Review

Role of Oxidative DNA Damage in Dietary Carcinogenesis

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Dietary factors are implicated in approximately 35% of cancers attributed to environmental factors. Although an extremely wide variety of dietary factors are considered to contribute to carcinogenesis, its precise mechanism remains to be clarified. We focused on the role of oxidative DNA damage in carcinogenesis mediated by various dietary factors. We investigated the mechanism of oxidative DNA damage induced by a) amino acid metabolites, in relation to carcinogenesis caused by protein intake and amino acid imbalance, b) heterocyclic amines formed during cooking meat and fish, c) sugar and hyperglycemia-related aldehydes, d) carcinogens contained in fermented foods, such as urethane, e) phytoestrogens including soy isoflavones, f) carcinogens in edible plants, such as caffeic acid and isothiocyanate, g) food additives, such as potassium bromate and benzoyl peroxide. These dietary factors and their metabolites induced metal-mediated oxidative damage to DNA in human cultured cells and ³²P-labeled DNA fragments obtained from human cancer-relevant genes. On the basis of our results, it is concluded that polycyclic compounds, such as heterocyclic amines, can cause oxidative DNA damage, although they appear to mainly form DNA adduct. On the other hand, monocyclic and aliphatic compounds, such as amino acid metabolites and urethane, may mainly cause oxidative DNA damage whereas they do not appear to form DNA adduct. Soy isoflavones may cause carcinogenesis through initiation *via* oxidative DNA damage caused by their metabolites and promotion *via* cell proliferation induced by themselves. In this review, we discuss the role of oxidative DNA damage as the common mechanism of dietary carcinogenesis.

Key words: dietary factor, oxidative DNA damage, reactive oxygen species, DNA adduct, carcinogenesis

Introduction

The causal relationship between dietary factors and human cancers is substantial (1,2). Dietary factors are implicated in approximately 35% of cancers attributed to environmental factors (3). Several possible mechanisms whereby dietary factors could be causally associated with induction of human cancer have been proposed. (i) Ingestion of carcinogens contained in food. Aflatoxin and other dietary contaminants are known to be carcinogens. (ii) Exogenous and en-

dogenous production of carcinogens from dietary constituents. Heterocyclic amines are exogenously formed from amino acids and proteins during cooking meat and fish (4,5). Carcinogens are also formed endogenously from dietary constituents. Carcinogenic nitrosamines are endogenously formed by the nitrosation of secondary amines *via* the reaction with nitrite. (iii) Alteration in metabolic activation of carcinogens. In some instances, as with ethanol, its cocarcinogenic effect is to enhance the production of neoplasia by a mechanism of the activation of carcinogens. (iv) Promoting agents to act on initiated cells. The most common mechanism of diet-associated carcinogenesis in the human is considered to be through the action of major dietary constituents such as fat, carbohydrate and protein, as promoting agents (3). However, although an extremely wide variety of dietary factors are likely to contribute to carcinogenesis, the precise mechanism of carcinogenesis remains to be clarified.

Reactive oxygen species (ROS) are known to be involved in various human diseases including cancer. ROS are capable of causing damage to various cellular constituents, such as nucleic acids, proteins and lipids, leading to carcinogenesis. Although numerous chemicals are considered to cause carcinogenesis *via* the formation of DNA adduct, oxidative DNA damage may serve as the common mechanism for carcinogenesis induced by a wide variety of dietary factors. We have investigated the mechanism of oxidative DNA damage induced by various dietary factors, including a) amino acid metabolites, in relation to carcinogenesis caused by protein intake and amino acid imbalance, b) heterocyclic amines formed during cooking meat and fish, c) sugar and hyperglycemia-related aldehydes, d) carcinogens contained in fermented foods, such as urethane, e) phytoestrogens including soy isoflavones, f) carcinogens in edible plants, such as caffeic acid and isothiocyanate, g) food additives such as potassium bromate and ben-

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Table 1. Mechanism of DNA damage induced by dietary factors

Dietary factors	Suspected carcinogens	DNA damage		Ames test	Ref. No.
		*Adduct	†Oxidation		
Amino acid metabolites	3-Hydroxyanthranilic acid	—	+	?	(22)
	Homogentisic acid	—	+	+	(28)
Heterocyclic amines	PhIP	++	+	+	(46)
	MeIQx	++	+	+	(57)
	IQ	++	+	+	(65)
Sugar	Glyceraldehyde	+	+	?	(68)
Fermented foods	Urethane	—	+	—	(91)
Phytoestrogens	Genistein	—	+	—	(99)
Plant components	Allyl isothiocyanate	—	+	—	(120)
	Caffeic acid	—	+	—	(112)
	4-Hydrazinobenzoic acid	+?	+	?	(102)
Food additives	Potassium bromate	—	+	+	(124)
	Benzoyl peroxide	—	+	—	(125)

*Adduct: —, no evidence; +, limited evidence provided by only one report; ++, sufficient evidence provided by two or more reports.

