Forum Review

Evaluation for Safety of Antioxidant Chemopreventive Agents

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ABSTRACT

Antioxidants are considered as the most promising chemopreventive agents against various human cancers. However, some antioxidants play paradoxical roles, acting as "double-edged sword." A primary property of effective and acceptable chemopreventive agents should be freedom from toxic effects in healthy population. Miscarriage of the intervention by β-carotene made us realize the necessity for evaluation of safety before recommending use of antioxidant supplements for chemoprevention. We have evaluated the safety of antioxidants on the basis of reactivity with DNA. Our results revealed that phytic acid, luteolin, and retinoic acid did not cause DNA damage under the experimental condition. Furthermore, phytic acid inhibited the formation of 8-oxo-7,8-dihydro-2′-deoxyguanosine, an indicator of oxidative DNA damage, in cultured cells treated with a H_2O_2-generating system. Thus, it is expected that these chemopreventive agents can safely protect humans against cancer. On the other hand, some chemopreventive agents with prooxidant properties (α-tocopherol, quercetin, catechins, isothiocyanates, *N*-acetylcysteine) caused DNA damage via generation of reactive oxygen species in the presence of metal ions and endogenous reductants under some circumstances. Furthermore, other chemopreventive agents (β-carotene, genistein, daidzein, propyl gallate, curcumin) exerted prooxidant properties after metabolic activation. Therefore, further studies on safety should be required when antioxidants are used for cancer prevention. *Antioxid. Redox Signal.* 7, 1728–1739.

INTRODUCTION

CHEMOPREVENTION IS DEFINED as reduction of the risk of cancer development through the use of pharmaceuticals or micronutrients (33). Cancer preventive strategies are attractive from the viewpoint of public health. Many studies have addressed the role of antioxidant micronutrients in vegetables and fruits in protection against cancers (20). More than two-thirds of human cancers could be prevented through appropriate lifestyle modification. For example, consumption of fruits and vegetables is associated with a reduced risk of developing cancer (99). Numerous phytochemicals derived from edible plants have been reported to interfere with a specific stage of the carcinogenic process (99). Development of dietary compounds as potential cancer chemopreventive agents is highly desirable, because of their safety, low toxicity, and general acceptance as dietary supplements (9).

Phytochemical preparations are marketed as herbal medicines or dietary supplements for a variety of alleged nontoxic therapeutic effects. However, they have yet to pass controlled clinical trials for efficacy, and their potential for toxicity is an understudied field of research (22). Dietary supplementation of publicly available foods and the ingestion of specific supplements are usually applied for prolonged periods of time. Therefore, it is essential that the strategy is devoid of risks (103). The Alpha-Tocopherol Beta Carotene Cancer Prevention Study Group (1) and the Beta-Carotene and Retinol Efficacy Trial (76) supplied β-carotene and/or vitamin A to smokers and asbestos-exposed workers, who were high-risk groups for lung cancer. After follow-up for several years, higher incidences of

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lung cancer were observed in the intervention groups than the placebo groups. We have first demonstrated that β-carotene metabolites have prooxidant properties by the observation of reactivity with DNA (59). Therefore, we deeply realize the necessity for evaluation of safety before recommending use of antioxidant supplements for chemoprevention.

We have evaluated the safety of antioxidants on the basis of reactivity with DNA. Antioxidant chemopreventive agents without prooxidant properties inhibit the carcinogenic process because of chelating metals, scavenging reactive oxygen species (ROS) or interfering with a specific manner (Fig. 1A). On the other hand, some chemopreventive agents exert prooxidant properties (Fig. 1B), and some agents exert prooxidant properties after metabolic activation (Fig. 1C). Details are noted below.

MATERIALS AND METHODS

We have examined the reactivity of antioxidants with DNA in application to the screening of carcinogenicity for evaluation of safety. Primary procedures are as follows.

Preparation of ^{32}P-5'-end-labeled DNA fragments and detection of DNA damage

Exon-containing DNA fragments obtained from the human tumor-relative genes such as *p53* and *p16* tumor suppressor genes and c-Ha-*ras-1* protooncogene (8, 11, 88). A 5'-end-labeled DNA fragment was obtained by phosphorylation with [γ-^{32}P]ATP and T$_4$ polynucleotide kinase (111). The fragment was further digested with restriction enzyme to obtain a singly labeled fragment. A standard reaction mixture (in a 1.5-ml microtube) contained the test chemicals, metal ions and other endogenous compounds, ^{32}P-5'-end-labeled DNA fragment, and calf thymus DNA in sodium phosphate buffer. After incubation at 37°C, the DNA fragments were treated with piperidine or formamidopyrimidine-DNA glycosylase (Fpg) protein. Then, DNA was electrophoresed on an 8% polyacrylamide/8 *M* urea gel. The autoradiogram was obtained by exposing an x-ray film to the gel. The preferred cleavage sites were determined by direct comparison of the cleaved oligonucleotides with a standard Maxam-Gilbert sequencing reaction (55). The relative amounts of oligonucleotides from the treated DNA fragments were measured with a laser densitometer.

