Nutrition and dietary carcinogens

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Three major factors for human carcinogenesis are (i) cigarette smoking, (ii) infection and inflammation and (iii) nutrition and dietary factors. Nutrition and dietary factors include two categories, namely genotoxic agents and constituents including tumor promotion-associated phenomena. This article first describes the genotoxic agents as microcomponents. These are mutagens/carcinogens in cooked food, fungal products, plant and mushroom substance, and nitrite-related materials, polycyclic aromatic hydrocarbons and oxidative agents. Emphasis has been given to heterocyclic amines (HCAs) to which humans are continuously exposed in an ordinary lifestyle. HCAs in food are mainly produced from creatin(in)e, sugar and from amino acids in meat (upon heating). They are imidazoquinoline and imidazooxinozoline derivatives and phenylimidazopyridine. HCAs are pluripotent in producing cancers in various organs including breast, colon and prostate. Discussion is also given to plant flavonoids which are mutagenic but not carcinogenic. As a macrocomponent, overintake of total calories, fat and sodium chloride is discussed from the viewpoint of the increase of genetic alterations in tissues and of tumor promotion-associated issues. Studies of nutrition and dietary condition will eventually lead us to cancer prevention, namely delay of onset of cancer to the late phase of human life, which is called ‘natural-end cancer’ (Tenju-gann).

Introduction

Three major factors for human carcinogenesis are cigarette smoking, infection and inflammation, and nutrition and dietary carcinogens (1,2). Nutrition and dietary carcinogens relevant to carcinogenesis can be grossly divided into two categories, (i) microcomponents and (ii) macrocomponents and total calorie intake (3,4). From the mechanistic viewpoint, there are genotoxic agents causing genetic alterations related to carcinogenesis and constituents inducing tumor promotion-associated phenomena. Genotoxic agents are clearly defined as causing DNA damage resulting in gene point mutations, deletions and insertions, recombinations, rearrangements and amplifications, as well as chromosomal aberrations. Dietary tumor promoters are less distinctively defined in terms of their modes of action, but, generally speaking, they cause cell proliferation with or without accompanying chronic cell damage. Although tumor promoters represented by 12-O-tetradecanoylphorbol-13-acetate (TPA) exert their actions as microcomponents, most nutritional and dietary tumor promoters are of the macrocomponent type.

In this review, two important agent categories are discussed. First, microcomponents that are genotoxic are described. The major focus is on recent advances in our knowledge on heterocyclic amines (HCAs) produced by cooking proteinaceous food, like meat and fish. HCAs are unique both in that they are naturally occurring (5,6), so that complete avoidance of exposure is impractical, and in that they induce tumors of organs like the breast, colon (7) and prostate (8) which are increasing in advanced nations. Furthermore, the mutation spectra of tumors produced by some HCAs are similar to those of human cancers (9,10), and many, although not all, epidemiological studies have provided positive correlations between cancer incidence and consumption of heavily cooked meat (11–13). Other carcinogenic microcomponents, of which most human intake is irregular, are also reviewed concisely.

Carcinogenic processes are understood to involve multiple steps (14). It can be readily appreciated that, in the body of a healthy human, there are many cells which already have genetic alterations of cancer-related genes caused by various genotoxic substances, including dietary carcinogens. Moreover, genomic instability (15,16), frequently resulting from mutations in genes, encoding proteins related to DNA repair would be expected to be produced by mutational events. If a mutation occurs in the genes, more rapid accumulation of additional gene alterations would be yielded in other cancer-related genes. Therefore, the potential contribution of minute amounts of mutagens/carcinogens present in the diet cannot be overlooked with regard to the significance for carcinogenesis.

The second big category is constituted by macrocomponents whose excessive intake causes tumor promotion. These include fat for breast and colon carcinogenesis (17,18) and sodium chloride for gastric carcinogenesis (19,20).

A critical overview of the generally accepted understanding on nutrition and dietary carcinogens with regard to human cancers is presented. The recent setback with β-carotene (21) tells us that we have to be cautious in drawing oversimplified conclusions in a black or white fashion in nutrition and dietary carcinogen related issues. Nutrition and dietary carcinogens exist as one of the subjects among many trends in cancer research which need to receive critical appraisal.

