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Review

Heterocyclic amines formed in the diet: Carcinogenicity and its modulation by dietary factors

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Introduction

Food intake is a major source of carcinogenic factors.[1] On average, each human consumes roughly 15 tons of dry weight material in his or her life.[2] The relation between food and human cancers is highlighted in many reports on animal carcinogenesis experiments and in epidemiologic investigations.[1,3,4] There are two different methods by which food can contribute to human carcinogenesis. The first is the presence of genotoxic agents in food that is responsible for human cancer. A typical example is the mycotoxin aflatoxin B_1, which exists as a contaminant in foodstuff in which *Aspergillus flavus* grows. The second concerns the variety of food components that can enhance or suppress human carcinogenesis through somewhat more indirect ways than genotoxicity. Typical examples are enhancement of mammary cancer by excess intake of calories and fat and of gastric cancers by high salt consumption.[5–7]

In the mid-1970s, screening for environmental genotoxic substances was performed by monitoring for mutagenicity in *Salmonella* strains; for example, cigarette smoke was found to contain mutagenic and carcinogenic substances.[8] Most substances require metabolic activation by mammalian enzymes to exert their mutagenicity. We focused on the possibility that smoke produced by cooking fish and meat might contain genotoxic substances. We based our hypothesis on the premise that, if cigarette smoke condensate is mutagenic and carcinogenic, why not the smoke condensate in kitchens?

Smoke particles from broiling fish were collected on glass-fiber filters, and strong mutagenicity to *Salmonella typhimurium* TA98 was detected with a metabolic activation system (S9 mix).[9] By thus monitoring mutagenicity, we were able to isolate a series of new potent mutagenic substances from charred parts of fish and meat, condensed beef extract, and pyrolyzed amino acids and proteins.[10,11] We termed these substances *heterocyclic amines* (HCAs). Their structures are given in *Figure 1*.

The mechanism of formation of HCAs during heating of fish and meat was elucidated. Quantitative measurement of HCAs in food also was developed to allow estimation of the magnitude of exposure of humans to HCAs. Although the human intake of HCAs is minute, the risk of an HCA contribution to human cancer cannot be overlooked. Fortunately, various methods to reduce the formation of HCAs are now available. In addition, modulation of HCA formation and suppression of carcinogenicity by nutritional condition and supplementation offer great hope. In this article, we describe the biological properties of HCAs including carcinogenicity, their possible relevance to human cancer, prevention of HCA formation, and modulation of the carcinogenicity by other dietary factors.

Chemical properties of HCAs

More than 20 HCAs have been isolated as mutagens from various heated materials, and structures of 19 of these compounds are now known.[10–13] Among these, ten HCAs have been studied for their chemical and biological properties including their respective carcinogenicities. *Figure 1* shows their structures and *Table 1* lists their chemical names and sources of isolated HCAs.[10,11]

All the HCAs are soluble in methanol, acetone, chloroform, and dimethyl sulfoxide, as well as in acidic aqueous solution. They can be crystallized as free bases or as salts with hydrochloric acid, hydrogen bromide, or acetic acid, forming pale yellow or colorless crystals. HCAs are fairly stable in the dark and under cold conditions. The HCAs shown in *Figure 1* have been chemically synthesized on a large scale for use in long-term carcinogenesis experiments of rodents and monkeys.[11]