Measurement of 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) in cellular and isolated DNA

Human cultured cells were treated with test chemicals. Then, cells were washed three times with cold phosphate-buffered saline. Under anaerobic conditions, DNA was extracted using lysis buffer, RNase A, and proteinase K (61). For using isolated DNA, calf thymus DNA was incubated with test chemicals, metal ions, and other endogenous compounds at 37°C. After ethanol precipitation, DNA was digested to the nucleosides with nuclease P$_1$ and alkaline phosphatase, and then analyzed by high-performance liquid chromatography coupled with electrochemical detector (37).
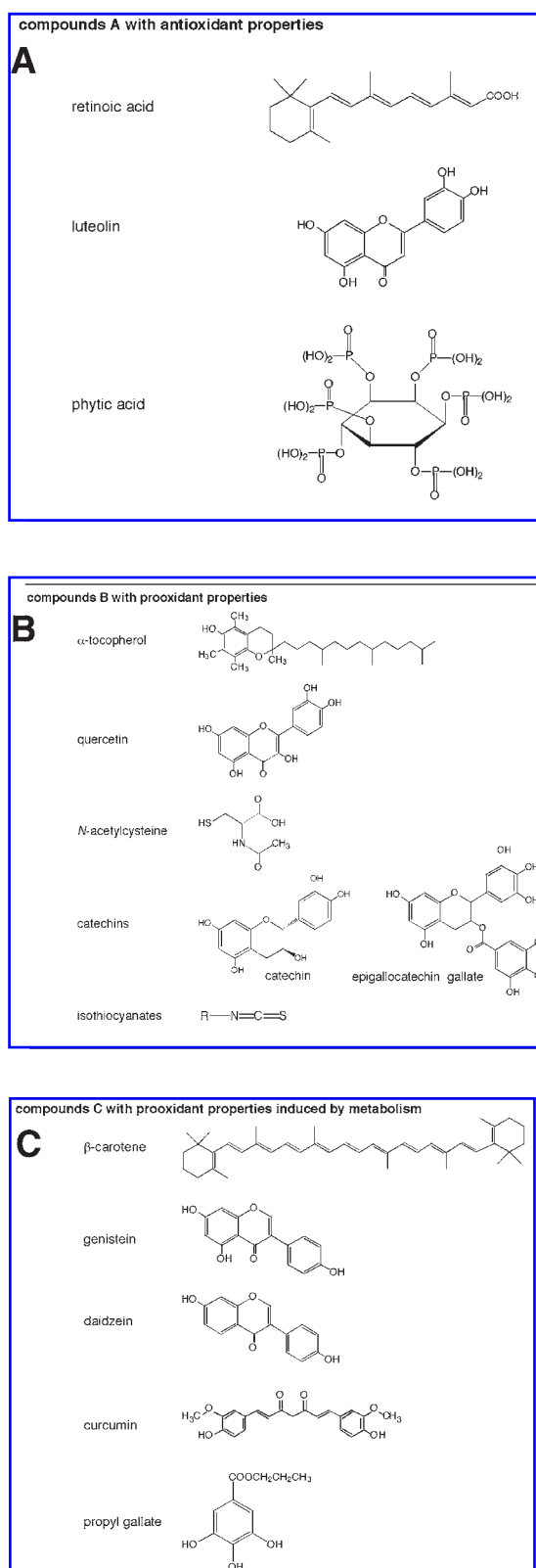

FIG. 1. Chemopreventive agents with antioxidant and prooxidant properties: compounds with antioxidant properties (A), compounds with prooxidant properties (B), and compounds with prooxidant properties induced by metabolism (C).

EVALUATION OF
ANTIOXIDANTS FOR SAFETY

Vitamins A and E

β-Carotene and vitamin A.   Many epidemiological studies showed that vitamin A intake decreased incidence of cancers of lung, bladder, upper gastrointestinal tract, and breast, as reviewed by Willett and MacMahon (110). It has been expected that the antioxidant potency of vitamin A and β-carotene may protect against cancer occurrence (24). However, intervention trials with β-carotene failed (1, 76). Regardless of the miscarriage of the intervention, attempts to use retinoids and carotenoids for cancer chemoprevention and therapy are ongoing (10, 63, 85). Therefore, the causal mechanisms should be elucidated to establish safe approaches in cancer chemoprevention. We examined the reactivity of β-carotene and its metabolites with DNA. It is known that β-carotene is metabolically converted to two molecules of retinal principally by central cleavage. Then, retinal is further oxidized to retinoic acid or reduced to retinol (Fig. 2). We determined that both retinol and retinal caused oxidative damage to cellular and isolated DNA (59). Retinoids significantly induced 8-oxodG formation in HL-60 cells, but did not significantly increase 8-oxodG in H_2O_2-resistant HP100 cells. Electron spin resonance spectroscopic studies using a trapping agent α-(4-pyridyl1-oxide)-*N-tert*-butylnitrone have demonstrated retinol- and retinal-derived radicals with six-line signals assigned as carbon-centered radicals. Using the cytochrome *c* reduction method, generation of superoxide (O_2^-) derived from the autooxidation of retinoids was detected. The generation of O_2^- was significantly correlated with 8-oxodG formation. We confirmed using isolated DNA that retinol and retinal induced DNA damage including 8-oxodG in the presence of Cu(II), whereas retinoic acid and β-carotene induced no or little DNA damage. Both retinol and retinal play important roles in carcinogenesis in the intervention studies using excess amounts of β-carotene. On the other hand, retinoic acid has no prooxidant property (59) but has the potential of regulating cell differentiation (28). A recent intervention study

FIG. 2. Metabolic conversion of β-carotene and the potential of DNA damage by β-carotene and its metabolites.

suggests 9-*cis*-retinoic acid has potential chemopreventive properties in former smokers (47).

