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Mutation Research 506–507 (2002) 9–20

Fundamental and Molecular Mechanisms of Mutagenesis

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Comments on the history and importance of aromatic and heterocyclic amines in public health☆

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Received 8 February 2002; received in revised form 3 June 2002; accepted 21 June 2002

Abstract

The carcinogenic risk of aromatic amines in humans was first discovered when a physician related the occurrence of urinary bladder cancer to the occupation of his patients. They were employed in the dyestuff industry, chronically exposed to large amounts of intermediate arylamines. Laboratory investigations disclosed that rats and mice administered specific azo dyes arylamines or derivatives developed cancer, primarily in the liver. Also, at that time, a possible pesticide, 2-aminofluorene, was tested for chronic toxicity, revealing that it rapidly induced cancers in several organs of rodents. This led to investigations on the mode of action of this class of chemicals, including their metabolic conversion. Biochemical activation to more reactive N-hydroxy compounds was found to occur, mostly in the liver, through what is now known as the cytochrome P450 enzyme systems, and also through prostaglandin synthetases. There were species differences. Guinea pigs were resistant to carcinogenesis because of the low titer of the necessary activating enzymes. In target tissues, a second essential reaction was necessary, namely acylation or sulfate ester formation. The reactive compounds produced display attributes of genotoxicity in appropriate test systems. Interest in this class of compounds increased when of Sugimura and colleagues discovered the formation of mutagens at the surface of cooked meat or fish, that were identified as heterocyclic amines (HCAs). These compounds undergo the same type of activation reactions, as do other arylamines. Epidemiological data suggest that meat eaters may have a higher risk of breast and colon cancer. HCAs induced cancer in rats in these organs and also in the prostate and the pancreas. In addition, there is some evidence that they affect the vascular system. The formation of HCAs during cooking can be decreased by natural and synthetic antioxidants, by tryptophan or proline, or by removing the essential creatine through brief microwave cooking prior to frying or broiling. The amounts of HCAs in cooked foods are small, but other components in diet such as ω-6-polyunsaturated oils have powerful promoting effects in target organs of HCAs. On the other hand, the action of HCAs may be decreased by foods containing antioxidants, such as vegetables, soy, and tea. Some constituents in foods also induce phase II enzymes that detoxify reactive HCA metabolites. Additional mechanisms involved decreased growth of neoplasms by intake of protective foods. Possibly, the carcinogenic effect of HCAs is accompanied by the presence of reactive oxygen species (ROS), which are also inhibited by antioxidants. World-wide, there have been many contributors to knowledge in this field. Adequate information may permit now to adjust lifestyle and lower the risk of human disease stemming from this entire class of aryl and HCA.

Keywords: Arylamines; Heterocyclic amines; Formation; Activation; Detoxification; Metabolism; Cancer; Vascular disease

☆ Presented at the 8th International Conference on Carcinogen/Mutagenic N-substituted Aryl Compounds, Washington, DC, November 13, 2001, organized by Drs. R. Sinha and E.G. Snyderwine.

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PII: S0027-5107(02)00147-1

1. Introduction

Human observations at the end of the 19th century by a skilled clinician in the then new dyestuff industry in the German Rhineland led to the report that a number of employees presented with urinary bladder cancer (reviewed in [1,2]). These pioneering findings were extended to workers in the dyestuff industry in Great Britain. Furthermore, the split products of the azo bond in such dyes, aromatic amines, also led to urinary bladder cancer. Thus, heavy occupational exposure to 4-aminobiphenyl and benzidine induced urinary bladder cancer in workers. The same 4-aminobiphenyl is also present in tobacco smoke, and it was noted that smokers were at high risk not only of heart disease and lung cancer, but also of urinary bladder cancer, demonstrated by Tannenbaum and Skipper [3]. These human observations were complemented by laboratory research in animal models initially, and by examination of the mechanism of action of the aromatic amines.

2. The carcinogenic azo dyes and arylamines

The field of the carcinogenic azo dyes was the subject of detailed research by investigators at the McArdle Laboratory, University of Wisconsin, led by Harold Rusch, Elizabeth and James Miller [4,5], beginning about 1944, and with important contributions by Van Potter and Henry Pitot, and their many students. These investigations led to an understanding of structure–activities correlations of the carcinogenicity of many types of azo dyes in rats.