Genotoxic agents as dietary carcinogens

**HCAs as food-borne mutagens/carcinogens**

The series of mutagenic and carcinogenic HCAs were discovered due to curiosity about daily life conditions. Smoke derived from grilling sun-dried fish in a kitchen was subjected to mutagenicity testing, because condensates of cigarette smoke are known to show mutagenic activity to *Salmonella typhimurium* strains (5,22).
Smoke condensates derived from grilling fish (sardine, horse mackerel and herring) and meat (beef) thereby exhibited potent mutagenic activity with S9 mix metabolic activation, especially to a frameshift type mutagen detector, S. typhimurium TA98. The mutagenic principle was found to be present in a basic fraction and different from polycyclic aromatic hydrocarbons (PAHs). Active principles were purified by organic solvent extraction and various column chromatographies, and their structures were finally determined (23). They were newly registered compounds. They have been chemically synthesized in large quantity and standard carcinogenic assays in rodents carried out. All HCAs so far examined have demonstrated positive carcinogenicity (24,25).

Chemical names, trivial abbreviations and years of isolation and structural determination of HCAs are listed in Table I. Carcinogenicity of HCAs in rats and mice and years of publication are given in Table II. Structures of HCAs are illustrated in Figure 1. They are divided into two classes, namely 2-amino-3-methylimidazo[4,5-b]indole (IQ)-type HCAs and non-IQ-type HCAs. The former are produced when mixtures of creatin(in)e, amino acids and sugars are heated. The aminimidazole moiety of IQ-type HCAs seems to be derived from creatin(in)e while the remaining parts such as the quinoline, quinoxaline and phenylpyridine moieties are Maillard reaction products of amino acids and sugars. Non-IQ-type HCAs are produced by pyrolysis of amino acids and proteins (26), being produced at higher temperature than IQ-type HCAs. Grilled meat, juice from heated meat, and stewed meat heated for a prolonged time all contain HCAs. Charred and black crust materials on the surfaces of proteinaceous foods, which are produced by contact with a naked flame, contain especially high levels of HCAs.

HCAs are converted to their hydroxymidamino derivatives by cytochrome P450s, especially CYP1A2 (27) and further activated by the esterification enzymes, acetyltransferase (27) and sulfotransferase (28). The reactive ultimate forms produce adducts with guanines mainly at their C8 positions (25), resulting in change in DNA sequences by base substitution, deletion and insertion. However, comparison of DNA adduct levels and in vivo mutant frequencies in various organs did not demonstrate a direct correlation (29) and, furthermore, mutant frequency and cancer incidence are not directly related (30). These findings indicate that other unknown factors are involved with in vivo carcinogenesis. One of the characteristics of the HCA series is their pluripotential to yield cancers in various organs (25), including breast and colon (7), prostate (8), skin, lymphoid tissue, ear ducts, blood vessels and liver (31).

Various approaches have been made to evaluate the risk of HCAs in humans. Carcinogenic doses of HCAs in rodents have been compared with daily human intake of HCAs (32), and effects of combination exposures of HCA with other HCAs or other carcinogens also studied (33). When HCAs having the same target were combined, their effects exceeded the sums of individual data, indicating complexity of risk (34). Moreover, production of genomic instability by IQ-type HCAs has been assessed by searching the database of human cancers (9,10,34). Moreover, production of genomic instability by IQ-type HCAs has been investigated (34). Epidemiological investigations along different lines revealed some positive (11,13) and negative (12) links between cancer risk and intake of well-
done meat or fish. It must be borne in mind that human beings are continuously exposed to HCAs together with large numbers of other genotoxic agents (35), although the level of exposure to each individual agent is very minute (36, 37). The roles of interactions among the many carcinogens existing in the environment need to be taken into account in considering the risk of a particular compound.

Formation of HCAs can be significantly reduced by inexpensive and practical measures like avoidance of exposure of meat surfaces to flames, usage of aluminum foil to wrap meat before oven roasting and the employment of microwave cooking (38). In addition, there are many substances which have been found to block HCA carcinogenesis. For example, diallyl disulfide, an organosulfur compound in garlic, and chlorophyllin suppress 2-amino-1-methyl-6-phenylimidazo-[4,5-b]pyridine (PhIP)-induced breast cancer development in rats (39–41).

