with non-melanoma skin cancer led to the unexpected discovery that Se protects against colon, lung, and prostate cancers; data on liver cancer were not presented [105, 106]. After extending the trial to 10 years, the resulting trend was still the same [107]: they found that Se significantly decreased the incidence of total cancer and prostate cancer, but the incidences of lung or colorectal cancers were not reduced significantly. However, Duffield-Lillico *et al.* [107] found that subjects with low plasma Se levels had a lower incidence of cancer, whereas those with high plasma Se levels did not correlate with cancer incidence. The results from this trial led to the initiation of other clinical intervention trials, including Se and Vitamin E Cancer Prevention Trial (SELECT) in the USA and Prevention of Cancers by Intervention with Se (PRECISE) in Europe [108, 109]. The SELECT trial has recently been published, but failed to detect inhibitory effects of Se on lung, colon, or prostate cancer; data on liver cancer were not presented [50]. The European clinical trials are currently ongoing [110].

Several studies have investigated the effect of Se on different phases of hepatocarcinogenesis using varying *in vivo* hepatocarcinogenesis protocols, initiating agents and tumor promoters (Table 2). The level of Se added to the AIN-93 diet is 0.15 mg Se/kg diet, with the total amount estimated to be about 0.18 mg/kg diet, due to background levels in the other ingredients of the diet [58]. The amounts of Se added to diets in the studies described below do not exceed the recommended level by as much as in some of the vitamin E studies; the toxicity of Se at higher doses limits the amount that can be added to diets. Several early studies observed that Se inhibited complete carcinogenesis in the liver [61–63, 111–114]. Two studies that used DEN as the initiator and PB as the promoting agent demonstrated that Se did not affect the induction of hyperplastic hepatic nodules or carcinomas when Se supplementation was given during either the initiation or promotion stages, or throughout the entire study [115, 116]. Milks *et al.* [117] found that feeding higher levels of Se only during AFB_1 administration inhibited the subsequent development of altered hepatic foci. Conflicting results on focal development were reported by Baldwin and Parker [118]: in AFB_1-treated rats supplemented with 6.0 mg Se/kg diet and fed high-fat diets during the initiation period, the mean volume and volume fraction of GGT-positive foci were decreased but the number of foci/cm^3 of liver was not affected; dietary Se did not affect the promotion of GGT-positive foci by PB. Baldwin and Parker [119] also observed that focal development was increased in a very low Se group in rats treated with AFB_1 with no further treatment. Using a Solt–Farber protocol, Bjorkhem-Bergman *et al.* [120] found that 1 and 5 mg/kg Se administered to rats during initiation had no effect on the number and volume of hepatic nodules, but Se administered during either the selection or 6-month progression stages decreased the volume occupied by the nodules in the liver. LeBoeuf *et al.* [121] also noted that Se (6 mg/kg diet) decreased focal growth (mean volume) with no corresponding effect on the number of GGT-positive foci in the liver when fed either after DEN or during AAF administration; however, when high (6 mg/kg diet) Se was fed between DEN initiation and PB promotion, the induction of altered hepatic foci was increased. Other hepatocarcinogenesis studies have shown that Se inhibits focal growth: Lei *et al.* [122] demonstrated that Se inhibited the induction of altered hepatic foci and tumors by AFB1, and

Glauert *et al.* [123] observed that Se inhibited the incidence of tumors as well as the number of altered hepatic foci induced by the peroxisome proliferator ciprofibrate in rats. Using *N*-nitrosobis(2-oxopropyl)amine to induce liver tumors in hamsters, Lee *et al.* [124] found that Se (as sodium selenite) injected i.p. (1 mg/kg twice *per* week for 12 wk) inhibited the volume and area of tumor foci. Stemm *et al.* [125] examined the effect of feeding both deficient and supplemental Se levels during the promotion of PGST-positive foci by 3,3′,4,4′-tetrachlorobiphenyl (PCB-77), a coplanar PCB and Ah receptor agonist; and 2,2′,4,4′,5,5′-hexachlorobipenyl (PCB-153), a di-ortho-substituted PCB and constitutive androstane receptor agonist. Feeding the highest level of Se was found to increase the number of PGST-positive foci but to decrease their volume.