†Oxidation: +, evidence provided by our study.

zoyl peroxide.

In our experiments, we used human cultured cells and ³²P-labeled DNA fragments obtained from human cancer-relevant genes. The advantage of using ³²P-labeled DNA fragments is that we can examine not only DNA-damaging abilities of test chemicals but also the site specificity of DNA damage. We have demonstrated that various chemicals induced double-base DNA damage at the 5'-ACG-3' sequence (damaged bases are underlined), complementary to a hot spot of the *p53* gene, in the presence of 20 μ M Cu(II) (6). To examine cellular DNA damage, we treated cultured cells with test chemicals alone, because these chemicals are capable of causing DNA damage by mobilizing endogenous copper ions. Copper is an essential component of chromatin (7,8). Normal plasma copper level in humans is approximately 20 μ M (9), and thus, the concentration of copper used in our cell-free system is physiologically relevant. On the basis of our results, we summarized the relative potential of each (suspected) dietary carcinogen to cause oxidative DNA damage and DNA adduct formation as shown in Table 1. In this review, we discuss the role of oxidative DNA damage as the common mechanism of carcinogenesis mediated by various dietary factors.

Amino Acid Metabolites

Epidemiological studies have suggested that high protein intake is a risk factor for several types of cancers, particularly colon, rectum and breast cancers (10). Significantly increased risks of renal cell cancer were observed with increasing consumption of high protein foods (11). An animal experiment has demonstrated that high protein intake enhanced the development of chemically-induced preneoplastic foci in the liver (12). Amino acid imbalance may participate in

dietary carcinogenesis. Methionine deficiency is reported to lower the threshold of chemical-induced toxicity and increase the risk of carcinogenesis (13). In experimental animals, threonine-imbalanced diet (basal diet supplemented with branched-chain amino acids or indispensable amino acids, which enhances threonine catabolism) induced accumulation of its metabolite aminoacetone (14), which is capable of causing DNA damage (15). However, the mechanism of carcinogenesis mediated by protein intake remains to be clarified. One possible mechanism of carcinogenesis caused by protein intake is DNA damage induced by amino acid metabolites. Several amino acid metabolites are proposed to be mutagenic and carcinogenic. Therefore, we examined the mechanism of oxidative DNA damage induced by amino acid metabolites.

Tryptophan metabolites: Tryptophan is metabolized to 3-hydroxyanthranilic acid (3-HAA) and 3-hydroxykynurenine (3-HKyn) as shown in Fig. 1A. These metabolites are carcinogenic to the bladder in mice (16,17). Higher concentrations of these metabolites were detected in urines of the patients with bladder cancer than those of normal subjects (18). Therefore, the possibility that abnormal tryptophan metabolism in some patients with bladder cancer might be of causal significance of this disease has been discussed (19). Subcutaneous injection of these metabolites induces leukemia in mice (20). These metabolites are capable of causing chromatid breakage and translocations in mammalian cells (21). We examined the mechanism of DNA damage induced by 3-HAA and 3-HKyn. These tryptophan metabolites induced DNA double-strand breaks in cultured human cells in the presence of Mn(II). The enhancing effect of a catalase inhibitor and the inhibitory effect of *o*-phenanthroline on the strand

Fig. 1. Metabolism of amino acids (tryptophan, tyrosine and threonine).

breaks indicated the involvement of H_2O_2 and endogenous metal ion. These metabolites induced damage to DNA fragments obtained from c-Ha-ras-1 protooncogene in the presence of $20\ \mu\text{M}$ Cu(II). The inhibitory effects of bathocuproine, a Cu(I)-chelating agent, and catalase on DNA damage suggest involvement of Cu(I) and H_2O_2 . Cu(II)-mediated DNA damage was enhanced by preincubation of 3-HAA with Mn(II). These results suggest that in the presence of Mn(II) or Cu(II), these tryptophan metabolites produce H_2O_2 , which is activated by transition metal ion to cause oxidative DNA damage (22).