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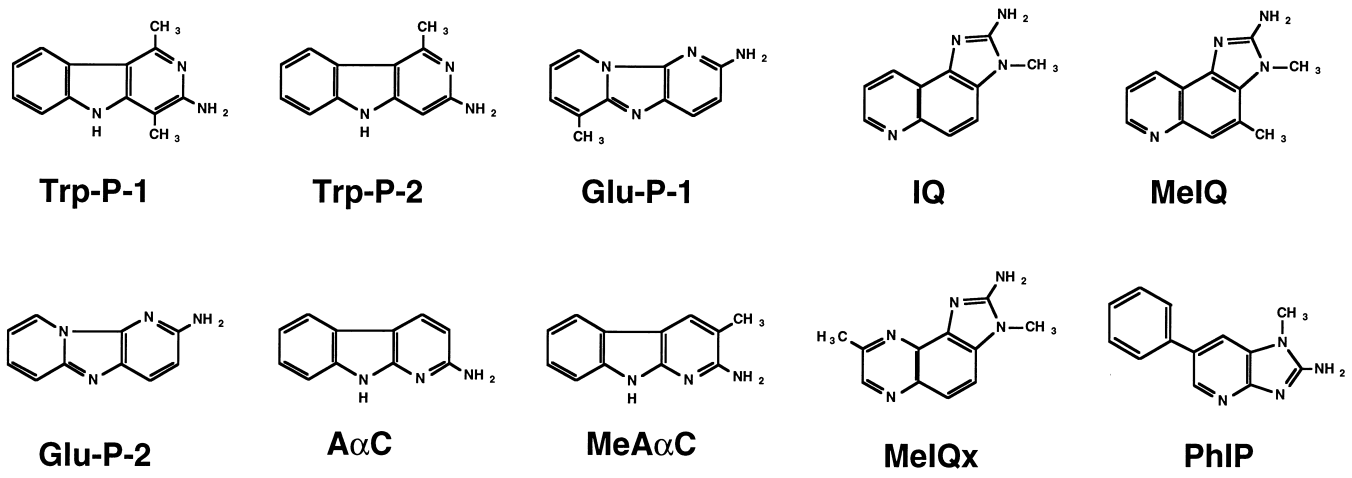


Figure 1 Structures of mutagenic and carcinogenic heterocyclic amines.

The aminoimidazole moiety of some HCAs is probably derived from creatin(in)e in meat and fish, and the other portions of the molecules presumably originate from reaction products of amino acids and sugars through Maillard reactions.[14] In fact, PhIP can be formed by heating a mixture of creatinine, phenylalanine, and glucose. MeIQx is produced when a mixture of creatinine, glycine, and glucose is heated, and IQ results from the combination of creatinine, glycine, and fructose. Some HCAs may be derived from free amino acids and amino acids in peptides in meat and fish. Trp-P-1 and Trp-P-2 were originally isolated as tryptophan pyrolysis products,[15] and Glu-P-1 and Glu-P-2 as products of glutamic acid pyrolysis.[16] AαC and MeAαC, originally isolated from soybean globulin pyrolysate,[17] were found to be produced by heating tryptophan.[18]

Genotoxic properties of HCAs

Mutagenicity of HCAs

HCAs show mutagenicity to *S. typhimurium* TA98 and TA100 with S9 mix, S9 of which was prepared from the livers of rats treated with polychlorinated biphenyls (PCB). TA98, a detector of frameshift type mutations, proved more sensitive than TA100, a detector of basepair change type mutations. The specific mutagenic activities of ten HCAs to TA98 and 100 are listed in *Table 2*.[10,11] It is noteworthy that the various HCAs' mutagenic activity per microgram to *S. typhirium* TA98 ranged from 200 for MeAαC to 661,000 for MeIQ.

HCAs can cause mutations in cultured mammalian cells such as Chinese hamster lung cells with a marker of resistancy against diphtheria toxin.[19] They also can induce chromosomal aberrations and sister chromatid exchange in cultured mammalian cells in vitro.[20–22]

Metabolism of HCAs and their DNA adduct formation

HCAs are metabolically activated by CYP1A2 to yield their hydroxylamino derivatives and further activated by esterification with acetic acid and sulfuric acid to ultimate forms, producing DNA adducts.[23,24] IQ, MeIQ, MeIQx, PhIP, Trp-P-2, Glu-P-1, and MeAαC have been reported to form mainly adducts at the C-8 position of the guanine base in DNA.[25–32] As examples, the structures of adducts of MeIQx and PhIP with guanine are shown in *Figure 2*.