Two studies have used transgenic models. In mice overexpressing both TGF-α and c-myc, both Se deficiency and high dietary Se (as sodium selenite) inhibited the development of tumors [126]. In Mdr2 knockout mice, the feeding of supplemental selenomethionine for 3 months followed by a latency period of 13 months resulted in a decrease in the incidence of large tumor nodules [127].

Xu *et al.* [128] used a transplantable tumor model using HepG2 human hepatoma cells. Mice were given drinking water containing sodium selenite or green tea extracts in which the tea had been grown with different levels of Se. Both selenite and high Se teas inhibited the growth of the transplantable tumors.

Several studies have examined whether changes in oxidative stress correlated with effects on carcinogenicity by Se. In the Glauert *et al.* [123] study in which ciprofibrate-induced carcinogenesis was reduced by dietary Se, high Se levels increased serum and liver GPx activity, but did not decrease the oxidative damage indices, such as TBARS and conjugated dienes, indicating that increased GPx activity may have no protection against oxidative damage. Hepatoma cells injected into Sprague-Dawley rat livers resulted in decreased GPx1 activity, but no significant effect on oxidative stress markers, TBARS and OHdG, were seen [129]. Se supplementation reduced lipid peroxide levels in tissues [130]. Se that was supplemented either before initiation or during initiation and selection/promotion phases of hepatocarcinogenesis was found to be effective in altering hepatic lipid peroxidation and antioxidant enzyme activities either in the hepatoma or in the normal liver tissues. Moreover, increased level of lipid peroxidation products and reduced levels of antioxidants, superoxide dismutase and catalase, were observed in non-tumor bearing organs; however, these conditions were reversed to normal upon Se supplementation. When AAF was used as selection agent in a modified Solt–Farber protocol, selenite did not produce an effect on GPx1 activity [120]. The liver GPx1 activity of rats given selenomethionine was also shown to be either not affected or only slightly increased as a result of long-term AAF feeding [131]. They also observed high glutathione levels in the nodules as well as the surrounding parenchyma.

In contrast, it was shown that a high Se diet (2.0 mg/kg as sodium selenite) given to rats administered DEN had no effect on DEN-induced OHdG and no correlation was observed between GPx activity and OHdG levels [132]. In fact, they found that high Se diet increased liver OHdG levels; therefore, instead of protecting against DNA oxidative damage, inorganic Se supplementation may be enhancing DNA oxidative damage *in vivo*. Using the Big Blue rat transgenic model, Zeng *et al.* [133] found that dietary Se did not affect the mutation rate either in the liver or in the colon in rats injected with 1,2-dimethylhydrazine, a colon carcinogen.

Thioredoxin reductase (TrxR) is another selenoprotein with antioxidant activity, which could mediate Se's effect on carcinogenesis. Berggren *et al.* [134] observed that hepatic TrxR activity of rats fed with high sodium selenite (1.0 mg/kg) diet was increased twofold; however, this increase was not sustained and was not accompanied by a corresponding increase in TrxR protein synthesis. They suggested that this may be caused by decreased Se incorporation, leading to decrease in TrxR protein synthesis. Increased TrxR proteins in tumors of TGF-α/c-myc transgenic mice was noted compared with normal liver tissues [135]. Stemm *et al.* [125], however, did not find any changes in TrxR activity in response to PCBs or dietary Se.