Tyrosine metabolites: Certain metabolites of tyrosine have been reported to be carcinogenic or mutagenic. Tyrosine is metabolized to homogentisic acid (HGA) and *p*-hydroxyphenyllactic acid (Fig. 1B). *p*-Hydroxyphenyllactic acid caused leukemic changes and hepatomas in mice (23,24). HGA is accumulated and high incidence of hepatocarcinoma occurs in patients with

chronic form of hereditary tyrosinemia (23,25). Recently, it has been reported that chronic liver disease in murine hereditary tyrosinemia type 1 induces resistance to cell death, which may lead to an accumulation of damaged cells and therefore enhance the risk for cancer (26). HGA has been reported to be mutagenic in *Salmonella typhimurium* and Chinese hamster V79 cells (27). Thus, HGA and its metabolites may be involved in carcinogenesis. We demonstrated that HGA caused damage to DNA fragments in the presence of Cu(II). DNA damage was enhanced by piperidine treatment, suggesting that HGA plus Cu(II) caused not only strand breakage but also base damage and liberation. Catalase and bathocuproine inhibited DNA damage, suggesting the involvement of H_2O_2 and Cu(I). The formation of 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) by HGA increased depending on its concentration in the presence of $20\ \mu\text{M}$ Cu(II). It is concluded that H_2O_2 is generated during Cu(II)-catalyzed HGA autoxidation

and reacts with Cu(I) to form the Cu(I)-peroxide complex, capable of causing oxidative DNA damage (28).

Threonine and glycine metabolites: Aminoacetone is an aminoketone, which is produced through oxidation of L-threonine or condensation of glycine and acetyl coenzyme A (Fig. 1C) (29). L-Threonine administration significantly increased urinary excretion of aminoacetone in humans (29). Consumption of threonine-imbalanced diet resulted in aminoacetone accumulation in experimental animals (14). Serum amino acid profiles are associated with malignant tumors. It has been reported that cancer patients had significantly decreased serum levels of threonine and glycine compared with the control group (30,31). The level of aminoacetone in tumor tissues was higher than that in normal tissues (32). These findings suggest that accumulation of aminoacetone caused by enhanced catabolism of threonine and glycine may contribute to carcinogenesis. In our study, aminoacetone caused DNA cleavage and 8-oxodG formation in human cultured cells. Aminoacetone caused damage to DNA fragments obtained from the human *c-Ha-ras-1* and *p53* genes in the presence of Cu(II). Analysis of the products generated from aminoacetone revealed that aminoacetone underwent Cu(II)-mediated autoxidation in two different pathways: the major pathway in which methylglyoxal and NH_4^+ are generated and the minor pathway in which 2,5-dimethylpyrazine is formed through condensation of two molecules of aminoacetone. These findings suggest that H_2O_2 generated during the autoxidation of aminoacetone reacts with Cu(I) to form reactive species capable of causing oxidative DNA damage (15).

We have demonstrated that various amino acid metabolites induce oxidative DNA damage mediated by endogenous metal ions. On the basis of these findings, it is concluded that oxidative DNA damage caused by amino acid metabolites may be involved in carcinogenesis induced by protein intake and amino acid imbalance.

Heterocyclic Amines

Heterocyclic amines are a family of mutagenic and carcinogenic compounds produced during pyrolysis of creatine, proteins and amino acids (4,5,33–35). Heterocyclic amines, such as 2-amino-1-methyl-6-phenylimidazo [4,5-*b*] pyridine (PhIP), 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx) and 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ), have been isolated from cooked beef, chicken, fish and pork (4,5,33,36). An epidemiological study showed a significantly increased risk for cancers at all sites and for gastric cancer associated with the consumption of cooked fish (4). Thus, most heterocyclic amines in food-pyrollysates were suggested to pose probable or possible

carcinogenic risk to humans (4).

Sugimura and coworkers have extensively investigated the mechanism of carcinogenesis induced by heterocyclic amines. DNA adduct formation has been considered to be a major causal factor of DNA damage by carcinogenic heterocyclic amines. However, Hayatsu and coworkers have demonstrated that an *N*-hydroxy derivative of 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-2) produces intracellular reactive oxygen species that can damage DNA in cultured cells (37,38). These reports led us to the idea that DNA adduct formation may not be sufficient for heterocyclic amine-induced carcinogenesis, and that ROS may participate in this process. Details of our studies on oxidative DNA damage induced by heterocyclic amines and their metabolites are described below.