The conclusion has been drawn that HCAs present a possible risk for human carcinogenesis and they have been assigned to the group 2 category in the classification of the IARC. For molecular risk estimation, tumors produced by HCAs in rodents show alterations in cancer related genes, H-ras, K-ras, p53 (34), Apc (42) and β-catenine (43). Apc in rat colon cancers exhibits a unique mutation, namely deletion of G from 5′-GGGA-3′ sequences (42), and studies of in vivo mutation in lacI gene demonstrated a validity of using this mutational pattern as a signature mutation of PhIP (9, 10). This pattern of genetic changes is present in the p53 gene in human cancers. Other dietary carcinogens in food

**Mycotoxins**

Foods may be contaminated with toxins of the mold *Aspergillus flavus*. A well known typical toxin is aflatoxin B₁ (AFB₁) which induces hepatic carcinomas in many species of experimental animals including rats, monkeys and fish (44). Rainbow trout is very sensitive to AFB₁-induced carcinogenesis, and used in a large scale experimental study such as the dose–response relationships between AFB₁–DNA adduct levels and cancer development (45). AFB₁ is metabolically activated to its 8,9-epoxide by cytochrome P450s, and modifies DNA with formation of 8,9-dihydro-8-(N²-guanyl)-9-hydroxylflatoxin B₁ adducts. Molecular epidemiological investigations revealed the presence of high levels of AFB₁–DNA adducts in areas where human diet is highly contaminated with AFB₁ (44). In addition, a mutation in the p53 tumor suppressor gene caused by transversion of GC to TA in human hepatomas has been noted at the third position of codon 249 (46, 47). Contamination of AFB₁, AFB₂, AFG₁ and AFG₂ in the diet was limited to <20 p.p.b. in the USA, and that in UK was 4 p.p.b. In the case of Japan, AFB₁ in the dietary supply must be <10 p.p.b. (48).

Another mycotoxin, fumonisin B₁ produced by the corn pathogen, *Fusarium moniliforme*, is reported to be carcinogenic in rats (49). In addition, sterigmatocystin, produced by *Penicillium, Aspergillus* and *Bipolaris*, induces hepatocellular carcinomas on oral administration and skin carcinomas by skin painting in rats (50, 51).

**Norsesquiterpene glucoside from bracken fern**

Bracken fern was called to the attention of scientists by the finding that cows pastured in fields where bracken fern was growing, developed hematuria with tumors of the urinary bladder (52, 53). Feeding rats with bracken fern yielded carcin-rats (39–41). Leucocyanidines and senkirkine are responsible for the carcinogenicity of two kinds of coltsfoot, *Pteridium* (58). The same compound named aquilide A was generated as a mutagenic compound after alkaline treatment (59). However, bracken fern has been eaten by people in East Asia like Japanese and Koreans for centuries, and the plant growing naturally was understood to be good for health with the psychology of herbal medicine. Bracken fern in early spring was a favorite dish for Japanese. Although the ferns are boiled and the water is discarded before eating, ptaquiloside is not totally removed. It is ironic that educated people in Japan still believe that bracken is a healthy food.

**Pyrolizidine alkaloids from plants**

Pyrolizidine alkaloids are present in various edible plants, and some of them have been shown to be mutagenic in *Salmonella* strains and carcinogenic in rodents (60, 61). Petasisenine is present in a kind of coltsfoot, *Petasites japonicus* Maxim, whose young flower stalks have been used as a food and for herbal remedies in Japan. Senkirkine is the main alkaloid component in another coltsfoot, *Tussilago farfara* L., the dried buds of which are also taken as a herbal remedy in China and Japan. Petasisenine and senkirkine are responsible for the carcinogenicity of two kinds of coltsfoot, *P. japonicus* Maxim and *T. farfara* L., respectively, inducing liver tumors.
in vegetables – and demonstrably carcinogenic. Dimethylamine in Flavonoid and related compounds in plants; fruits and Cycasin from cycad nuts