In addition, important advances were made by Boyland, who was initially concerned with industrial carcinogenesis, through studies of workers in the rubber industry, who had urinary bladder cancer [6,7]. Later on, he made major contributions, together with Haddow and colleagues at the Chester Beatty Institute in London, through investigations of the mechanisms of action of many types of arylamines, including some used in hair dyes. Wood and Bonser [8] were involved in this field, together with Clayson (reviewed in [1,9]). Walpole and Williams [10] undertook the synthesis of methyl analogs of 4-aminobiphenyl, used in the dyestuff and rubber industry, based on the hypothesis that methyl substituents would lower carcinogenicity. They discovered, however, that 3-methyl-4-aminobiphenyl and related compounds were actually more carcinogenic, and interestingly gave rise to chemicals that induced colon cancer. 2′,3-Dimethyl-4-aminobiphenyl was used extensively to induce colon cancer in rats for mechanistic studies [11–13]. It was also found that this chemical could cause cancer of the mammary gland and the prostate, and hence was a valuable addition to the list of experimental carcinogens [14,15]. On the other hand, Walpole discovered that 1,3-dimethyl-4-aminobiphenyl, or 3,5, 3′,5′-tetramethylbenzidine, with the 2-*ortho*-methyl groups hindering reactions on the significant amino group, are not carcinogenic, and he and other investigators noted they were not mutagenic [16–18]. Along those lines, Weisburger et al. [19] reported that 3-methyl-2-naphthylamine induced cancer in the colon and mammary gland in rats and formulated the hypothesis that *ortho*-methylarylamines might be carcinogens, verified by the finding that *ortho*-toluidine, the simplest compound of this class, was proven to be carcinogenic [20,21].

3. The carcinogenic 2-acetylaminofluorene and its mechanism of action

Toxicity studies of the proposed pesticide, 2-aminofluorene, were performed by Wilson et al. [22]. They reported that this compound displayed low acute toxicity, but continuing administration to laboratory rodents led to increasing toxicity, and unexpectedly demonstrated that this chemical was highly carcinogenic. Bielschowsky investigated the modification by endocrine factors of such experimental carcinogens, the acetyl derivative 2-acetylaminofluorene [23]. Research on this carcinogen extended by the group of the Millers [5] at the McCardle Laboratory for Cancer Research and by Weisburger and Weisburger [24] at the NCI. The Millers and the Weisburgers visualized the potency of arylamines based on experience with structure–activity correlations and examined the relationship between structure and carcinogenesis. The Weisburgers synthesized the isomeric 1-,2-,3-, and 4-acetylaminofluorene and found that only the 2-isomer was highly carcinogenic. Theoretical chemists in Paris, Bergmann and Pullman (cf. [25]),

and Bernard attempted to interpret structure–activity correlations with aminofluorene by examining electron structure in these ring systems, as they also did with the polycyclic aromatic hydrocarbons, utilizing elaborate calculations and specific assumptions, a heroic effort before the availability of computers. A current approach along those lines has recently appeared [26].

A major biochemical activation reaction was N-hydroxylation [27]. Ring-hydroxylation and conjugation led to the formation of detoxified metabolites [24]. A major element in the activation reaction is performed by cytochrome P450 1A2, although several other cytochromes can also generate reactive metabolites [28]. A second set of activation reactions is sulfation of the N-hydroxy compounds by a PAPS sulfotransferase, mainly with regard to liver carcinogenesis [29,30]. Of great relevance was the essential activation by several isomeric forms of N-acetylases in the extra-hepatic organs, in animal models and in humans [31–33]. Differences in sensitivity as a function of species or individuals are accounted for by distinct levels of the enzymes required. For example, guinea pigs are not sensitive to the carcinogenic action of the arylamines, because they have low titers of enzymes to perform N-oxidation [24]. In several organs, prostaglandin H synthases can catalyse the N-oxidation reaction, often associated with reactions such as peroxidation and peroxyl radical formation and the generation of reactive oxygen species (ROS) [34,35]. In addition to the sulfation and acetylation reactions, there are certain other activation reactions by phase II enzymes for some types of arylamines and species [36,37]. On the other hand, formation of glucuronides through the action of isozymes of UDP-glucuronosyl transferases, or that of glutathione *S*-transferases, yielding conjugated polar derivatives are important detoxification reactions [24,38]. Foods such as vegetables or the black or green tea polyphenols, that increase these phase II enzymes, may, in part, reduce carcinogenesis through such mechanisms [39,40] (Table 1).