PhIP: In investigations of foods for the presence of multiple heterocyclic amines, PhIP is usually found to be the most abundant. Oral administration of PhIP produces lymphomas in mice (39), adenocarcinomas of the small and large intestine, and mammary adenocarcinomas in rats (40). Thus, PhIP has been estimated to possess possible carcinogenic risk to humans (Group 2B) by International Agency for Research on Cancer (IARC) (4). Heterocyclic amines are metabolically activated to DNA-binding products *via* the exocyclic amino group. PhIP is oxidized to a *N*-hydroxy derivative [PhIP(NHOH)] in the liver by cytochrome P450 enzymes, and the latter is esterified by acetyltransferases or sulfotransferases to its ultimate carcinogen (4,5,36). The DNA-adduct formation is thought to be crucially important in carcinogenesis and PhIP-DNA adducts have been detected in human tissues (36). However, there are several reports indicating no straightforward relationship between PhIP-DNA adduct formation and carcinogenicity (41–43). It has been reported that oral administration of PhIP to rats increased 8-oxodG level in mammary gland (44). Furthermore, it has been reported that several antioxidants significantly inhibited PhIP-induced mutagenicity (45) and carcinogenicity (41), suggesting involvement of ROS.

We demonstrated that PhIP(NHOH) induced Cu(II)-dependent 8-oxodG formation, which was enhanced by addition of NADH. Experiments with ^{32}P -labeled DNA fragments showed that PhIP(NHOH) formed formamidopyrimidine-DNA glycosylase (Fpg)-sensitive and piperidine-labile lesions at the 5'-site guanine of GG and GGG sequences. Interestingly, the antioxidant enzyme superoxide dismutase (SOD) enhanced the formation of piperidine-labile lesions at thymine residues. A UV-visible spectroscopic study indicated that Cu(II) and SOD catalyze the autoxidation of PhIP(NHOH). These results suggest that Cu(II)-dependent autooxidation of PhIP(NHOH) coupled with

Fig. 2. DNA adduct formation and oxidative DNA damage induced by PhIP.

NADH-mediated reduction of its oxidized product forms redox cycle, resulting in oxidative DNA damage (46) in addition to DNA adduct formation (Fig. 2).

MeIQx: Oral administration of MeIQx produces hepatocellular carcinomas and lung tumors in mice (47,48), and hepatocellular carcinomas and squamous cell carcinomas of the Zymbal gland in rats (49,50). MeIQx is oxidized to the *N*-hydroxy derivative [MeIQx(NHOH)] in the liver by cytochrome P450 1A2 isozyme, and the latter is esterified by *O*-acetyltransferase to the *N*-acetoxy derivative, which reacts with DNA to form adducts (51,52). DNA adducts generated by MeIQx were found *in vitro* and *in vivo* by means of the ³²P-postlabeling method (53). On the other hand, it has been reported that several antioxidants significantly inhibited MeIQx-induced hepatocarcinogenesis in rats (54,55). 8-oxodG level in rat liver increased dose-dependently with the concentration of MeIQx in the diet (56). These reports lead us to consider that reactive oxygen species may participate in MeIQx-induced carcinogenesis.

In our study, MeIQx(NHOH) caused Cu(II)-mediated DNA damage, including 8-oxodG formation, and DNA damage was greatly enhanced by NADH. A UV-visible spectroscopic study showed that rapid decompo-

sition of MeIQx(NHOH) occurred in the presence of Cu(II), suggesting that Cu(II) catalyzes the autoxidation of MeIQx(NHOH) and NADH reduces the oxidized product back to MeIQx(NHOH). These results suggest that a copper-peroxo intermediate, derived from the reaction of Cu(I) with H₂O₂, participates in DNA damage by MeIQx(NHOH), and NADH enhances DNA damage *via* a redox cycle (57).

IQ: IQ is a representative member of mutagenic and carcinogenic heterocyclic amines. 2-Nitro-3-methylimidazo[4,5-*f*]quinoline (nitro-IQ) is an analogue of IQ with a nitro group in place of the amino group and detected in urine as an IQ metabolite (34). IQ is chemically converted to nitro-IQ by treatment with nitrite (58). Exposure to sunlight causes oxidation of IQ to form nitro-IQ (59). Thus, nitro-IQ may be present by photoactivation in airborne particles from cigarette smoke and frying meat, and humans are likely to be exposed to nitro-IQ. Nitro-IQ exhibits mutagenicity in bacterial systems (60–62) and genotoxic activities (63,64), as well as IQ.