Overall, the role of Se in human and experimental liver cancer is not clear. Three clinical trials found a chemopreventive effect of Se in human liver cancer; however, one additional trial did not. Two major clinical trials held in the United States (Nutritional Prevention of Cancer and SELECT) did not quantify liver cancer. In experimental animal studies, a wide variety of animal models have been used, including complete carcinogenesis models, the Solt–Farber model, and initiation–promotion models. Although some studies have shown that feeding higher amounts of Se inhibits complete carcinogenesis, initiation, and promotion, others show no effect or even an enhancement of carcinogenesis. Increasing dietary Se has been shown to increase the activity of selenoenzymes, such as GPx, but this increase does not necessarily lead to reductions in endpoints of oxidative damage, such as lipid peroxidation or oxidative DNA damage.

4 Vitamin C

Vitamin C is an essential nutrient in humans and other primates as well as several other species, such as guinea pigs. Vitamin C functions as an electron donor for several mammalian enzymes, including dopamine β-monooxygenase, prolyl 3- and 4-hydroxylase, and trimethyllysine hydroxylase, and also may function as an antioxidant, although under certain conditions it may have a prooxidant effect [136].

One epidemiological study has examined the role of dietary vitamin C in liver cancer etiology. In that prospective study, Kurahashi *et al.* [137] examined the effect of the consumption of fruits, vegetables, and some antioxidants on

Table 2. Effect of dietary Se on experimental liver carcinogenesis

Reference	Form and dose of Se	Species, strain, and sex	Induction/initiation regimen	Promotion regimen	Endpoint	Results	
						Initiation (or complete carcinogenesis)	Promotion
Harr *et al.* [111]	Sodium selenite, 0 (18 μg/kb in basal diet), 0.1, 0.5, and 2.5 mg/kg diet	Female OSU-brown rats	AAF, 150 mg/kg in diet	None	Tumors	Inhibition of complete carcinogenesis by increased dietary Se	Not studied
Griffin *et al.* [112]	Sodium selenite, 0 or 6 mg/kg in diet or in drinking water	Male Sprague-Dawley rats	0.5 g/kg 3′-methyl-4-dimethylaminoazo-benzene in diet for 8 wk	None	Tumors	Inhibition of complete carcinogenesis by either route of administration	Not studied
Marshall *et al.* [113]	Sodium selenite, 0 or 4 mg/kg in drinking water	Male Sprague-Dawley rats	AAF, 0.3 g/kg in diet for 14 wk	None	Tumors	Inhibition of complete carcinogenesis	Not studied
Daoud and Griffin [114]	Sodium selenite, 0 or 4 mg/kg in drinking water	Male Sprague-Dawley rats	0.5 g/kg 3′-methyl-4-dimethylaminoazo-benzene in diet for 9 wk	None	Tumors	Inhibition of complete carcinogenesis	
Aquino *et al.* [116]	Sodium selenite, 0.17 or 2 mg Se/kg diet	Male Wistar rats	DEN (40 mg/kg) in drinking water for 4 wk	PB, 0.05% of diet for 19 or 24 wk	Tumors and preneoplastic lesions	No effect	No effect
Dorado *et al.* [115]	Sodium selenite, 0.16, 4, or 6 mg Se/kg diet	Female Sprague-Dawley rats	PH/DEN (40 mg/kg)	0.05% PB in the diet for 19 or 46 wk	Tumors and preneoplastic lesions	No effect	No effect
Milks *et al.* [117]	Sodium selenite, 0, 0.2, 2.0, or 5.0 mg Se/kg drinking water	Male Sprague-Dawley rats	2.0 μmol/kg AFB_1	0.05% PB in drinking water for 1 wk; 0.01% PB in drinking water for 7 wk	GGT-positive foci	Inhibition	Not studied
LeBoeuf *et al.* (three studies) [121]	Sodium selenite, 0.1, 3.0, or 6.0 mg/kg diet	Female Sprague-Dawley rats	PH/DEN (100 mg/kg, p.o.)	None	GGT-positive foci	Not studied	Focal volume decreased; focal number not affected
	Sodium selenite, 0.1 or 6.0 mg/kg diet	Female Sprague-Dawley rats	PH/DEN (25 mg/kg, p.o.)	0.1 or 6.0 ppm Se feeding for 8 wk, followed by 0.05% PB	GGT-positive foci	Not studied	Increased focal volume and number
	Sodium selenite, 0.1 or 6.0 mg/kg diet	Female Sprague-Dawley rats	AAF (0.2 g/kg in diet), four 4-wk cycles	None	GGT-positive foci	Focal volume decreased; focal number not affected	Not studied
Baldwin and Parker [119]	Sodium selenite, <0.02 or 0.15 mg/kg diet	Male Sprague-Dawley rats	10 daily p.o. doses of 0.4 mg/kg AFB_1	None	GGT-positive foci	Focal development increased in very low Se group	Not studied
Baldwin and Parker [118]	Sodium selenite, <0.02, 0.15, 1.9 (promotion study only) or 2.5 (initiation study only) mg/kg diet	Male Sprague-Dawley rats	10 daily p.o. doses of 0.4 mg/kg AFB_1	0.03% PB in diet	GGT-positive foci	Focal development decreased by Se in high-fat but not low-fat groups	Focal size increased in Se-deficient groups
Nyandieka *et al.* [62]	"Se element" in diet (1 mg/kg body weight)	Male Wistar rats	AFB_1 (500 μg/kg)	None	Tumors	Inhibition of tumor development in rats receiving Se	Not studied