Vitamin E.   α-Tocopherol is widely recognized as being the most important biological antioxidant of the lipid phase (35). There is considerable interest in the possibility that vitamin E (α-tocopherol) may be protective against cancer and cardiovascular diseases (106). On the other hand, several studies have demonstrated that the vitamin can act as a carcinogen, at both the initiation and promotion stages (58, 67). We have indicated that α-tocopherol in the presence of Cu(II) can induce extensive DNA damage, including base modification and strand breakage (113). The predominant DNA cleavage sites were thymine and cytosine residues. Inhibitory effects of catalase and bathocuproine on DNA damage suggest that H_2O_2 and Cu(I) are required for the DNA damage. An electron spin resonance spin-trapping study showed the formation of hydroxyl radical (·OH) generated from α-tocopherol and Cu(II). This suggests that ·OH, generated via the reaction of Cu(I) with H_2O_2, is responsible for the induction of DNA damage. Cu(II) ions are known to bind tightly to DNA, where they can interact with reducing agents (ascorbic acid, glutathione, phenolics, or NADH) and H_2O_2, resulting in oxidative damage to the nucleic acid, including base modification and strand breakage (7, 41). Our experiments have shown that α-tocopherol incorporated into liposomes is able to induce DNA damage in the presence of Cu(II). It is reasonably considered that vitamin E may have not only anticarcinogenic effects but also carcinogenic potentials.

Polyphenols

Isoflavones.   Epidemiological and experimental studies have shown that soy products can reduce the risk of cancer and provide other benefits, including lowering cholesterol and blood pressure and preventing cardiovascular diseases and osteoporosis (40, 44, 48, 65, 84, 94). The soy isoflavones, genistein (5,7,4′-trihydroxyisoflavone) and daidzein (7,4′-dihydroxyisoflavone), are representative phytoestrogens (95), and act as chemopreventive agents against cancers, cardiovascular disease, and osteoporosis. Because of these health benefits, the consumption of soy food and the use of isoflavone supplements have been increasing (56). However, recent studies revealed that genistein and/or daidzein induced cancers of reproductive organs in rodents, such as the uterus (66) and vulva (105). These reports led us to consider that soy isoflavones may have a carcinogenic effect on female reproductive organs. We showed that genistein and daidzein exerted cell proliferative activity on estrogen-sensitive MCF-7 cells (62), as reported previously, while their metabolites had little or no activity. In accordance with the data on cell proliferation, the surface plasmon resonance sensor showed that genistein and daidzein induced higher affinity binding of estrogen receptor (ER) to estrogen response element (ERE), while the metabolites had little or no binding activity. Isoflavones such as genistein and daidzein may induce cell proliferation through ER-ERE binding. On the other hand, the isoflavone metabolites orobol (5,7,3′,4′-tetrahydroxyisoflavone)   and   7,3′,4′-trihydroxy-isoflavone significantly induced 8-oxodG formation in MCF-
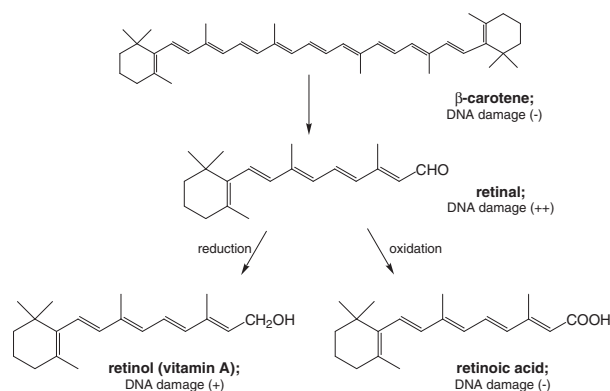
10A cells (Fig. 3). Interestingly, Fpg treatment revealed that orobol induced significant cleavage of the guanine residue of the ACG sequence complementary to codon 273, a well-known hotspot (49) in the *p53* gene (Fig. 4A). Piperidine treatment cleaved cytosine and guanine residues at the ACG (Fig. 4B). Similar results were obtained with 7,3′,4′-trihydroxy-isoflavone. Oxidative DNA damage by isoflavone metabolites plays a role in tumor initiation, and cell proliferation by isoflavones via ER-ERE binding induces tumor promotion and/or progression, resulting in cancer of estrogen-sensitive organs. Our study raises the possibility that genistein and daidzein are carcinogenic in estrogen-sensitive organs, even though isoflavones are generally regarded as chemopreventive agents.