Mutational spectra induced by HCAs in vivo

Big Blue® mice carrying the *lacI* transgene are convenient for examination of mutational spectra of chemicals. MeIQ, PhIP, and AαC were added to the diets of these mice, and their mutational spectra were analyzed in the colon, where

Table 1 Chemical names, abbreviations, and sources of isolated heterocyclic amines

Chemical name	Abbreviation	Source
3-Amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole	Trp-P-1	Tryptophan pyrolysate
3-Amino-1-methyl-5*H*-pyrido[4,3-*b*]indole	Trp-P-2	Tryptophan pyrolysate
2-Amino-6-methyldipyrido[1,2-*a*:3′,2′-*d*]imidazole	Glu-P-1	Glutamic acid pyrolysate
2-Aminodipyrido[1,2-*a*:3′,2′-*d*]imidazole	Glu-P-2	Glutamic acid pyrolysate
2-Amino-9*H*-pyrido[2,3-*b*]indole	AαC	Soybean globulin pyrolysate
2-Amino-3-methyl-9*H*-pyrido[2,3-*b*]indole	MeAαC	Soybean globulin pyrolysate
2-Amino-3-methylimidazo[4,5-*f*]quinoline	IQ	Broiled sun-dried sardine
2-Amino-3,4-dimethylimidazo[4,5-*f*]quinoline	MeIQ	Broiled sun-dried sardine
2-Amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline	MeIQx	Fried beef
2-Amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine	PhIP	Fried beef

Table 2 Mutagenicities of heterocyclic amines and typical carcinogens in *Salmonella typhimurium* TA98 and TA100

Compound	Revertants/μg	
	TA98	TA100
Trp-P-1	39,000	1,700
Trp-P-2	104,200	1,800
Glu-P-1	49,000	3,200
Glu-P-2	1,900	1,200
AαC	300	20
MeAαC	200	120
IQ	433,000	7,000
MeIQ	661,000	30,000
MeIQx	145,000	14,000
PhIP	1,800	120
Aflatoxin B_1	6,000	28,000
AF-2	6,500	42,000
4-Nitroquinoline 1-oxide	970	9,900
Benzo[a]pyrene	320	660
MNNG	0.00	870
N-Nitrosodiethylamine	0.02	0.15
N-Nitrosodimethylamine	0.00	0.23

AF-2—2-(2-Furyl)-3-(5-nitro-2-furyl)acrylamide. MNNG—*N*-Methyl-*N*′-nitro-*N*-nitrosoguanidine.

high mutation frequencies were observed.[33] The three HCAs predominantly caused mutations at G:C basepairs to produce G:C → T:A transversions.

Moreover, G:C basepair deletions in the 5′-GGGA-3′; sequence of the *lacI* gene were detected in DNA samples after treatment with PhIP at a significantly higher rate than with MeIQ or AαC.

Carcinogenicity data for HCAs in experimental animals

Most carcinogenesis experiments using HCAs have been performed employing F344 rats and CDF_1 mice of both sexes under standard in vivo conditions, with chronic feeding of commercial pellet diet containing HCAs at concentrations of 100 to 800 ppm.[10,11,34–37] The results are summarized in *Table 3*. Target organs in rats include the liver, urinary bladder, small and large intestines, Zymbal gland, clitoral gland, skin, oral cavity, mammary gland, and prostate. Target organs in mice are the liver, blood vessels, forestomach, lung, hematopoietic system, and lymphoid tissue. It is interesting to note that PhIP efficiently induces colon cancers in male rats and mammary gland cancers in female rats.[35] Furthermore, recent investigations revealed PhIP to be a carcinogen for the ventral lobe of the rat prostate.[36] Urinary bladder cancers developed in rats fed diets containing Trp-P-2.[37] In addition, MeAαC caused severe atrophy in the salivary glands and pancreas of rats.[38]

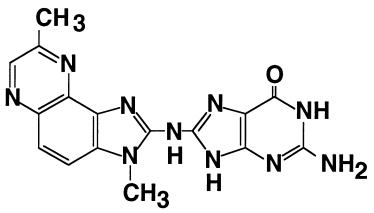
IQ induced hepatocellular carcinomas in cynomolgus monkeys.[39] The time period for liver cancer induction was similar to that of *N*-nitrosodiethylamine in cynomolgus monkeys.