Table 2. Continued

Reference	Form and dose of Se	Species, strain, and sex	Induction/initiation regimen	Promotion regimen	Endpoint	Results	
						Initiation (or complete carcinogenesis)	Promotion
Lei *et al.* [122]	Sodium selenite, 0, 3, or 6 mg/kg in tap water for 79 wk	Male Wistar rats	AFB_1, 126 μg/wk in drinking water for 27 wk	None	Basophilic, eosinophilic, and clear cell foci and nodules; HCCs	Inhibition of carcinogenesis by higher Se levels	Not studied
Glauert *et al.* [123]	Sodium selenite, 0.04, 0.2, or 1.0 mg/kg diet	Female Sprague-Dawley rats	Ciprofibrate, 0.25 g/kg in diet, for 6 or 21 months	None	Tumors; GGT-positive, and ATPase- and G6Pase-negative foci	Tumor incidence and focal development decreased by Se at 21 months	Not studied
Nyandieka *et al.* [61]	"Se element" in diet (1 mg/kg body weight)	Male Wistar rats	AFB_1 (500 μg/kg)	None	Tumors	Inhibition of tumor development in rats receiving Se	Not studied
Nyandieka and Wakhisi [63]	"Se compound" in diet (1 mg/kg body weight)	Male Wistar rats	AFB_1 (500 μg/kg, p.o.)	None	Tumors	Inhibition of carcinogenesis by Se	Not studied
Mukherjee *et al.* [131]	Selenomethionine, 8 mg/kg in drinking water	Male Sprague-Dawley rats	AAF, 0.5 g/kg in diet for 16 wk	None	Preneoplastic lesions and hyperplastic nodules	Number of preneoplastic lesions and incidence and size of hyperplastic nodules decreased in rats given Se before, during, and before and during AAF administration	Not studied
Bjorkhem-Bergman *et al.* [120]	Sodium selenite, 0, 1 or 5 mg/kg in drinking water	Fischer-344 rats	DEN, 200 mg/kg	0.02% AAF in diet for 4 days followed by PH; then two p.o. injections of AAF (20 mg/mL) 2 and 4 days later. Rats then maintained up to 12 months	Glutathione-*S*-transferase 7-7 positive foci and nodules	No effect	Inhibition in rats fed higher levels of Se
Novoselov *et al.* [126]	Sodium selenite, 0, 0.1, 0.4, or 2.25 mg/kg in diet; or triphenylselenonium chloride, 30 ppm	Double transgenic TGF-α/c-Myc mice	Hepatic overexpression of TGF-α and c-myc	None	Foci, adenomas, and carcinomas	Complete carcinogenesis by TGF-α/c-myc overexpression inhibited by deficient and high levels of dietary Se	Not studied
Katzenellenbogen *et al.* [127]	Selenomethionine, 8 mg/L in drinking water	Mdr-2 knockout mice	Mdr-2 knockout	None	Liver tumors	Decrease in the incidence of large tumor nodules in mice given Se	Not studied
Xu *et al.* [128]	Sodium selenite or Se-enriched green tea extracts p.o. (5.0, 10.0, and 20.1 μg Se/kg bodyweight/day)	Kunming mice	HepG2 transplantable tumors	None	Transplantable tumor growth	Both selenite and high Se teas inhibited the growth of the transplantable tumors	Not studied