We have found that nitro-IQ caused DNA damage including 8-oxodG formation in the presence of NADH and Cu(II). Catalase and bathocuproine inhibited DNA damage, suggesting the involvement of H₂O₂ and Cu(I).

A UV-vis spectroscopic study showed that rapid spectral change was observed in the presence of Cu(II). These results suggest that nitro-IQ is reduced nonenzymatically by NADH in the presence of Cu(II) to form the redox reaction, resulting in oxidative DNA damage mediated by the copper-oxygen complex derived from Cu(I) and H₂O₂. We conclude that nonenzymatic reduction of nitro-IQ and resulting oxidative DNA damage can play a role in carcinogenesis of IQ (65).

We demonstrated that heterocyclic amines and their metabolites cause oxidative DNA damage. On the basis of these findings, it is concluded that oxidative DNA damage may be involved in the carcinogenic process of heterocyclic amines in addition to DNA adduct formation.

Sugar

Epidemiological and experimental studies show that diets high in refined sugars possibly increase the risk of colorectal cancer (66). Moreover, a link between sugar consumption and pancreatic carcinoma has been suggested (66). A frequent consumption of sugars may lead to insulin resistance, and cause an increase in insulin-like growth factors (IGF), which are promoters of carcinogenesis. Increased risks of cancers and oxidative DNA damage have been observed in diabetic patients (67). Many endogenous aldehydes such as 3-deoxyglucosone and glyceraldehyde increase under hyperglycemic condition. We demonstrated that these endogenous aldehydes induced oxidative DNA damage as described below, and postulated that this type of DNA damage participates in diabetes-associated carcinogenesis (68) in addition to IGF-mediated tumor promotion. Our experiments with ³²P-5'-end-labeled DNA fragments showed that these aldehydes induced Cu(II)-mediated DNA damage, which was enhanced by the addition of H₂O₂. Glyceraldehyde significantly increased 8-oxodG formation in human HL-60 cultured cells, and the addition of glucose oxidase, as an H₂O₂ generating system, significantly increased 8-oxodG formation. We conclude that oxidative DNA damage by hyperglycemia-related aldehydes, especially glyceraldehyde, and marked enhancement by H₂O₂ may participate in diabetes-associated carcinogenesis, with reference to high sugar intake.

Alcohol and Carcinogens in Fermented Foods

Alcohol drinking was classified as carcinogenic to humans (Group 1) by IARC on the basis of sufficient epidemiological evidence (69). The occurrence of malignant tumors of the oral cavity, pharynx, larynx, esophagus and liver is causally related to the consumption of alcoholic beverages (70). IARC evaluated acetaldehyde, the major intermediary metabolite of ethanol, as possibly carcinogenic to humans (Group 2B)

(71). Acetaldehyde reacts with DNA to form DNA adducts, such as 1,N²-propano-2'-deoxyguanosine, which is formed in the presence of histones and other basic molecules. This adduct can exist as a ring-closed or ring-opened aldehyde form. Whereas the ring-closed form is mutagenic, the ring-opened form can participate in the formation of DNA-protein and DNA interstrand cross-links (72). In addition, acetoaldehyde can inhibit DNA repair. It has been reported that various aldehydes inhibit O⁶-methylguanine-DNA methyltransferase (MGMT) in cultured human cells (73), and that an intraperitoneal injection of ethanol inhibited the activity of MGMT in rats (74). On the other hand, alcohol also induces oxidative stress, leading to oxidative DNA damage, lipid peroxidation, protein carbonyl formation, and the decrease in hepatic antioxidant defense (75,76), which may participate in carcinogenesis. Ethanol increased the formation of 8-oxodG in human mammary epithelial cells (77). *Trans*-4-hydroxy-2-nonenal, a product of lipid peroxidation, is known to react with DNA to form promutagenic etheno-DNA adducts (78). In addition, CYP2E1 is inducible by ethanol, and ethanol exerts an enhancing effect on metabolic activation by CYP2E1 of procarcinogens, such as nitrosoamines (75,79). Therefore, ethanol may exhibit a cocarcinogenic effect by enhancing the production of ultimate carcinogens via metabolic activation.