Clarification of genetic alterations in HCA-induced tumors in animals should help us understand the roles of these environmental carcinogens in the development of human cancers. Mutations of *Ha-ras*, *Ki-ras*, and *p53* have been observed in Zymbal gland tumors in F344 rats induced by IQ, MeIQ, and MeIQx.[40–43] In forestomach tumors of CDF_1 mice given MeIQ, *Ha-ras* and *p53* mutations also were detected.[43] Moreover, *p53* alterations were observed in liver tumors of F344 rats induced by MeIQx.[43] In the case of PhIP-induced colon cancers in F344 rats, a specific mutation of the *Apc* gene—deletion of a guanine base at 5′-GGGA-3′ site—was observed in five of eight rat colon cancers tested.[44] It should be stressed that mutations in *Ha-ras*, *Ki-ras*, and *Apc* genes in HCA-associated tumors correlated well with the specific mutational spectra in the *lacI* gene in Big Blue mice treated with HCAs, as described in the previous section. Recently, PhIP-induced colon cancers without *Apc* mutations were found to possess a mutation in gene for β-catenin.[45]

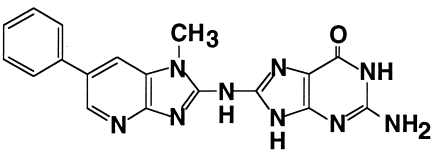
Alterations in repeat lengths of microsatellite DNA sequences, which are composed of mono- to tetra-nucleotide repeats, also have been frequently observed, especially in colon tumors and mammary gland tumors induced by PhIP, and less often in those induced by IQ and 7,12-dimethylbenz[a]anthracene, respectively.[46,47]

Quantification of HCAs in cooked food and human urine

In general, HCAs are present in cooked foods at minute levels. There are now many clean-up methods available to determine precisely their content in specific foods; for example, acid/base partition, cation exchange TIN-100 H05E fiber column, XAD-2 column, Extrelut-20 cartridge, Bond-Elut C_{18} cartridge, Sep-Pak C_{18} cartridge, blue cotton, and blue rayon.[48–51] Among these, blue cotton and blue rayon, which consist of cotton and rayon, respectively,



Guanine-C8-MeIQx



Guanine-C8-PhIP

Figure 2 Structures of adducts of MeIQx and PhIP with guanine.

Table 3 Carcinogenicities of heterocyclic amines in rats and mice

Chemical	Species	Concentration in diet (%)	Duration of feeding (weeks)	Target organs
Trp-P-1	Rats	0.015	52	Liver
	Mice	0.02	89	Liver
Trp-P-2	Rats	0.01	112	Liver, urinary bladder
	Mice	0.02	89	Liver
Glu-P-1	Rats	0.05	67	Liver, small and large intestines, Zymbal gland, clitoral gland
	Mice	0.05	68	Liver, blood vessels
Glu-P-2	Rats	0.05	104	Liver, small and large intestines, Zymbal gland, clitoral gland
	Mice	0.05	84	Liver, blood vessels
AαC	Mice	0.08	98	Liver, blood vessels
MeAαC	Rats	0.02, 0.01	100	Liver
	Mice	0.08	84	Liver, blood vessels
IQ	Rats	0.03	72	Liver, small and large intestines, Zymbal gland, clitoral gland, skin
	Mice	0.03	96	Liver, forestomach, lung
MeIQ	Rats	0.03	40	Large intestine, Zymbal gland, skin, oral cavity, mammary gland
	Mice	0.04, 0.01	91	Liver, forestomach
MeIQx	Rats	0.04	61	Liver, Zymbal gland, clitoral gland, skin
	Mice	0.06	84	Liver, lung, hematopoietic system
PhIP	Rats	0.04	52	Large intestine, mammary gland, prostate
	Mice	0.04	82	Lymphoid tissue

covalently bound to the blue pigment copper phthalocyaninetrisulfonate, can specifically adsorb multicyclic planar compounds including HCAs, and they are thus very useful and efficient for concentration of HCAs from crude material.[49] HCAs in partially purified materials can be determined by high performance liquid chromatography (HPLC) with ultraviolet, electrochemical, and fluorescence detection, liquid chromatography–mass spectrometry, gas chromatography-mass spectrometry with selected-ion monitoring, and enzyme-linked immunosorbent assays.[48,50,51] Recently, it was reported that HCAs can be accurately and sensitively detected by gas chromatography with *N*-dimethylaminomethylene derivatives.[52]