Table 2. Continued

Reference	Form and dose of Se	Species, strain, and sex	Induction/initiation regimen	Promotion regimen	Endpoint	Results	
						Initiation (or complete carcinogenesis)	Promotion
Lee *et al.* [124]	Sodium selenite, i.p. (1 mg/kg i.p. twice *per* week for 12 wk)	Male Syrian hamsters	BOP (10 mg/kg) s.c. 2 ×/wk for 10 wk	None	Tumors and PGST-positive foci	Se injections inhibited the volume and area of tumor foci	Not studied
Stemm *et al.* [125]	Sodium selenite, 0.02, 0.2, and 2.0 mg Se/kg diet	Female Sprague-Dawley rats	DEN, 150 mg/kg p.o.	PCB-77, or PCB-153 (300 μmol/kg) i.p., 4 injections administered every 14 days	PGST-positive foci	Not studied	Feeding the highest level of Se was found to increase the number of PGST-positive foci but to decrease their volume

Abbreviations: ATPase, adenosine triphosphatase; BOP, *N*-nitrosobis(2-oxopropyl)amine; G6P, glucose-6-phosphatase; GGT, g-glutamyl transpeptidase; PCB-77, 3,3′,4,4′-tetrachlorobiphenyl; PCB-153, 2,2′,4,4′,5,5′-hexachlorobipenyl.

the risk of HCC. Intakes of vitamin C in the middle and highest tertile were found to significantly increase the risk of developing HCC in smokers, whereas its effect in non-smokers was not significant.

Several studies have found that vitamin C inhibits experimental hepatocarcinogenesis. Administering 5% sodium ascorbate in the diet inhibited liver tumors induced by *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine, 0.2% *N*-bis(2-hydroxypropyl)-nitrosamine and 0.2% *N*-ethyl-*N*-hydroxyethylnitrosamine in rats [138]. Vitamin C was also found to inhibit the development of liver tumors induced by DEN [139] or DEN/AAF [140]. The lipophilic ascorbic acid and 2-*O*-octadecylascorbic acid (CV-3611) inhibited the development of spontaneous tumors in male C3H/HeNCrj mice [140]; both ascorbic acid and CV-3611 inhibited the induction of altered hepatic foci by a choline-deficient diet in rats [141]. Hemicalcium ascorbate, CV-3611, and ascorbyl palmitate were all found to inhibit the induction of HCCs by 3′-methyl-4-dimethylaminoazobenzene in ODS rats (a strain that cannot synthesize ascorbic acid) [142].

However, other studies have observed that vitamin C does not affect or actually enhances hepatocarcinogenesis. Dietary vitamin C did not affect the induction of liver tumors by s.c. nitrosamine [143] or p.o. AFB_1 [144] injections in Wistar rats. Sodium ascorbate also did not affect the development of PGST-positive foci induced by DEN [145]. In rats administered 0.05% *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine, feeding 5% sodium ascorbate did not affect the development of altered hepatic foci [146]. In rats receiving kojic acid as a tumor promoting agent, Takabatake *et al.* [147] found that 5000 ppm ascorbic acid in the drinking water increased the induction of altered hepatic foci.