Urethane (ethyl carbamate) is produced by chemical reaction of urea and ethanol present in wine and sake, when these beverages are heated under the acidic conditions of fermentation (80). Urethane is also present in fermented food products such as bread, yogurt, and cheese (81–83). In rodents, urethane is a multipotential animal carcinogen, producing lung tumors, lymphomas, hepatomas, and melanomas (84,85) and thus evaluated as a Group 2B carcinogen by IARC (85). The genotoxic effects of urethane, however, remain unclear as *in vitro* mutagenesis studies, such as the Ames test (86). Urethane has been reported to form DNA adducts in the liver of rats and mice (87–89). Methyl carbamate, structurally analogous to urethane, is carcinogenic to rats (90), although it can not form DNA adduct. Thus, we investigated whether urethane metabolites can induce oxidative DNA damage.

We have shown that *N*-hydroxyurethane, a urethane metabolite, induced damage to ³²P-labeled DNA fragments and 8-oxodG formation in the presence of Cu(II). DNA damage was inhibited by catalase and bathocuproine, suggesting the involvement of H₂O₂ and Cu(I). When treated with esterase, *N*-hydroxyurethane induced 8-oxodG formation to a similar extent as that induced by hydroxylamine, which is generated by treatment of *N*-hydroxyurethane with esterase (Fig. 3). Enhancement of DNA cleavages by endonuclease IV suggests that hydroxylamine induced depurination.

Fig. 3. Proposed mechanisms of DNA damage induced by urethane metabolites.

Furthermore, hydroxylamine induced a significant increase in 8-oxodG formation in HL-60 cells but not in its H_2O_2 -resistant clone HP 100 cells. Interestingly, electron spin resonance (ESR) spectroscopy, utilizing $\text{Fe}[N\text{-(dithiocarboxy)sarcosine}]_3$, demonstrated that nitric oxide (NO) was generated from hydroxylamine and esterase-treated *N*-hydroxyurethane. In conclusion, urethane may induce carcinogenesis through oxidative DNA damage and, to a lesser extent, NO-mediated DNA depurination by its metabolites, in addition to DNA adduct formation (Fig. 3) (91).

Phytoestrogens

Epidemiological and experimental studies have shown that soy products can reduce the risk of cancer (92,93) and provide other benefits. The soy isoflavones, genistein (5,7,4'-trihydroxyisoflavone) and daidzein (7,4'-dihydroxyisoflavone), are representative phytoestrogens (94) and act as chemopreventive agents against cancers, cardiovascular disease, and osteoporosis. However, recent studies revealed that genistein and/or daidzein induced cancer of reproductive organs in rodents, such as the uterus (95) and vulva (96). Genistein also has an enhancing effect on breast (97) and colon cancer (98). These reports led us to consider that soy isoflavones may have a carcinogenic effect on female reproductive organs.

To clarify the molecular mechanisms of carcinogenesis by soy isoflavones, we examined the ability of genistein, daidzein, and their metabolites, 5,7,3',4'-tetrahydroxyisoflavone (orobol), 7,3',4'-trihydrox-

isoflavone (7,3',4'-OH-IF), and 6,7,4'-trihydroxyisoflavone (6,7,4'-OH-IF), to induce DNA damage and cell proliferation (99). We performed the E-SCREEN assay to examine the estrogenic activity of these soy isoflavones and their metabolites by comparing the number of estrogen-dependent human breast cancer cell line, MCF-7 cells, in the absence of estrogens (negative control) and in the presence of these compounds (100). This assay revealed that genistein and daidzein enhanced proliferation of MCF-7 cells, while their metabolites had little or no effect. A surface plasmon resonance sensor showed that binding of isoflavone-liganded estrogen receptors (ER) to estrogen response elements (ERE) was largely consistent with cell proliferative activity of isoflavones. Orobol and 7,3',4'-OH-IF significantly increased 8-oxodG formation in human mammary epithelial MCF-10A cells, while genistein, daidzein, and 6,7,4'-OH-IF did not. Experiments using isolated DNA revealed that metal-dependent oxidative DNA damage was induced by orobol and 7,3',4'-OH-IF. DNA damage was enhanced by the addition of NADH. These findings suggest that oxidative DNA damage by isoflavone metabolites plays a role in tumor initiation and that cell proliferation by isoflavones via ER-ERE binding induces tumor promotion and/or progression, resulting in cancer of estrogen-sensitive organs (Fig. 4) (99). Our results suggest that soy isoflavones, genistein and daidzein, have the ability to produce not only beneficial actions but also adverse effects including carcinogenesis in a similar manner to estrogens (101).