Cycad plants grow in tropical areas. Their nuts have been used as a source of starch by the inhabitants of Guam, and the Amami Oshima and Miyako Islands of Japan. Cycad nuts have been shown to induce tumors in rats with the β-d-glucoside of methylazoxymethanol, cycasin, as the responsible agent (62,63). However, cycasin shows no carcinogenicity in germ-free rats, and it has been established that hydrolysis of cycasin by intestinal microflora is required to yield the ultimate form, methylazoxymethanol. This latter acts as a methylating agent and induces colon cancers in rats (64). Nowadays, the natives of Guam, and the Amami Oshima and Miyako Islands of Japan have almost no occasion to eat cycad nuts, and therefore the possibility of human tumor induction is limited. Hydrazines from mushrooms

The commonly eaten cultivated mushroom, Agaricus bisporus, contains a hydrazine compound, agaritine (β-N-[γ-L(+)]-glutamyl]-4-hydroxymethylphenylhydrazine), and its decomposition products. Three breakdown products of agaritine are known to be carcinogenic in mice: the N′-acetyl derivative of 4-hydroxymethylphenylhydrazine, 4-methylphenylhydrazine hydrochloride and the tetrafluoroborate form of 4-(hydroxyethyl)benzenediazonium ion (65,66). In addition, uncooked cultivated mushrooms themselves are carcinogenic in mice, inducing tumors in the bone, stomach, liver and lung. Agaritine is also present in the Japanese forest mushroom, Cortinellus shiiitake (67). The edible false morel mushroom, Gyromitra esculenta (68), contains several other carcinogenic hydrazine derivatives, such as the gyromitrin shown to be converted into carcinogenic N-methyl-N-formylhydrazine and methylhydrazine under acidic conditions such as in the stomach (65,68).

Flavonoid and related compounds in plants; fruits and vegetables

When extracts from various plants were subjected to mutation tests, many of them showed positive mutagenicity with or without metabolic activation (69–72). The active principles were identified as quercetin (70), kaempferol orisorhamnetin (71). Rutin and astragalin, glycoside compounds of quercetin and kaempferol, respectively, are not mutagenic before digestion by glycosidase (69). Quercetin and rutin are abundant in citrus fruit, and sulfate esters of isorhamnetin and quercetin are found in large quantities in dill weed (72). The specific mutagenic activity of these flavonoid compounds roughly corresponds to that of polycyclic aromatic hydrocarbons (73). Fortunately, carcinogenicity tests of flavonoids by several laboratories gave negative results (74,75). An NTP study showed only adenomas in the kidney of male rats which are known to be related to a high urinary level of α2u-globulin and not relevant to human carcinogenesis (76).

Quercetin produces diphertheria-toxin-resistant mutants (77), but does not induce hgprt mutants or sister chromatid exchanges in in vitro cultured mammalian cells (78). In vivo, one report indicated micronuclei formation (79) and other studies proved negative (80). The fact that flavonoids are genotoxic in vitro using microbes, but are not carcinogenic at all gives a warning about the simple statement that mutagens are carcinogens and vice versa. Thus, short-term microbial tests to screen environmental carcinogens already lost their scientific basis when flavonoids were proven to be mutagenic in bacteria but non-carcinogenic in vivo in the early 1980s (74,75). Therefore, regulatory agencies are now very careful about interpreting results of microbial assays. In any case, the data from the long-term animal experiments are useful and reliable to indicate the presence or absence of carcinogenicity.

Genistein is a kind of isoflavone abundant in soybeans, with genistein as its glycoside. Genistein is not mutagenic but has been found to promote carcinogenesis in some cases. For example, azoxymethane (AOM)-induced colon cancer was enhanced by genistein (81). On the other hand, induction of breast cancer in female rats by dimethylbenz[a]anthracene administration was significantly suppressed by genistein (82). Nitrite, nitrate and nitrosatable mutagen precursors and carcinogens

Sodium nitrite has been used as a food additive for preservation and as a coloring substance in meat. Dialkylnitrosamines such as dimethylnitrosamine can be produced from dialkylamines such as dimethylamine and nitrite under weakly acidic conditions. This reaction proceeds in the stomach and therefore nitrite and nitrosatable dialkylamines are important components in food. The accepted levels of sodium nitrite in food are under the control of regulatory agencies; for instance, in ham and sausage 50 p.p.m. is allowed in Japan and 200 p.p.m. in the USA.