Quercetin and luteolin. Flavonoids, particularly flavonol and flavone, are commonly found in many vegetables and herbs. Quercetin is the most widely distributed flavonol, and luteolin is one of the most widely distributed flavones in the plant kingdom. Onions are rich in quercetin, which has perceived benefits to human health, including anticarcinogenic properties (26). On the other hand, quercetin has been reported to be carcinogenic (13, 70, 77). Considerable evidence suggests that quercetin has prooxidant activity (6, 86), and DNA-damaging ability in cultured cells (18, 23). Previously, we showed that quercetin induced DNA damage by prooxidative effects, but luteolin did not, in a simplified *in vitro* model (114). Some flavonoids, including quercetin and luteolin, are topoisomerase II (topo II) inhibitors and act as anti-tumor agents (4, 115). Topo II inhibitors can induce apoptosis by means of DNA-replication-associated damage involving formation of a stable drug–topo II–DNA ternary complex, called a cleavable complex (64). We showed that both quercetin and luteolin induced DNA cleavage to 1–2-Mb and less than 200-kb DNA fragments, followed by DNA ladder formation in
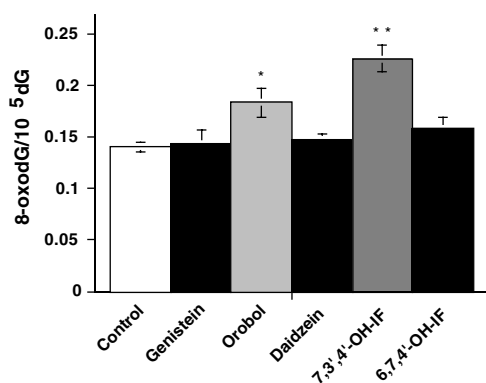

FIG. 3. Intracellular 8-oxodG formation by isoflavone metabolites in MCF-10A cells. Human mammary epithelial MCF-10A cells were treated with 10 μ*M* isoflavones or their metabolites in the experimental medium at 37°C for 1 h. Results are expressed as means ± SE of values obtained from three independent experiments. OH-IF, trihydroxyisoflavone. *$p < 0.05$, **$p < 0.01$, significantly different compared with the control by Student's *t* test.
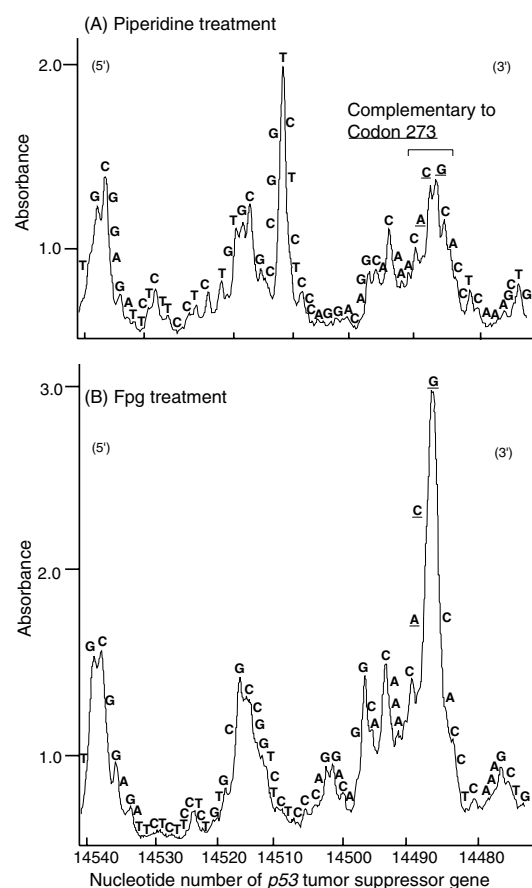

FIG. 4. Site specificity of DNA cleavage induced by isoflavone metabolites in the presence of Cu(II) and NADH. The reaction mixture contained the ^{32}P-5′-end-labeled 443-bp DNA fragment (*Apa*I 14,179–*Eco*RI 14,621*), 20 μ*M* per base of calf thymus DNA, 5 μ*M* orobol, 20 μ*M* CuCl$_2$, and 200 μ*M* NADH in 10 m*M* phosphate buffer (pH 7.8) (*^{32}P label). After incubation for 1 h at 37°C, the DNA fragments were treated with piperidine (**A**) or Fpg protein (**B**) and electrophoresed by the Maxam-Gilbert sequencing reaction (55). The relative amounts of DNA fragments were measured by scanning the autoradiogram with a laser densitometer. The horizontal axis shows the nucleotide number of the human *p53* tumor suppressor gene, and underscoring shows the complementary sequence to codon 273 (nucleotide numbers 14,486–14,488).

HL-60 cells (112). The significant increase in 8-oxodG formation in HL-60 cells and no increase in their H$_2$O$_2$-resistant clone HP 100 cells were observed after treatment with quercetin. It indicated that quercetin induced oxidative DNA damage and that H$_2$O$_2$ was the main mediator of such damage. Luteolin inhibited topo II activity more strongly than quercetin in crude nuclear extract. Inhibition of topo II activity by luteolin was associated with DNA cleavage by cleavable complex formation. Luteolin-induced DNA cleavage and DNA ladder formation in HP 100 cells were similar to those in HL-60 cells. These results suggest that H$_2$O$_2$-mediated DNA damage is the

main pathway in quercetin-induced apoptosis, and topo II-mediated DNA cleavage is that in luteolin-induced apoptosis. Several papers indicated that luteolin does not have mutagenicity and carcinogenicity (13, 70, 77). Luteolin can be expected as a comparatively safe chemopreventive agent.