Fig. 4. Proposed mechanisms of carcinogenesis induced by soy isoflavones.

Carcinogens in Edible Plants

Some naturally occurring substances in edible plants are carcinogenic. For example, cycasin in cycad and ptaquiloside in bracken are such compounds. 4-Hydrazinobenzoic acid contained in the mushroom *Agaricus bisporus* is also carcinogenic. We demonstrated that 4-hydrazinobenzoic acid induced DNA damage *via* not only H_2O_2 production but also phenyl radical production, suggesting that both oxidative DNA damage and DNA adduct formation play important roles in carcinogenesis (102). On the other hand, epidemiological studies have shown a marked reduction in the risk of developing a variety of malignancies by large consumption of vegetables and fruits (103,104). However, the supplementation with β -carotene and/or vitamin A to smokers and asbestos-exposed workers increased the incidence of lung cancer (105,106). We have demonstrated that several phytochemicals, known as antioxidants such as β -carotene and α -tocopherol, induced oxidative DNA damage due to their prooxidant properties (107–109). We examined the mechanisms of DNA damage induced by carcinogens in edible plants, which have antioxidant properties, such as caffeic acid and isothiocyanates.

Caffeic acid: Coffee is evaluated as possibly carcinogenic to the human urinary bladder (Group 2B) whereas there is some evidence of an inverse relationship between coffee drinking and cancer of the large bowel (110). Caffeic acid is one of the coffee polyphenols, which have antioxidant properties. Oral ad-

ministration of caffeic acid in combination with known carcinogens resulted in enhancing or inhibiting effects (111). We found that caffeic acid induced alkali-labile DNA strand breaks in cultured human cells in the presence of Mn(II). The strand breakage was increased by the treatment with buthionine sulfoximine (a GSH synthesis inhibitor) and 3-aminotriazol (a catalase inhibitor), indicating the involvement of H_2O_2 . Experiments with isolated DNA showed that caffeic acid caused DNA damage in the presence of Cu(II). Cu(II)-mediated DNA damage was enhanced by preincubation of caffeic acid with Mn(II). We suggested that caffeic acid produced H_2O_2 in the presence of Mn(II) or Cu(II) to cause damage to cellular and isolated DNA (112).

Isothiocyanates: Organic isothiocyanates ($R-N=C=S$) are widely distributed in plants, many of which are consumed by humans. Vegetables, including broccoli and cauliflower, contain substantial quantities of isothiocyanates, mostly in the form of their glucosinolate precursors (113). Several studies revealed that preventive effects of isothiocyanates against chemical carcinogen-induced carcinogenesis *in vivo* and *in vitro* (114–116). On the other hand, it has been proposed that isothiocyanates exhibit carcinogenic potential to humans. The National Toxicology Program (NTP) has evaluated that allyl isothiocyanate (AITC) is carcinogenic to rats (117). AITC caused transitional-cell papillomas in the urinary bladder of male rats, and fibrosarcomas in the subcutaneous tissue in female rats (117). Benzyl isothiocyanate (BITC) and phenethyl isothiocyanate (PEITC) exhibited promotion potential during the postinitiation stage (118,119). We demonstrated that AITC caused Cu(II)-mediated DNA damage and 8-oxodG formation more strongly than BITC and PEITC. AITC significantly induced 8-oxodG formation in HL-60 cells, but not in H_2O_2 -resistant HP100 cells, suggesting the involvement of H_2O_2 in DNA damage. A UV-visible spectroscopic study revealed an association between the generation of superoxide and the yield of SH group from isothiocyanates. We conclude that oxidative DNA damage may play important roles in carcinogenic processes induced by AITC (120).

Sulforaphane (4-methylsulfinylbutyl isothiocyanate) and other isothiocyanates are thought to exhibit an anticarcinogenic effect by inducing Phase II metabolizing enzymes and antioxidant enzymes through the activation of the transcription factor Nrf2 (121,122). On the other hand, we have demonstrated that isothiocyanates are capable of causing oxidative DNA damage, although isothiocyanates with a long chain have a weaker ability to produce SH group compared with AITC. If their DNA-damaging ability exceeds the anticarcinogenic effect, they would exhibit a carcinogenic potential. Proposed mechanism of carcinogenesis induced by isothiocyanates is shown in Fig. 5.