The National Center for Toxicological Research, FDA, in Arkansas, conducted an extensive program on dose–response relationships with 2-acetylaminofluorene, attempting to visualize in large numbers of rats the lowest dose of a carcinogen that would have minimal effects, the so-called ED_{01} studies [41]. Recently, the group of Williams demonstrated that certain genotoxic carcinogens, including arylamines, displayed a

Table 1
General conclusions after 100 years of research on arylamines and heterocyclic arylamines

No.	Conclusion
1	The title compounds can induce cancer at specific sites in animal models and in humans.
2	The appropriate chemical structure determines whether a chemical is a potent carcinogen.
3	A methyl group *ortho* to amino group often increases the carcinogenicity, and may alter organotropism.
4	Arylamino compounds or a N-acetyl derivatives have similar effects, since acetylation or deacetylation can occur in vivo.
5	Biochemical oxidation, to form N-hydroxy compounds, mainly by cytochrome P450 1A2, but also other cytochrome P450 enzymes, and by prostaglandin H. synthases is required to elicit not only carcinogenicity, but other actions such as methemoglobin formation.
6	Biochemical reduction by enzymes such as xanthine oxidase reductase of nitro aryl compounds can yield the proximate N-hydroxy intermediate as a function of structure.
7	The N-hydroxarylamines require a second step biochemical activation via *ortho*-acetylation, sulfate ester formation (mainly for liver as target) or other reactive ester biosynthesis.
8	The reactive compounds are genotoxic, i.e. react with DNA and genes at specific codons, yielding a mutated gene.
9	Those compounds also increase the rate of cell duplication through specific mechanisms. The effect is to introduce the mutated gene into daughter cells, the classic initiation reaction.
10	Some chemopreventive agents decrease the rate of cell duplication, permitting the operation of DNA repair systems and restoring the integrity of the normal cell DNA and genes.
11	Other chemopreventive agents increase the level of mainly phase II enzymes, such as glucuronosyltransferases or glutathione transferases, serving to eliminate the reactive carcinogens as inactive metabolites.
12	The intestinal microflora can split phase II conjugates such as glucuronides, and the N-hydroxy compound liberated can be subjected to acetylation to form a DNA reactive, genotoxic metabolite affecting the colon. The N-hydroxy compound liberated can also undergo enterohepatic cycling and metabolisms, or be reduced to the parent amine, that can also be reabsorbed, or, if formed in the lower intestinal tract, to be excreted.

dose–response with a definite threshold [42,43]. Although threshold level of exposure for arylamine carcinogenicity is apparent in animal models, in humans, other environmental factors including diet, as well as specific genetic factors may potentially affect susceptibility to arylamine carcinogenesis. For example, the customary high-fat Western diet may have a promoting action on arylamine-induced cancers and thereby may alter the risk of carcinogenesis at specific organs such as breast, colon, prostate, or pancreas [44].

4. The heterocyclic arylamines, carcinogens in cooked meat

Sugimura in 1976 asked the question about what might be in the fumes emitted during cooking of meat or fish, although Widmark thought there might be "cancer-producing substances in fried meat" in 1939 (cf. [45]). Ames provided an easy and sensitive assay to detect genotoxic carcinogens by determining their mutagenicity in a bacterial system, the now well-known, reliable *Salmonella typhimurium* test [46]. This was used by Sugimura and colleagues, who discovered mutagenicity in fumes from frying meat or fish. An analysis of the surface of the brown part of the cooked foods was found to be even more mutagenic [47,48]. Weisburger, at the American Health Foundation and his associates had been studying the role of dietary fats in essential roles in the development of high incidence types of neoplasia such as in the breast, colon, prostate and pancreas [49]. Dietary fats could act as promoters, but the potential genotoxic carcinogens in the diet were not known at that time. It seemed possible that these mutagens formed during cooking might be likely candidates. Weisburger and a post-doctorate fellow, Spingarn, convinced a fast food restaurant to fry 50 kg of ground, lean meat to the well-done state, and then a pharmaceutical house to extract the fried meat in 2000 l of methanol, and to provide his laboratory with a lyophilized product [50]. This was utilized to develop a separation method by HPLC, employing mutagenicity to locate peaks of interest. Spingarn obtained a brief fellowship to work with Sugimura and associates, where the chemical structure of one of the mutagens, namely 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ), was established [51]. IQ was a newly discovered chemical, a heterocyclic aromatic amine (HCA), an *ortho*-methylamino structure. Systematic studies later provided information on other such chemicals present in cooked meat or fish. Felton and associates made a key discovery based on the use of specialized analytical techniques, using multiple chromatographic steps, combined with mutagenicity [52]. They reported the presence of another HCA, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine, PhIP, in relatively large amounts, but that had lower specific mutagenicity and thus, was missed by previous investigators. This group later developed procedures to minimize HCA formation during cooking and still kill any *Escherichia coli* present in ground beef [53]. They reported on the production of HCAs as a function of heat flow during frying [54]. Gravy and meat drippings contained elevated levels of HCAs [55,56].