Catechins. Catechins, including catechin, epicatechin, and epigallocatechin gallate (EGCG), are a class of flavonoids with potent antioxidant and cancer chemopreventive properties. Catechins are believed to be an active constituent of green tea. Several epidemiological studies suggest that green tea consumption is associated with a reduced risk of several forms of cancer in human populations (34, 46). On the other hand, several human cohort and case-control studies have indicated significant positive relationships between green tea consumption and cancers of various organs (50, 102, 116). In addition, green tea catechins have been reported to enhance colon carcinogenesis in rats (31). We showed that the content of 8-oxodG of DNA in HL-60 cells treated with 1 mM catechin was significantly increased in comparison to untreated cells, whereas catechin did not cause a significant increase in the amount of 8-oxodG in HP100 cells (74). Addition of bathocuproine significantly decreased the amounts of 8-oxodG induced by 1 mM catechin, suggesting the possible role of endogenous cellular copper in the activation of catechin to a DNA damaging species. Epicatechin appears to be more potent than catechin in the induction of 8-oxodG in cells. To clarify underlying mechanisms, we examined DNA damage by catechins by using isolated DNA. In the presence of Cu(II), the amount of 8-oxodG increased with increasing concentration of catechins. The addition of 100 μM NADH enhanced Cu(II)-mediated 8-oxodG formation induced by catechin and epicatechin. NADH is a reductant existing at high concentrations (100–200 μM) in cells (53). Amounts of 8-oxodG formed by epicatechin were larger than those by catechin in the presence of NADH. The calculation has indicated that both the highest occupied molecular orbital of these catechins and the lowest unoccupied molecular orbital of their corresponding quinones are localized on their B rings. The calculated highest occupied molecular orbital energy of epicatechin (8.08 eV) is lower than that of catechin (8.12 eV), suggesting that epicatechin is more easily oxidized than catechin. The lowest unoccupied molecular orbital of the quinone form of epicatechin is larger (-0.65 eV) than that of the quinone form of catechin (-0.67 eV), suggesting that the quinone form of epicatechin is easily reduced by NADH than that of catechin. These differences between catechin and epicatechin can be explained by a steric effect of the OH group at the 3-position of their C rings.

Catechins, especially EGCG, can ameliorate free radical damage to DNA, under certain conditions (2, 3). To assess the safety, we compared the intensity of DNA damage in the presence of metal ions by four catechins: catechin, epigallocatechin (EGC), epicatechin gallate (ECG), and EGCG (21). DNA fragments treated with various concentrations of catechins in the presence of metal ions were detected by autoradiography. Oligonucleotides were detected as a result of DNA damage. In the presence of an Fe(III) complex such as Fe(III)EDTA and Fe(III)ADP, EGC and EGCG induced DNA

damage. In addition, ECG also caused mild DNA damage. Catechin induced only slight DNA damage. The order of DNA damaging ability was EGCG ≈ EGC > ECG >> catechin. In the presence of Cu(II), all four catechins induced DNA damage; the order of DNA damaging ability was EGC > catechin > EGCG > ECG. Piperidine treatment revealed that catechins induced not only direct breakage of the deoxyribose phosphate backbone but also base modification in the presence of metal ions.

At the present time, human trials for green tea catechins are already in progress some countries (82, 109). Catechins may have the dual function of carcinogenic and anticarcinogenic potentials. These findings require further studies on safety and risk assessment of catechins.

Propyl gallate (PG). PG is widely used as an important synthetic antioxidant in the food industry. In contrast, the National Toxicology Program (68) reported that PG induced preputial gland tumors, islet-cell tumors of the pancreas, and pheochromocytomas of the adrenal glands in male rats. PG also induced malignant lymphoma in male mice. Accumulation of PG may contribute to carcinogenesis. However, the mechanism leading to carcinogenesis has not yet been clarified. We demonstrated that PG significantly increased 8-oxodG formation in HL-60 cells (45). However, PG itself did not increase the level of 8-oxodG in isolated calf thymus DNA in the presence of metal ions. PG is hydrolyzed enzymatically to gallic acid (GA) by cellular carboxylesterase. We demonstrated that GA increased the amounts of 8-oxodG in the presence of Cu(II), Fe(III)EDTA, and Fe(III)ADP. From these results, it is considered that GA, produced from PG by esterase, may be involved in oxidative DNA damage in cultured human cells. High-performance liquid chromatography analysis of the products generated from PG incubated with esterase revealed that PG converted into GA. On the basis of these results, the possible mechanisms of metal-mediated DNA damage induced by GA, a metabolite of PG, are proposed as follows: Metal-mediated autoxidation of GA generates the semiquinone radical. In the presence of metal ion (M^n), H$_2$O$_2$ is generated by O$_2^-$ dismutation with concomitant reduction of M^n to M^{n-1}. In the presence of Cu(II), GA induces DNA damage by the interaction of Cu(I) and H$_2$O$_2$ to form a Cu(I)–hydroperoxo complex such as Cu(I)OOH. Fe(III)EDTA-mediated DNA damage resulting from exposure to GA is caused by ·OH generated from the Fenton reaction. The ·OH is extremely short-lived and travels a very short distance in water (72, 101). This can be one of the reasons that Cu(II)-mediated DNA damage caused by GA is stronger than Fe(III)EDTA-mediated damage, although autoxidation of GA mediated by Fe(III)EDTA is faster than that by Cu(II). It is concluded that GA plays an important role in PG carcinogenicity.