Fig. 5. Proposed mechanisms of induction and suppression of carcinogenesis by isothiocyanates.

Food Additives

Most manufactured foods and drinks contain chemicals that added deliberately with the intention to have certain functions, such as preservatives, colors, flavors and antioxidants. Experimental evidence has shown that some food additives are mutagenic or carcinogenic.

Potassium bromate: Potassium bromate ($KBrO_3$) is allowed only for bread baking as a food additive because of its oxidizing property, and should not be detected in the final food products. $KBrO_3$ is potentially mutagenic and a well-known renal carcinogen to rats (123). Our previous study indicated that $KBrO_3$ induced 8-oxodG formation in human leukemia cell line HL-60 as well as in its H_2O_2 -resistant clone, HP100, suggesting no involvement of H_2O_2 (124). Depletion of GSH by buthionine sulfoximine (BSO) had a little inhibitory effect on $KBrO_3$ -induced 8-oxodG formation, because of the presence of cysteine. Experiments with ^{32}P -labeled DNA fragments obtained from the human cancer-relevant genes suggested that $KBrO_3$ induced 8-oxodG formation at 5'-site guanine of GG and GGG sequences of double-stranded DNA in the presence of GSH, since the treatment with Fpg protein led to chain cleavages at the guanine residues. $KBrO_3$ required glutathione and cysteine in guanine-specific oxidation of DNA. It is speculated that reduction of $KBrO_3$ by SH compounds yields bromine oxides and bromine radicals, which cause guanine oxidation, leading to carcinogenesis of $KBrO_3$.

Benzoyl peroxide: Benzoyl peroxide (BzPO), a free-radical generator, has a tumor-promoting activity. BzPO is widely used in variety of applications, including a food additive. BzPO induced piperidine-labile sites at

the 5'-site guanine of GG and GGG sequences of double-stranded DNA in the presence of Cu(I) (125). BzPO plus Cu(I) induced 8-oxodG formation in double-stranded DNA more effectively than that in single-stranded DNA. Furthermore, we observed that BzPO increased the amount of 8-oxodG in human cultured cells. It is concluded that benzoyloxy radicals generated by the reaction of BzPO with Cu(I) may lead to 8-oxodG formation and piperidine-labile oxidative lesions at the 5'-guanine of consecutive guanine sequences in double-stranded DNA, and the damage seems to be relevant to the tumor-promoting activity of BzPO.

Conclusion

We have investigated the mechanism of DNA damage induced by various dietary factors. We summarized the mechanism of DNA damage induced by dietary factors (Table 1). Polycyclic compounds, including heterocyclic amines, are likely to mainly form DNA adduct, whereas they can cause oxidative DNA damage. On the other hand, monocyclic and aliphatic compounds, including amino acid metabolites and urethane, mainly cause oxidative DNA damage whereas these metabolites do not appear to form DNA adduct.

ROS are considered to play an important role in carcinogenesis through oxidative DNA damage (126). ROS can induce the formation of various oxidative DNA lesions (127–130). Guanine is most easily oxidized among the four DNA bases, because the oxidation potential of guanine is lower than the other three DNA bases, adenine, cytosine and thymine (129,131). 8-oxodG is considered to be a mutagenic DNA lesion. It was reported that misincorporation of adenine occurs

opposite 8-oxodG during DNA synthesis, leading to G→T transversions (132,133). The mutational spectra induced by other oxidative DNA lesions have been investigated. 2,5-Diamino-4*H*-imidazol-4-one (Iz) and 2,2,4-triamino-5-(2*H*)-oxazolone (Oz) can be generated by oxidation of guanine and 8-oxodG (129). Similarly to 8-oxodG, Oz induced G→T transversions (134), whereas Iz induced G→C transversions (135,136).

We demonstrated that various dietary factors caused metal-mediated oxidative DNA damage. On the basis of our results, oxidative DNA damage may serve as a common mechanism of carcinogenesis mediated by various dietary factors. On the other hand, some dietary carcinogens are known to form DNA adduct. Each carcinogen forms characteristic DNA adduct with different nature and mutagenic potential (137). Thus, dietary factors may participate in carcinogenesis *via* DNA adduct formation as a specific mechanism in cooperation with oxidative DNA damage as a common mechanism. These studies would provide an insight in the mechanism of dietary carcinogenesis and its prevention.

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