This new class of chemicals seems to be formed by Maillard reactions in the presence of creatinine, as reported initially by Grivas et al. [57], Jägerstad et al. [55], Skog [58], and Taylor et al. [59]. These findings accounted for the fact that only foods containing creatine, mostly meat and fish, would give rise to HCAs during cooking, and leading to the *ortho*-aminomethylimidazo ring present in the HCAs. It also explained why cooking of other types of foods does not yield typical HCAs. This research required technical developments, including essential in vitro bioassay systems, the test of Ames [46] for mutagenicity in *S. typhimurium*, a modified *S. typhimurium* more sensitive to HCAs established by Kamataki et al. [60], and of the test of Williams [43], examining DNA repair in hepatocytes. Very valuable are specific adsorption procedures of HCAs from dilute aqueous solutions with blue cotton or blue rayon, to concentrate these compounds, developed by Hayatsu et al. [61]. Structural elements bearing on mutagenicity were reviewed by Hatch et al. [62].

The finding that this new class of mutagens could cause cancer was by the Tokyo group of Ohgaki et al. [63] in mice, of Takayama et al. [64] in rats, and of Tanaka et al. [65] and Weisburger [66] who administered IQ to female rats and discovered a high incidence of cancer in the mammary gland, and adenomas in the pancreas. PhIP is also a mammary gland carcinogen in rats [67,68]. A major series of bioassays were conducted by Ito and coworkers [69,70,72]

and associates in Nagoya (cf. [71]). In rats, certain HCAs induced cancer in mammary gland, colon, pancreas and prostate, target sites in humans that are often linked to dietary factors. This research in rats suggested that the HCAs may be the genotoxic carcinogens associated with human cancer in these target organs. It was documented by Adamson et al. [73] at the NCI that IQ induced primary liver cancer in non-human primates in as little as 3 years, one of the shortest latent periods in tests of chemicals in that system. The proceedings of two international conferences record these and related findings, including discussions of the relevant mechanisms [71,72].

Studies implicating the role of HCAs in human cancer were not far behind, when Gerhardsson de Verdier et al. [74] reported that regular consumers of well done fried meat led to cancer in colon, rectum, and pancreas, mimicking the animal findings. Findings by Sinha and Rothman further supported the involvement of well-done cooked meat consumption and specifically PhIP and MeIQx exposure in human breast and colon cancers [75], and Sinha [Mutat. Res., this issue]. In Sweden, and at IARC similar findings were made and reviewed [76–78]. The problem in interpreting epidemiologic results for colorectal cancer as a function of meat intake is two-fold. Some reports fail to examine the extent of cooking. It is clear that only well-done to very well done meat contains adequate amounts of HCAs [55–58,77]. Secondly, colorectal cancer is a complex disease involving multiple etiologic and genetic factors. Nutritional habits, including type and amount of fats and HCA intake affect mainly the induction of distal colon cancer and to some extent of rectal cancer (in the latter, alcohol also plays a role). For proximal colon cancer, nutrition has little influence, and inclusion of proximal colon cancer is a confounding factor. Biochemical approaches suggested that the aorta and heart are also possible targets [79–81]. Life-long vegetarians have a demonstrated lower incidence of heart disease and several types of cancer, such as in the breast or colon [82,83]. A number of investigators have discussed the presence of HCAs in cooked foods, and the risk of several important types of cancer in consumers (cf. [84–87]). The general consensus from these studies is that intake of well-done meats is associated an increased risk of certain cancers.