Miscellaneous

Curcumin. Curcumin is the major yellow pigment in turmeric, curry, and mustard, and has also been widely used in cosmetics and drugs (17). A major source of human consumption of curcumin is turmeric, which is used as a coloring agent

and spice in many foods (27). Studies on the chemopreventive efficacy of curcumin have shown that it possesses both anti-initiating and antipromoting activities in several experimental systems (17). Animal studies have demonstrated that curcumin inhibits carcinogenesis in various tissues, including skin (32), colorectal (83), oral (100), forestomach (92), and mammary (93) cancers. In contrast, the National Toxicology Program study (71) showed that dietary administration of turmeric oleoresin with a high curcumin content (79–85%) induced clitoral gland adenomas in female rats. There is also evidence for carcinogenic activity of the turmeric oleoresin in mice based on an increased incidence of hepatocellular adenoma. We demonstrated the prooxidant property of curcumin with metabolic activation by CYP enzymes (CYP 2D6, 1A1, 1A2, 2E1) (87). Figure 5 shows mechanisms of curcumin-induced anticancer and carcinogenic effects. In a metabolic pathway, curcumin is converted by hydrogenation to tetrahydrocurcumin, the more promising chemopreventive agent (75). In contrast, curcumin undergoes *O*-demethylation catalyzed by certain CYPs to *O*-demethyl curcumin (36). *O*-Demethyl curcumin is then autoxidized into the *O*-demethyl curcumin radical, leading to the production of the corresponding *o*-quinone form. Several studies indicate that NAD(P)H may non-enzymatically reduce *o*-quinones to catechols through two-electron reduction (29). The formation of the NAD(P)H-dependent redox cycle results in enhanced O_2^- generation, leading to enhancement of oxidative DNA damage. Thus, the NADH-dependent redox cycle of *O*-demethyl curcumin may continuously generate ROS and mediate oxidative DNA damage. The anticarcinogenic effect of curcumin is associated with its influence on metabolizing enzymes (96, 104). High CYP activity may yield much *O*-demethyl curcumin, leading to a carcinogenic effect.

Isothiocyanates (ITCs). Organic ITCs (R-N=C=S), also known as mustard oils, are widely distributed in plants, many of which are consumed by humans. Vegetables belonging to the family Cruciferae and genus *Brassica* (*e.g.,* broccoli and cauliflower) contain substantial quantities of ITCs, mostly in the form of their glucosinolate precursors (117). Extracts of broccoli sprouts were effective in reducing tumors in carcinogen-treated rats (19). The National Toxicology Program has evaluated that allyl ITC (AITC) is carcinogenic to rats (69). It has been reported that benzyl ITC (BITC) and phenethyl ITC (PEITC) exhibit promotion potential during the post-initiation stage (30, 51). Figure 6A shows carcinogenic potential and the intensity of DNA damage by ITCs in our system (60). The highly electrophilic central carbon atom of the -N=C=S group (117) should be hydrolyzed to give rise to the SH group. Figure 6B shows a proposed mechanism of DNA damage induced by ITCs (60). Autooxidation of the SH group is coupled with generation of O_2^- from O_2. The generation of H_2O_2 by O_2^- dismutation and O_2^--mediated reduction of Cu(II) to Cu(I) occur. The ability to yield an SH group from ITCs may depend on the
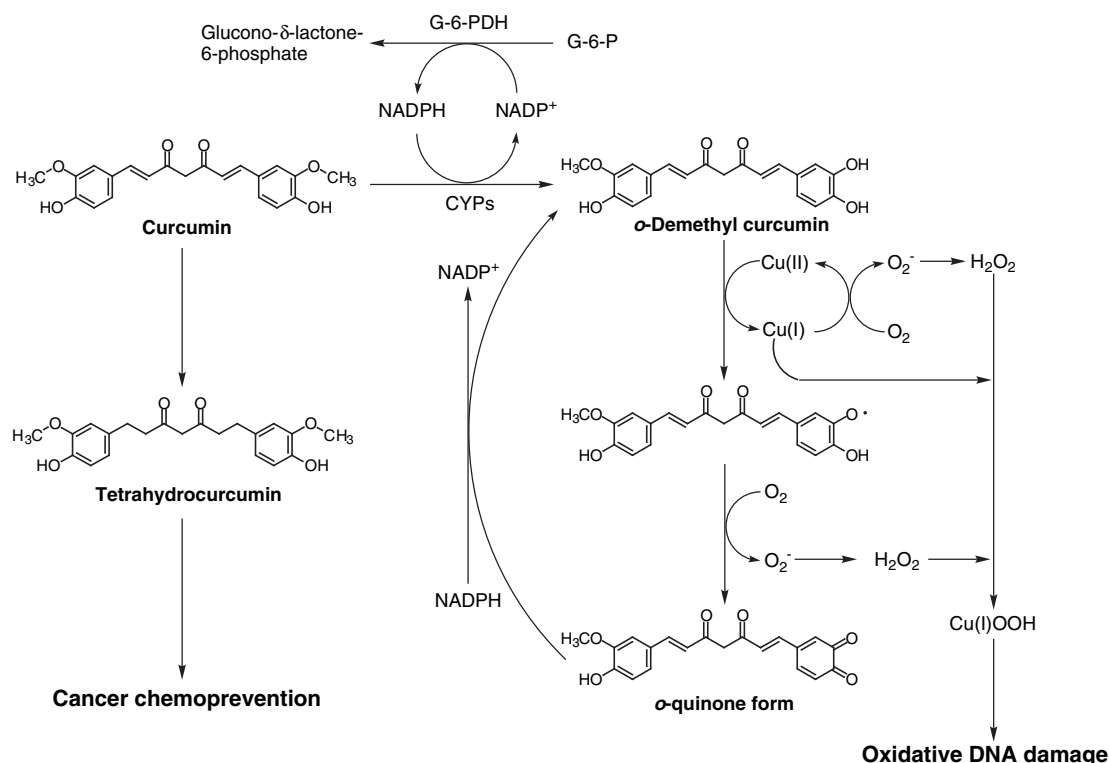