Overall, the studies on vitamin C also do not support a clear chemopreventive role. Only one epidemiological study, a prospective study, has been performed; it observed that consuming higher amounts of vitamin C led to a higher risk of developing HCCs in smokers, with no effect in non-smokers. In experimental animal studies, a number of different models have observed that supplemental vitamin C or lipophilic derivatives of vitamin C inhibited carcinogenesis. But other studies, some of which used similar models, observed no effect or an actual enhancement of carcinogenesis in animals administered higher amounts of vitamin C.

5 Antioxidant phytochemicals

Other major sources of antioxidants in the diet are non-nutritive phytochemicals. These agents are not essential nutrients but are found in high concentrations in some foods. Over 5000 phytochemicals have been identified, and include chemicals classified as carotenoids, phenolic acids, flavonoids, alkaloids, coumarins, and organosulfur compounds [148]. Several of these phytochemicals have been examined for their effects on carcinogenesis in the liver [149]. Many phytochemicals have antioxidant activity,

and several of these have been examined for their ability to influence hepatocarcinogenesis. Those phytochemicals with antioxidant activity are discussed below, as well as one synthetic antioxidant, *N*-acetyl cysteine (NAC), which is also taken as a dietary supplement. We have not discussed synthetic antioxidants used as food additives to prevent the oxidation of unsaturated fatty acids, such as butylated hydroxyanisole and butylated hydroxytoluene [150]. Of the antioxidant phytochemicals, β-carotene and epigallocatechin gallate (EGCG) (or tea extracts containing EGCG) had the most consistent preventive effects.

5.1 β-Carotene

The carotenoid β-carotene has been demonstrated to quench singlet oxygen and also scavenge peroxy radicals [151]. Several studies have examined the effect of β-carotene on liver carcinogenesis and most, but not all, observed a protective effect [152–167]. In experimental carcinogenesis studies in other tissues, most studies have observed a protective effect of β-carotene [168–170]. However, in the human α-Tocopherol, β-Carotene (ATBC) Trial and β-Carotene and Retinol Efficacy Trial (CARET), β-carotene was found to enhance the development of lung cancer in smokers [171–173]. In the ATBC Trial, participants received α-tocopherol and/or β-carotene for an average of 5.5 years; in the CARET, participants received β-carotene and vitamin A for an average of 4.5 years.

5.2 Ellagic acid

Ellagic acid is a naturally occurring plant phenol that has strong scavenging ability for hydrogen peroxide, superoxide anion, and hydroxy anion *in vitro* [174, 175]. Tharappel *et al.* [167] found that ellagic acid increased the number of PGST-positive foci, but decreased their mean volume in rats treated with DEN and PCB-77. Ellagic acid was similarly found to increase the number of PGST-positive foci in a multi-organ carcinogenesis model [176]. Ellagic acid, however, was found to inhibit AAF-induced liver tumors [177]. Ellagic acid additionally has been found to be anti-carcinogenic in other tissues [178–180].

5.3 Curcumin

Curcumin, derived from the spice turmeric, has antioxidant activity against free radicals, and also increases the activity of antioxidant enzymes [181]. In previous studies in the liver, curcumin has been found to inhibit the induction of altered hepatic foci by DEN or by DEN/AAF [182, 183]. In rats treated with DEN and PCB-77, curcumin did not affect the number of foci induced, but significantly decreased their mean volume [167]. However, curcumin was found to enhance the promotion of altered hepatic foci by 2-amino-3,4-dimethylimidazo[4,5-f]quinoline [184]. Dietary curcumin did not affect the incidence of spontaneous liver tumors in Long-Evans Cinnamon rats [185], which could be related to an elevation in lipid-peroxidation-related DNA adducts in curcumin-fed rats [186]. Curcumin was found to prevent colon carcinogenesis in several studies [187–189]. Most other carcinogenesis studies in other tissues have also observed an inhibition in tumor induction after curcumin feeding [181, 190].