Based on the original discovery of Swedish researchers [55,57,58], that the key amino-methylimidazo ring stemmed from creatinine, only muscle meats that contain creatinine, give rise to HCAs upon cooking. The Felton et al. [84,88] recorded that brief microwaving of meats in a vessel that allowed run off of the juices led to the elimination of most of the creatinine. Then, ordinary cooking procedures could follow and produced significantly lower amounts of HCAs. Addition of creatinine produced more HCAs, and thus, the amount of creatinine seems to be a limitating factor. Skog et al. [89] found that carbohydrates lowered mutagen formation. Kikugawa et al. [90] reported similar findings with higher levels of glucose, ascorbate or erythorbate. Our group observed that many types of antioxidants interfered with the production of HCAs. This includes the synthetic BHA, or those present in soy protein products, or the antioxidant polyphenols present in green or black tea [91,92] (Table 2). Also, tryptophan and proline decreased the formation of these mutagens [93].

Virtually all studies in vitro demonstrating genotoxicity of heterocyclic amines (HCA), and of aromatic amines, as a function of chemical structure, have established the general attributes of these chemicals as likely human carcinogens. It has been established that HCAs are carcinogenic in many animal models, not only in those generally used in carcinogenicity bioassays, but also in non-human primates. Interestingly, whereas IQ is carcinogenic, MeIQx was negative in non-human primates apparently because of low N-hydroxylation of MeIQx in this species and preferential glucuronide formation [94]. Currently, a number of new approaches utilizing transgenic animals provide an accelerated and specific means of demonstrating carcinogenicity, and are the basis for investigations at the molecular level [95–98]. Carcinogenesis involves the formation of reactive metabolites that form DNA adducts and induce mutations in specific genes [99–115]. In addition, the expression (or lack of expression) of specific genes may also affect susceptibility to HCA carcinogenesis. For example, doses as low as 2 or 10 ppm of IQ in sensitive heterozygous p53-deficient mice yielded a small, but significant increase in foci of aberrant crypts in the colon. Importantly, a dosage of 0.4 ppm had no effect, possibly providing evidence for a practical threshold [113]. Genes regulating apoptosis, a process that

Table 2
Prevention of the formation of heterocyclic amines

No.	Process
1	Mixing 7–15% weight of soy protein concentrate with ground beef decreases formation of mutagens during frying by about 90% [91].
2	Mixing 2.5 g pectin or 4.0 g textured ProComm® or 4.0 g. Bontrac® (soy protein products) to 50 g beef patties decreased formation of mutagens during frying by 50–60% [91].
3	Mixing 1-3 mM chlorogenic acid or of 10–20 mM butylated hydroxyanisole (BHA) decreased mutagenicity of fried 50 g beef patties by 50–60%, and by 80–90%, respectively [91].
4	Applying a 0.5, 2, 5 or 7% solution in water of a commercial green tea polyphenol, polyphenon 60® to the 2 surfaces of 30 g beef patties led to a dose-related lowering of mutagenicity after frying [89]. Similar tests, but using a black tea polyphenol, polyphenon B® using 159, 175, 521 and 589 mg applied to both sides of 30 g beef patties, decreased mutagenicity by 70,70,90 and 95%, respectively [92].
5	Applying L-tryptophan or L-proline to the surface of ground meat produced a dose-related inhibition of mutagenicity. Also, such inhibition was found in the liquid model reactions of Jägerstad and colleagues [89].

serves to eliminate abnormal cells, may be modulated by specific HCAs as well as by chemopreventive agents and thus may influence arylamine carcinogenesis [116–118]. Future research with techniques such as cDNA microarrays and proteomics may provide further insight into the critical genes affecting susceptibility to HCA carcinogenesis.

5. Modification of the action of heterocyclic amines

Just as it was determined for arylamines, the heterocyclic compounds undergo hydroxylation on the nitrogen to generate the N-hydroxy compounds, proximate carcinogens. This reaction occurs mostly in the liver of diverse species. In the target organs, such as intestinal tract or mammary gland, a conversion of the proximate to the ultimate carcinogen occurs with the biosynthesis of an acetyl ester or similar ester forms. In contrast, C-hydroxylation is a detoxification reaction, and the resulting phenolic compounds undergo conjugation by phase II enzymes such as sulfotransferase, glutathione transferase, or glucuronosyl transferase. Extracts of vegetables, and green or black teas induce these phase II enzymes, and therefore, increase the detoxification reactions [119–126] (Table 3). Specific bacterial systems in the intestinal tract participate in the metabolism of HCA, both the chemical reaching the gut directly, and more frequently, the chemicals and their metabolites being secreted into the intestinal tract through the bile [127–129]. Polymorphisms and differences in expression of genes involved in metabolic processing, such as enzymes involved in the production of reactive metabolites, including cytochrome P4501A2, and other cytochrome P450 enzymes, sulfotransferases, or acetylases may influence genotoxicity [28–33,130–135] (Table 1). With many types of genotoxic carcinogens, there are parallel findings in man and animals, given similar chronic exposure and concentration, although with HCAs, humans seem to be more sensitive [136,137]. This