FIG. 5. Proposed mechanisms of curcumin-induced anti-cancer and carcinogenic effects. G-6-P, glucose 6-phosphate; G-6-PDH, glucose 6-phosphate dehydrogenase.
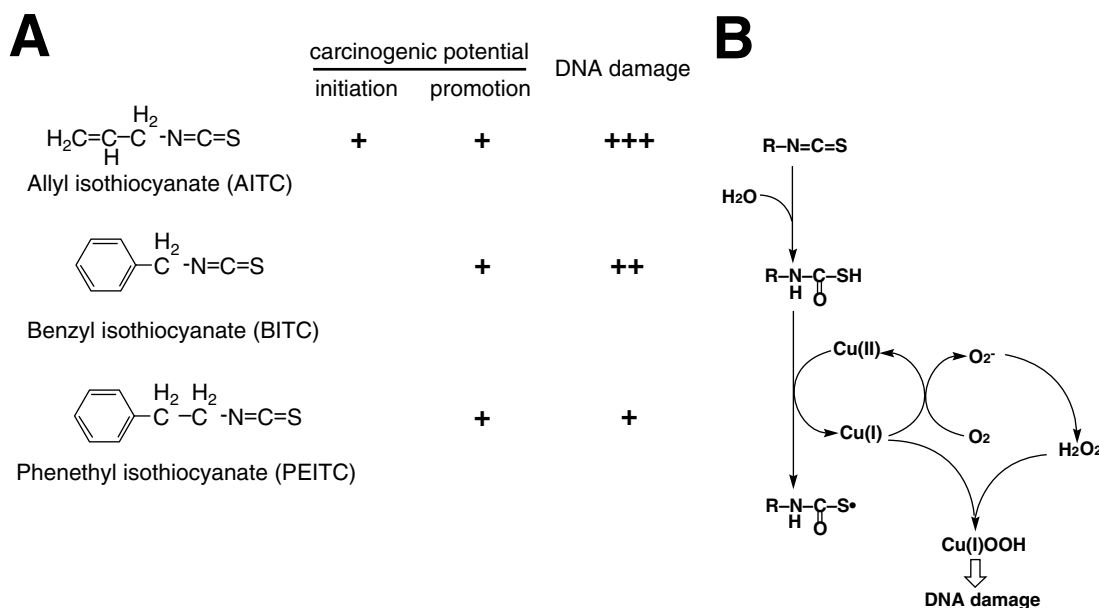
A

	carcinogenic potential		DNA damage
	initiation	promotion	

H$_2$C=C–C$^{H_2}_{H}$-N=C=S
Allyl isothiocyanate (AITC)   +   +   +++

C^{H_2}-N=C=S
Benzyl isothiocyanate (BITC)   +   ++

C^{H_2}–C^{H_2}-N=C=S
Phenethyl isothiocyanate (PEITC)   +   +

B

R–N=C=S

H$_2$O

R–N–C–SH
   H   O

Cu(II) ← → Cu(I)   O$_2^-$ → O$_2$ → H$_2$O$_2$

R–N–C–S•
   H   O

Cu(I)OOH

DNA damage

FIG. 6. Carcinogenic potential and DNA damage induced by ITCs (A and B).

length of the methylene chain, *i.e.,* ITCs with shorter methylene chains can yield greater amounts of the SH group, resulting in stronger DNA damage through O$_2^-$ generation. We demonstrated that the order of DNA damaging ability is AITC > BITC > PEITC. AITC is carcinogenic, while BITC and PEITC alone have tumor-promoting activities. ITCs with certain length of methylene chains may have anti-tumor activities alone.

N-Acetylcysteine. Cancer prevention by *N*-acetylcysteine has been shown to be effective in several animal experiments (5, 14). The oral administration of *N*-acetylcysteine completely prevented the induction of DNA alterations of various natures in rat lung cells (38). In addition, *N*-acetylcysteine prevented the *in vivo* formation of carcinogen–DNA adducts, and suppressed the development of tumors in rodents (14). Thus, numerous studies indicate that *N*-acetylcysteine can prevent mutation and cancer through a variety of mechanisms (15, 16). EUROSCAN, a randomized trial of a 2-year supplement of retinyl palmitate and/or *N*-acetylcysteine in patients with head and neck cancer or lung cancer, resulted in no benefit (107). Relevantly, Sprong *et al.* (97) have reported that low-dose *N*-acetylcysteine protects against endotoxin-mediated oxidative stress by scavenging H$_2$O$_2$, while higher doses may have the opposite effect. We demonstrated that the content of 8-oxodG in HL-60 cells was increased by the *N*-acetylcysteine treatment, but not in HP100 cells (73). Therefore, it is considered that generation of H$_2$O$_2$ plays an important role in *N*-acetylcysteine-induced 8-oxodG formation. Numerous studies have indicated that the formation of 8-oxodG causes misreplication of DNA that may lead to mutation or cancer (90). The formation of 8-oxodG in cellular DNA induced by *N*-acetylcysteine is noteworthy in relation to the report that 8-oxodG results in GT transversions. There is growing evidence

that compounds that are antioxidants at some concentrations become prooxidants at other concentrations.