Analytical data, measured by a combination of blue cotton treatment and HPLC, are available for nine HCAs in broiled beef, chicken, and mutton, fried ground beef and fish, and food-grade beef extract.[50] Among the HCAs detected, PhIP was the most abundant, present at levels of 0.56 to 69.2 ng/g. The level of MeIQx was second highest, at 0.64 to 6.44 ng/g, and the other HCAs were found at levels of 0.03 to 2.50 ng/g.

Using a similar quantification method, levels of unmetabolized HCAs in human urine have been determined. Four HCAs—MeIQx, PhIP, Trp-P-1, and Trp-P-2—were detected in 24 urine samples from ten healthy volunteers living in Tokyo and eating an ordinary diet.[53] However, they were not detected in urine samples of inpatients receiving parenteral alimentation. Because 1.2 to 4.3% of an oral dose of MeIQx is reported to be excreted unchanged in the urine,[54] the daily exposure of the volunteers was estimated to be 0.3 to 3.9 μg per person. This is in the same range that has been calculated for other carcinogens such as *N*-nitrosodimethylamine and benzo[a]pyrene.[50] Urinary levels of MeIQx also are reported to be positively associated with frequencies of cooked meat intake in residents of Los Angeles.[55] These observations strongly suggest that humans are continuously exposed to HCAs derived from foods in their daily life.

Factors to be considered for risk evaluation

Human livers can metabolically activate HCAs.[56] Healthy volunteers eating an ordinary diet excrete HCAs in urine.[53,55] MeIQx- and PhIP-DNA adducts have been identified in DNA from human colon, rectum, and kidney.[57,58]

Although it is evident that, in comparison with the dose necessary to induce tumors in rodents in long-term feeding experiments, the magnitude of HCA exposure in humans may not be sufficient to itself produce cancers, we need to pay attention to the presence of somatic cells that already have mutations due to various other genotoxic xenobiotics and autobiotics. Because human carcinogenesis is understood to be a multi-step process that involves multiple genetic alterations, additional mutations could be critical to complete the conversion of normal to malignant cells.[10] Mutations causing genomic instability because of impaired control of DNA replication and DNA repair are particularly important in this respect, because an explosive increase in genetic alterations may result. Cancer cells arise from stem cell populations and the number of these cells is proportional to the total number of cells in the body. Because a human weighs 250 times more than a rat, the target cell number size of a human is 250 times greater than that of a rat, meaning that the chance of being targeted by genotoxic agents is 250 times greater in humans than in rats.[59] A long life span also increases the chance of a single somatic cell acquiring a sufficient number of mutations. From these observations, HCAs clearly cannot be assumed to be without significance based on the ratio of the HCA intake dose to the HCA carcinogenic dose extrapolating from animal experiments.

Epidemiology

Epidemiologic surveys have indicated positive links between consumption of cooked meat and fish and an elevated risk of various kinds of cancers, as detailed below. Consumption of well-browned meat was found to be associated with an increased risk of colorectal cancers.[60,61] Two reports have documented a link between high intake of cooked meat and fish and risk of stomach cancer; both reports indicate that length of cooking time is an important factor in cancer risk.[62,63] For example, compared with rare beef, odds ratios were 2.4 for ingestion of medium and 3.2 for ingestion of well-done beef. The relative risk of cancer mortality associated with frequent (at least twice per week or more) consumption of broiled fish compared with less frequent consumption was 1.3 for cancer in all sites and 1.7 for gastric cancer. There have been four other studies that showed a positive relationship between intake of cooked meat and increased risk of pancreatic, urothelial, mammary, and esophageal neoplasms.[63–66] However, one study showed no correlation between HCA intake and cancer development.[67]