5.4 Lycopene

Lycopene is one of the major carotenoid antioxidants in tomatoes and is known to have a significant anticancer effect with its single oxygen quenching ability [191, 192]. Studies that have examined the role of lycopene in hepatocarcinogenesis saw mixed results. Astorg and colleagues found that lycopene decreased the initiating activity of DEN but not that of 2-nitropropane or AFB_1 in rats [161, 193]. Dietary lycopene was found to decrease the incidence and multiplicity of spontaneous liver tumors in C3H mice [159], but did not significantly affect the incidence of spontaneous liver tumors in Long-Evans Cinnamon rats [194]. Tharappel *et al.* [167] found that lycopene decreased the number of PGST-positive foci induced by DEN/PCB-77 but slightly increased their volume. Breinholt *et al.* [195], however, found that lycopene induced a low level of PGST-positive foci in rats. In other tissues, most studies have observed a chemopreventive effect of lycopene [196].

5.5 Coenzyme Q_{10}

Coenzyme Q_{10} is a lipophilic–redox-active substance found in hydrophobic area of the phospholipid bilayer of virtually all biological membranes [197]. The quinol form of coenzyme Q_{10} is a potent antioxidant in the inner mitochondrial membrane. It inhibits lipid peroxidation by either scavenging free radicals or reducing α-tocopheryl radical [198–200]. In the only study that has examined the effect of coenzyme Q_{10} on liver carcinogenesis, co-enzyme Q_{10} decreased the mean volume of PGST-positive foci in DEN/PCB-77-treated rats but did not affect the number induced [167]. In studies in other tissues, coenzyme Q_{10} was found to reduce the volume of dimethylbenzanthracene-induced mammary tumors [201], the number of colon tumors induced by dimethylhydrazine [202], and the number of aberrant crypt foci induced by azoxymethane [203].

5.6 EGCG

EGCG and other polyphenols present in green tea have antioxidant activity and also prevent oxidation by chelating metal ions such as iron or copper [204]. EGCG has also been

found to modulate signal transduction pathways that inhibit cell proliferation and increase apoptosis [205]. Most studies have found tea extracts and EGCG to be inhibitory in liver carcinogenesis models. Both green and black tea were found to inhibit the induction of hepatic tumors by DEN in C3H mice, but no association between EGCG content and chemopreventive effect was observed [206]. Green tea was found to prevent the promotion of hepatic tumors by pentachlorophenol in mice [207]. Green tea catechins were found to inhibit the promotion of altered hepatic foci by 2-amino-3,4-dimethylimidazo[4,5-f]quinoline or 2-amino-6-methyldipyrido[1,2-a:3′,2′-d]imidazole (Glu-P-1) in rats [156, 184]. In addition, Mao [208] found that an epicatechin complex inhibited the initiation of hepatocarcinogenesis by DEN. Purified EGCG as well as other purified catechins and tea extracts were found to inhibit the induction of PGST-positive foci by DEN and PB [209]. However, green tea catechins increased the induction of PGST-positive foci using a multi-organ rat carcinogenesis model [210]. A tea extract did not affect the promotion of PGST-positive foci by PCB-77 [167]. A green tea extract was found to inhibit the number but not the size of DEN-induced tumors in rats but did not affect tumors induced by choline deficiency [211]. In other tissues, EGCG as well as tea or tea extracts generally were found to inhibit carcinogenesis [212].

5.7 NAC

NAC is a precursor of glutathione and increases glutathione levels; it also can scavenge free radicals itself [213, 214]. NAC has also been shown to modulate transcriptional activities through pathways involving c-fos/c-jun, NF-κB, and cyclin inhibitors [214]. In liver carcinogenesis models, NAC inhibited the induction of PGST-positive foci by 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline in rats but did not affect the induction of liver tumors in rats treated with DEN and diethyldithiocarbamate [215, 216] or DEN and PCB-77 [167]. Several investigators have found that NAC prevents the induction of DNA damage and progression to cancer in smokers [217], and blocks tumor growth and angiogenesis in nude mice [218].