Discovery of endogenous formation of nitrate (83), followed by demonstration of the presence of nitric oxide synthase (NOS) by purification in 1990 (84), made the situation regarding nitrite much more complex. Endothelial NOS (eNOS), neuronal NOS (nNOS) and inducible NOS (iNOS) yield NO from L-arginine, and the endogenous formation of nitrite produced by the NOS pathway is now understood to be roughly equal to the sum of exogenous nitrite intake (85) and reductive formation of nitrite by bacteria from nitrite. The significance of exogenous intake of nitrite is not diminished, in spite of the discovery of NOS. Dialkynitrosamines are mutagenic with metabolic activation and demonstrably carcinogenic. Dimethylamine in fish meat is the most abundant nitrosatable precursor (86). Feeding of secondary amines and nitrite in combination in vivo results in carcinogenicity (87). There are in fact many nitrosatable compounds. One new example is tyramine, produced by fermentation of soybeans, which reacts with nitrite under acidic conditions yielding mutagenic 3-diazoxyamine (88). Rats given this compound developed oral cavity squamous cell carcinoma (89), 1-Methyl-1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid (MTCA), found in soy sauce produced by fermentation, has two isomers, (−)-1S,3S-MTCA and (−)-1R,3S-MTCA, the former predominating in soy sauce (90). MTCAs with nitrite show strong mutagenicity but carcinogenicity has not been proven. Yang et al. (91) isolated a chlorinated indole derivative from fava beans and its nitrite treatment yielded mutagens but again carcinogenicity was not proven. There is a possibility that many other nitrosatable compounds exist in raw fruits, vegetables, fish and fermented material (92–94) and an extensive survey of this area is warranted.

Pyrolysis products with mutagenicity and/or carcinogenicity other than HCs

Heating food materials results in the formation of PAHs; benzol[a]pyrene is formed in charred parts of biscuits (95) and PAHs in broiled steak (96). There is also a study describing the presence of PAHs in roasted coffee beans (97,98). The roasting process yields mutagenicity, which is not found in raw beans. Materials other than PAHs responsible for
mutagenicity in the pyrolysate are methylglyoxal, glyoxal and diacetyl (99,100). Methylglyoxal and glyoxal are mutagenic to *S.typhimurium* TA100 and especially to *S.typhimurium* TA104, both of which are base pair mutagen detectors. Their potential carcinogenicity has not been fully examined. On the other hand, brewed coffee was found to generate hydrogen peroxide upon leaving it in a cup in the presence of atmospheric oxygen (101,102). Even instant coffee, lyophilized coffee powder, produces hydrogen peroxide after dissolving in hot water, the concentration reaching 200 p.p.m. (102). Hydrogen peroxide is mutagenic and shows weak carcinogenicity with production of adenocarcinomas in the duodenum of mice (103). When hydrogen peroxide was added together with methylglyoxal, a synergistic effect on the mutagenicity was observed (102). The mutagenic activity of instant coffee could be explained by the action of hydrogen peroxide plus methylglyoxal. In contrast, cancer suppressing activities of coffee are available in an experimental animal study (104) and also in the human population in an epidemiological study (105).