CYP1A2 and acetyltransferase-2 (NAT-2) are involved in metabolic activation of HCAs. Both are known to be polymorphic in humans and it has been demonstrated that the highest risk phenotype (rapid CYP1A2 and rapid NAT-2) combined with a dietary preference for well-done red meat has a relative odds ratio of 6.45 for colorectal cancer risk.[68] It also has been reported that the risk of colorectal adenoma and carcinoma increased with intake of cooked meat in people classified as fast acetylators but not in those classified as slow acetylators.[69]

Suppression of HCA formation during cooking

Because HCAs are produced by normal cooking methods and are present in a variety of cooked foods, complete avoidance of exposure to HCAs is impossible and we must accept some ingestion in daily life. Therefore, it is very important to find ways to reduce the formation of HCAs as much as possible.

Formation of mutagenic and carcinogenic HCAs increases with temperature and cooking time.[70,71] For example, the level of PhIP was shown to be increased from 0.2 ng/g fried bacon at 175°C to 4.5 ng/g fried bacon at 225°C.[71] Significant increase of HCA formation with high temperature also has been observed in other dishes. As a practical measure, preventing direct contact of meat and fish with a naked gas or a charcoal flame reduces HCA formation. Wrapping meat and fish in aluminum foil before putting them in an oven is also an effective way to reduce HCA formation. Moreover, the mutagenicity is much lower when foods are cooked in a microwave oven.[72]

Creatin(in)e plays an essential role in generation of HCAs, and its removal and decrease is effective to lessen HCA formation. Microwave treatment for 1 minute to remove the juice containing creatin(in)e before normal cooking is reported to reduce HCA formation.[73] In addition, inclusion of soybean protein containing no creatin(in)e is effective for decreasing mutagen formation in fried beef hamburger.[74]

Supplementation with antioxidants also is considered beneficial in this respect, because free radical involvement in HCA formation through the Maillard reaction would be expected. In fact, the addition of butylhydroxyanisole to beef patties reduces mutagen formation due to frying.[74] Among the 14 kinds of antioxidants tested, green tea catechins and the major component [(−)-epigallocatechin gallate], two flavonoids (luteolin and quercetin), and caffeic acid clearly suppressed generation of both MeIQx and PhIP in heated mixtures of creatine, sugars, and amino acids.[75]

Modulation of mutagenicity and carcinogenicity of HCAs by dietary factors

Dietary fibers have been shown to reduce the mutagenicities of HCAs by adsorbing those compounds *in vitro*.[76,77] Adding 10% wheat bran to the diets of rats suppressed the development of aberrant crypt foci (ACF), which are putative preneoplastic lesions in the colon that are induced by administration of IQ by gavage.[78] The mutagenicity in extract of the feces from rats given IQ plus wheat bran was much higher than that from rats given IQ without wheat bran. These data are consistent with adsorption of the carcinogen to wheat bran fiber and the enhanced carcinogen excretion.

Hemin and other tetra-pyrrole compounds suppress the mutagenicity of HCAs, probably due to interactions between these two types of planar molecules.[79] Chlorophyllin, a stable and soluble derivative of chlorophyll, is an inhibitor of HCA-induced carcinogenesis in rats. IQ-induced liver, small intestine, and Zymbal gland tumors were suppressed by administration of chlorophyllin to rats.[80] Administration of chlorophyllin and IQ through gavage resulted in significant inhibition of covalent binding of IQ to DNA in the liver and small intestine.[81,82] Chlorophyllin reduced the absorption of IQ from the intestine, and removal of unmetabolized IQ through the feces was increased. Similar results were observed regarding development of ACF in the colons of rats receiving PhIP.[83] Excretion of unmetabolized PhIP in the feces also was increased. Moreover, the incidence of mammary adenocarcinomas induced by PhIP in female F344 rats was reduced by chlorophyllin coadministration.[84]