Table 3
Effect of green tea on percent of the total metabolites of 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ) in 24 h

Metabolites	Males		Females	
	Control	Tea	Control	Tea
5-OH-IQ sulfate	13.5 ± 0.2	16.0 ± 0.3[a]	10.7 ± 0.2	14.1 ± 0.2[a]
5-OH-IQ-glucuronide	24.0 ± 1.0	30.0 ± 3.0[a]	18.0 ± 0.2	25.1 ± 0.2[a]
N-OH-IQ-N-glucuronide	21.8 ± 0.3	24.0 ± 0.4[a]	15.4 ± 0.3	20.3 ± 0.4[a]
IQ sulfamate	31.0 ± 3.0	20.2 ± 0.2[a]	50.2 ± 1.1	35.3 ± 0.4[a]

Urine of male and female F344 rats.

[a] Student's *t*-test, $P < 0.05$, tea vs. control. The data are the mean ± S.D. for the groups of 10 rats for each of the four series. Increases in N- and C-hydroxylation, but especially of glucuronide formation by tea intake led to the results shown; from [126].

is especially true when there are promoters involved, such as those associated with certain dietary fats at these target organs. Although analyses of risk stemming from the intake of HCAs reported relatively low risk assessments, the potentially powerful promotion by ω-6-polyunsaturated oils on HCA carcinogenesis, paralleling that of other carcinogens, deserves further explanation [49,138–140]. Nevertheless, a series of epidemiologic reports suggested that saturated fats were promoters, when the fats were consumed with the fried or broiled meats, a source of HCAs [49,139,140]. Interestingly, fried and broiled fish have been reported to contain various HCAs. However, fish consumption is associated with a lower risk of certain cancers such as colorectal cancer [49,49a]. Although further studies are required, the ω-3-polyunsaturated oils in fish appear to be protective [49].

6. Reactive oxygen species and heterocyclic amine metabolism

The formation of ROS associated with the metabolism of fats and oils, and also that of the HCAs [141–146]. It may well be that the documented effects of the HCAs in damaging the vascular system and the heart, as well as formation of oxidized forms of DNA, may stem from the simultaneous formation of ROS. In contrast, the effect of antioxidants in protecting against HCAs may also be the result of their action in inhibiting the formation and action of ROS [124,145,146].

At a level of DNA, there are adducts at N-2 and C-8 positions of guanylic acid, formed through the reactive metabolites of the HCA. These can undergo DNA repair, and the rate of cell cycling is of great importance in that regard. If cell cycling is enhanced, as it is often as a result of cell damage from the action of exogenous toxicants such as carcinogens, or of organ-specific promoters, DNA damage cannot be repaired. The cell bearing an altered, mutated DNA is the likely precursor to tumor formation. On the other hand, a decreased cell cycling, as is observed with some products like the tea polyphenols, may hinder the fixation of carcinogen-induced mutation and genotoxicity [117,118]. Stimulation of apoptosis by tea polyphenols and other chemopreventive agents would also serve to eliminate tumor cells [116–118].

7. Conclusions

The discovery of the HCAs in the food chain of a large proportion of people in the world has provided impetus in research on the formation and mode of action of these chemicals, as well as practical application to reduce disease risk by modifying their production or increasing their detoxification, as reviewed in this paper, and at the 8th International Conference in general. A number of reviews and papers at conferences have appeared [37,71,72,147], including the 23rd International Symposium of the Princess Takamatsu Cancer Research Fund [148]. Of great relevance is an excellent, recent multi-author monograph edited by Nagao and Sugimura [149]. A fair understanding in this field came about by world-wide collaboration between a number of research groups that testifies to the rapid development possible through multi-disciplinary interactive research. Prevention of chronic diseases is the obviously the ideal means for disease control. Healthy people require little medical care, and thus, preventive public health approaches would lower medical care expenses.

Acknowledgements

I am indebted to Ms. Nancy Rivera for her excellent administrative support and to the editors of the Conference papers for their gracious, helpful advice. I am also grateful to the Friends Against Cancer Team for support of research in my laboratory.

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