5.8 Resveratrol

Resveratrol is found in grapes and other plants and is a phytoalexin (antifungal agent) with strong antioxidant activity [219]; it is also a competitive inhibitor of Ah receptor ligands and an inhibitor of transcription of cytochrome P-450 1A1 and other phase 1 enzymes *in vivo* [220, 221]. Resveratrol did not affect the induction of PGST-positive foci by DEN/PCB-77 in rats [167]. Resveratrol, however, was found to decrease the growth of a transplantable hepatomas [222, 223]. In other tissues, resveratrol inhibited chemically induced carcinogenesis in the skin, mammary gland, and colon, but had no effect in the lung [224].

6 Summary and conclusions

In summary, there are limited epidemiological data linking increased or adequate dietary vitamin E with liver cancer prevention. Although limited in number, intervention studies using supplemental vitamin E also do not indicate any overall benefit for liver cancer prevention. However, there is some evidence that supplemental vitamin E in individuals with pre-existing liver conditions (*e.g.* hepatitis and HCC) may have some benefits. The most convincing anti-cancer data for vitamin E come from animal studies. As indicated in this review, many animal studies have observed a reduction in hepatocarcinogenic indicators with increased dietary vitamin E, although other studies have reported increased altered hepatic foci with some levels of dietary vitamin E. One explanation for the inconclusive benefit to supplemental vitamin E may be that antioxidant requirements may be individualized. For example, antioxidants may be beneficial for someone with greater production of reactive oxygen species; however, for individuals with low levels of reactive oxygen species, antioxidants could be harmful by possibly decreasing apoptosis [225]. Thus, at this point, there is inconclusive evidence to recommend vitamin E supplements for liver cancer prevention, but more randomized, placebo-controlled intervention trials are necessary to definitively know the relationship between vitamin E and liver cancer prevention.

Studies examining the effect of dietary Se on human liver cancer or experimental hepatocarcinogenesis have shown contradictory results. Several studies have suggested that Se supplementation could inhibit the development of liver cancer in humans, but other studies do not support this. In experimental studies, a wide variety of models have been used. Again, it has been difficult to observe consistent effects. Increasing dietary Se leads to an increase in hepatic GPx activity; however, this did not lead to expected decreases in oxidative stress, such as lipid peroxidation or oxidative DNA damage. It is possible that mechanisms other than GPx and TrxR activities and these enzymes' anti-oxidant properties are mediating the cancer protective effects of Se.

Fewer studies have examined vitamin C. The only published epidemiological study found that consuming higher amounts of vitamin C may enhance the development of liver cancer. Experimental animal studies are inconsistent and most of the animal studies have used species in which vitamin C is not an essential nutrient.

Several studies have examined the effect of antioxidant phytochemicals on experimental hepatocarcinogenesis. Again, most of the effects observed are inconsistent between studies. The phytochemicals showing the most consistent inhibitory effects are β-carotene, and tea extracts or EGCG.

Overall, it is difficult to recommend the consumption of higher amounts of antioxidants to prevent liver cancer. Neither the human nor the animal studies provide compelling evidence that consuming higher amounts of the antioxidants studied would decrease one's probability of developing HCC. However, much remains to be determined about these agents' molecular mechanisms, including their effects on genes that are known to be overexpressed or mutated in hepatocarcinogenesis, and subsequent studies may provide a clearer picture as to whether any of these antioxidants have the capability to prevent liver cancer. These future studies should focus on molecular aspects of the antioxidant agents. Animal and cell culture models in which oncogenes are overexpressed and/or mutated or tumor suppressor genes are deleted will provide important information about antioxidant actions. Transgenic and knockout models that target the metabolism of the antioxidants will also be important in determining the role that specific antioxidants play in the prevention of hepatocarcinogenesis.

The authors have declared no conflict of interest.

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