Indole-3-carbinol, which is present in broccoli, cabbage, cauliflower, and other cruciferous vegetables, has demonstrated anticarcinogenic activity in a number of carcinogenesis experiments using rodents.[85,86] It was shown to suppress ACF development in rats given PhIP or IQ, both of which are metabolically activated to produce hydroxyamino derivatives by CYP1A2, while being hydroxylated at C-position in the ring by CYP1A1.[83,87,88] Dietary supplementation with indole-3-carbinol induced CYP1A1 to a much greater extent than CYP1A2 in the rat liver and colon mucosa. As a consequence, excretion of 4′-hydroxy-PhIP and 5-hydroxy-IQ and their corresponding *O*-glucuronides and *O*-sulfates in the urine was increased.[83,87,88]

It is well known that green tea catechins and green tea extract can prevent tumor development in various organs due to a variety of carcinogens.[89,90] This was also the case for HCA-induced tumorigenesis; for example, reduction of ACF formation in the colons of rats given IQ.[87] Green tea

catechins also suppressed Glu-P-1-induced hepatocarcinogenesis, using glutathione S-transferase placental form (GST-P) positive liver foci as the end point lesions.[91] Furthermore, at 1% in the diet, the catechins tended to lower the incidence and multiplicity and significantly reduce the mean size of mammary adenocarcinomas in F344 female rats fed diet containing 200 ppm PhIP.[92] Although the mechanisms underlying anticarcinogenesis by green tea catechins have not been fully elucidated yet, these agents may possess wide-spectrum chemopreventive potential in rodents, irrespective of the carcinogen and the stage of carcinogenesis.

Epidemiologic studies suggest that various dietary factors combine to cause colon cancer.[93,94] Animal fat consumption is positively associated with the risk of colon cancer, but no association has been found for vegetable fat.[93–95] Moreover, it is reported that colon cancer is linked more strongly with red meat consumption than with fat consumption, suggesting that other components of red meat may be responsible.[95] One experiment with rodents demonstrated that high-fat diets promote colon carcinogenesis induced by PhIP.[96] On the other hand, high intake of fish is suggested to be associated with a low risk of colon cancer.[95,97] This may be because fish oils are rich in polyunsaturated ω3 fatty acids including docosahexaenoic acid (DHA). In fact, DHA significantly reduced PhIP-induced ACF formation in the colon of rats.[98] The expression of cyclooxygenase 2 and formation of prostaglandin E_2 have been shown to be clearly elevated in colon tumors of rats and humans,[99–101] and DHA is known to reduce prostaglandin formation.[102] Therefore, decreased levels of prostaglandin E_2 are probably involved in the beneficial effects of DHA. Conjugated linoleic acids (CLA), which are present in several foods including milk, cheese, and cooked meat, have similarly shown anticarcinogenic properties; for example, against polycyclic aromatic hydrocarbon-associated tumors, as well as IQ-induced ACF development in the rat colon.[103–105]

Because fermented dairy products such as fermented milk and yogurt play an important role in human nutrition, the effects of *Bifidobacterium longum* on IQ-induced carcinogenesis were investigated in F344 rats. Supplementation with a diet containing 0.5% lyophilized cultures of *B. longum* resulted in inhibition of colon, mammary, and liver carcinogenesis due to 125 ppm IQ.[106]

Conclusion

HCAs are a group of environmental carcinogens that are produced with cooking under routine conditions, which makes human exposure to them almost unavoidable. This is in contrast to other environmental carcinogens that are generated as the outcome of industrial activity. In the latter case, reduction could be targeted by improved technology. However, human exposure to endogenous (autobiotic) carcinogens, naturally occurring carcinogens, and food-borne carcinogens will possibly remain largely as it is at present. Therefore, knowledge of how to minimize the formation of HCAs and to suppress their in vivo carcinogenicity by effectors added to the diet is a high priority. A simple but efficient method to lessen HCA exposure is the mechanical removal of charred parts of cooked meat and fish with a knife and fork. Suppression of metabolic activation and increasing detoxification are also important ways. Examples of effectors such as those described above are antioxidants such as flavonoids and catechins, chlorophyll, indole-3-carbinol, fiber, and DHA. In addition, a healthy and balanced nutritional diet should enhance resistance to development of tumors by HCAs, the study of which poses further challenges.

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