**Dioxins in food and alcoholic beverages**

Contamination of food by dioxins is now a hot topic. The compounds are toxic, and toxicity is highly variable from one compound to another and varies greatly among different species of animals. For instance, LD_{50} values of 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD) for guinea pig and DBA mice are 0.6 and 2570 µg/kg, respectively (106). Experiments with chronic skin application of 2,3,7,8-TCDD in female Swiss–Webster mice at a dose of 5 ng/animal, three times per week for 104 weeks revealed the development of fibrosarcoma in the integumentary system. When male hamsters received six i.p. or s.c. injections of 100 µg/kg body wt of 2,3,7,8-TCDD at 4 week intervals for 1 year, squamous cell carcinomas of the skin developed. The receptor for dioxin (AHR) is the same as that for PAHs. There are no data for dioxins but knockout mice for AHR do not develop any tumors with benzo[a]pyrene (107). The large exposure of workers to dioxin in factories occurred in the USA, the Netherlands and Germany. In an accident at the plant at Seveso, Italy, the population of the surrounding area was exposed to dioxin. The high exposure to dioxin has been shown to be linked to a slight increase in cancer incidence in various organs, with a relative risk for all sites of 1.4 (106). Based on the data on animal experiments, and accidental large amount exposure to humans, and considerations of mechanism of action, the IARC defines that 2,3,7,8-TCDD is carcinogenic to humans (106). This is a qualitative statement that one dioxin, TCDD, is a hazard; it is not a quantitative risk analysis and does not consider the relative potency of dioxins in relation to other known carcinogens. In the general public, the two lines of information are mixed up; that dioxin is classified as a human carcinogen and that dioxin is carcinogenic to mice at a low dose. This confusion is translated incorrectly that dioxin is a very potent human carcinogen. It is likely that people believe naturally occurring substances to be safe, but industrially derived materials to be dangerous. On the other hand, epidemiological studies have indicated an association between risk of breast cancer and colon cancer and the intake of well-done meat with relative risks of 4.6 and 2.8, respectively (13,108). These relative risk values are much higher than that for dioxin in Seveso. It should be noticed that the relative risk for cancer by smoking shows much higher values: the relative risk for lung cancer in heavy smokers is around 20 (2). Thus, we need a comprehensive view for the risk evaluation of the compound to human health.

Organizations such as governmental regulatory agencies use the information from the IARC and must make practical decisions regarding control of dioxin exposure in daily life. The IARC is not responsible for the present awkward situation, but it is to be hoped that more attention will be paid in future to the consequence of IARC statements. The fear that minute amounts of dioxin are carcinogenic to humans has resulted in the loss of budgetary resources which could be used for other constructive research.

Alcoholic beverages have also been identified as human carcinogens by the IARC, and excessive consumption of alcoholic spirits results in enhanced occurrence of esophagus, pharynx and liver cancers (109). Avoidance of heavy drinking is recommended by many organizations, but there is no worry about the presence of tiny amounts of alcohol in food and people enjoy reasonable amounts of alcoholic beverages.

**Nutritional macrocomponents and carcinogenesis**

**Total calorie intake and cancer**

Nutrition plays important roles in carcinogenesis through a variety of mechanisms. It is well established that excess calorie intake, resulting in fat deposits, is a risk factor. Animals with diet restriction develop much fewer of tumors than *ad libitum* fed animals (3,4). Digestion, absorption, metabolism and excretion of excess nutrients require oxidative metabolism and produce more active oxygen species which cause DNA damage (110).

**Fat intake and carcinogenesis**

Fat intake, especially animal fat intake has been blamed for increase in cancer incidence. Several epidemiological investigations have suggested that a positive correlation exists between fat intake and incidences of breast, colon and prostate cancers (2,17,18). Enhancing effects of fat on cancer development could be partly explained by the included calories. However, many experiments with alteration of fat quantity and quality under isocaloric conditions tell us that fat intake itself is important for carcinogenesis.

In spite of the long history of studies on fat and cancer, there remains some controversy. It is generally understood that animal fat rich in saturated fatty acids is more closely related to enhancement of carcinogenesis than plant-derived oils. Among the PUFAs, 6 PUFAs such as arachidonic acid serve as substrates for constitutive cyclooxygenase-1 and inducible cyclooxygenase-2, yielding various prostanooids. One of them, prostaglandin E_2 (PGE_2) seems to be involved in colon carcinogenesis. When EP1, one of the receptors for PGE_2, was knocked out in mice, the mice showed resistance to induction of colon lesions by AOM (114). Down stream signal transduction due to PGE_2 stimulation is now being elucidated since this might be crucial for understanding of colon neoplasia.
Heterocyclic Amines: Possible Human Carcinogens.

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Received August 2, 1999; accepted September 20, 1999