Phytic acid. Phytic acid (*myo*-inositol hexaphosphoric acid) is present in plants, particularly in cereals, nuts, oil seed, legumes, pollen, and spores (25, 89). In mammalian cells, phytic acid and the lower inositol phosphates are present as intracellular molecules (98). It has been reported that phytic acid is chemopreventive in rodent colon and mammary carcinogenesis models, and in transplanted fibrosarcoma models (39, 91). Phytic acid has been used as an antioxidant and could conceivably be a protective agent in the human diet (78). Our previous study (57) revealed that phytic acid efficiently inhibited oxidative DNA damage. Phytic acid decreased the formation of 8-oxodG in cultured cells treated with the H$_2$O$_2$-generating system using glucose oxidase, whereas phytic acid did not influence the accumulation of H$_2$O$_2$. The formation of 8-oxodG in calf thymus DNA by H$_2$O$_2$ and Cu(II) was decreased by phytic acid. Experiments using ^{32}P-labeled isolated DNA demonstrated that phytic acid inhibited DNA damage by H$_2$O$_2$ and metals, although *myo*-inositol did not inhibit oxidative DNA damage. Phytic acid and its derivatives bind metal ions such as Cu(II), Zn(II), and Cd(II) *in vitro* (54). The present study has revealed that the molar ratio between Cu(II) and phytic acid on inhibition of oxidative DNA damage is about 3. This result is supported by the work of Vohra *et al.* (108), who found that the binding ratio of the moles of metal/moles of phytic acid was about 3.5 at pH 7.5. These findings have led us to consider that phytic acid containing six phosphates functions as a metal chelator to inhibit the generation of highly reactive species such as ·OH and Cu(I)–hydroperoxo complex from H$_2$O$_2$. Binding of metals to phytic acid could facilitate the elimination of potentially toxic heavy metals from the organism. Phytic acid is expected to effectively inhibit oxidative DNA damage

by chelating Cu(II) as well as Fe(II) and Fe(III). We have concluded that phytic acid may inhibit the generation of highly reactive species from H_2O_2 by chelating transition-metal ions, resulting in prevention against cancer.

CONCLUSION

We summarize the conception about carcinogenesis and chemoprevention on the basis of our results and literatures (Fig. 7). Most of the carcinogens yield ROS in the presence of endogenous substances such as metal ions, NADH, and photosensitizers (42, 43). In addition, infection and inflammation induce several cancers via nitrative DNA damage with generation of reactive nitrogen species (52, 79–81). Relevantly, chemoprevention with aspirin and other anti-inflammatory agents should only be considered established (33), *via* inhibition of cyclooxygenase-2 and inducible nitric oxide synthase (12). ROS and reactive nitrogen species induce DNA damage, resulting in mutation and carcinogenesis, if DNA damage is not repaired. Antioxidant chemopreventive agents without a prooxidant property inhibit the carcinogenic process (Fig. 1A). Phytic acid can protect DNA from ROS by chelating metals. Luteolin and retinoic acid exert anticarcinogenic potency by scavenging ROS and/or by specific manners. From the point of view of safety, compounds lacking a prooxidant property are suitable for chemoprevention, even if the antioxidant efficacy is mild. However, some chemopreventive agents exert prooxidant properties after metabolic activation (β-carotene, curcumin, etc.) or without metabolic activation (α-tocopherol, quercetin, catechins, etc.). Trials to establish chemopreventive activity by antioxidants have been inconclusive (33). Furthermore, dietary supplementation of publicly available foods and the ingestion of specific supplements are usually applied for prolonged periods of time. Therefore, we would like to emphasize the importance of assessment for safety of chemopreventive agents.
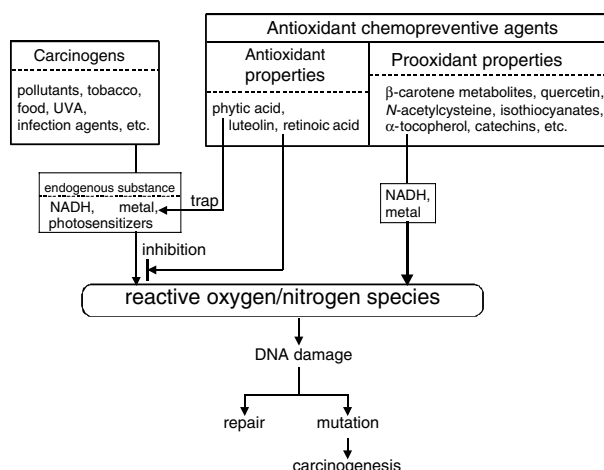

FIG. 7. **Chemopreventive agents have both antioxidant properties and prooxidant properties.** UVA, ultraviolet A.

ABBREVIATIONS

AITC, allyl isothiocyanate; BITC, benzyl isothiocyanate; ECG, epicatechin gallate; EGC, epigallocatechin; EGCG, epigallocatechin gallate; ER, estrogen receptor; ERE, estrogen response element; Fpg, formamidopyrimidine-DNA glycosylase; GA, gallic acid; ITC, isothiocyanate; O_2^-, superoxide; ·OH, hydroxyl radical; 8-oxodG, 8-oxo-7,8-dihydro-2′-deoxyguanosine; PEITC, phenethyl isothiocyanate; PG, propyl gallate; ROS, reactive oxygen species; topo II, topoisomerase II.

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