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RISK OF CARCINOGENESIS FROM EXPOSURE TO DIETARY MUTAGENS

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Summary. - *Epidemiological studies revealed the crucial role of dietary habits in human cancer. This review will summarize literature data on naturally occurring mutagens and carcinogens. The risk of carcinogenesis related to dietary mutagens will be discussed.*

Riassunto (Rischio di cancro per esposizione a mutageni nella dieta). - *Gli studi epidemiologici hanno messo in evidenza il ruolo cruciale delle abitudini alimentari nell'induzione di cancro. Questa rassegna riassume i dati di letteratura sui composti mutageni e cancerogeni presenti negli alimenti. Il rischio di cancerogenesi associato alla dieta verrà particolarmente discusso.*

Introduction

Epidemiological data have shown that diets and lifestyles are closely related to human cancer. Many immigrant studies revealed the importance of dietary habits in inducing cancers of digestive tract. Food contain various types of mutagens and carcinogens, and contain both initiators and promoters of carcinogenesis [1-4].

Trials to detect known carcinogens in food were made by Lijinsky and Shubik [5] and Kuratsune [6] and they found the presence of aromatic hydrocarbons in some cooked food. However, it was difficult to search for new carcinogens in food because animal experiments are very time consuming and investigations need good analytical chemical background.

In general mutagens and carcinogens in food may be: a) naturally occurring constituents especially in edible plants or spices; b) N-nitroso compounds which are formed from precursors in food and nitride by nitrosation reaction during cooking and processing or in the gastrointestinal tract; c) heterocyclic amines and polycyclic aromatic hydrocarbons, mainly pyrolysis products of amino acids and proteins; d) mutagenic dicarbonyl compounds produced by heating carbohydrates or by fermentation; e) mutagens formed by (amino carbonyl reactions) browning reaction.

In my present paper I do not want to deal with the role of food additives [7-9] (f) and contaminants [10, 11] (g) in the experimental carcinogenesis.

The Table 1 demonstrates the most important naturally occurring mutagens and carcinogens.

Pyrrolizidine alkaloids [12] are present in thousands of plant species. About 30 alkaloids are recognized as being hepatotoxic. Some of them are ingested by humans, particularly in herbs and herbal teas and occasionally in honey and can cause lung and liver lesions [13]. Mutagenic activity and carcinogenic effect have been described for the two most important representatives e.g. lasiocarpine and retrorsine.

Other pure alkaloids have been found to be hepatocarcinogenic in rats and some others have also been shown to be carcinogenic in different experimental animals [14].

Particularly, diester and cyclic diester pyrrolizidine alkaloids which contain necine, retronecine, and otonecine, are strongly animal hepatocarcinogens.

Among the most widespread of the known naturally occurring mutagens of plant origin are the *flavonoids* [15]. Among the flavonoids *quercetin*, *kaempferol* and *galangin* have been shown to be mutagenic [16]. Quercetin occurs in conjugated or free forms in many edible plant products, including fruits, vegetables (e.g. onion), tea, red wine, dillweed, sumac, and bracken fern. They are carcinogenic for the jejunal and bladder epithelium of the rats [17, 18].

Bracken fern is consumed in certain areas of the world as a food delicacy and salad green. Recently [19], bracken carcinogen ptaquiloside was successfully isolated form bracken fern by following the active principle with a carcinogenicity test. All ptaquiloside treated rats had mammary cancer. Multiplex ileal adenocarcinomas and urinary bladder tumors were also observed.

Table 1. - *Naturally occurring mutagens and carcinogens in edible plants*

1.	*Pyrrolizidine alkaloids*	(Hirono, 1986)
	Lasiocarpine	(M, C)
	Retrorsine	(M, C)
	Satidine	(M, C)
	Necine	(M, C)
	Retronecine	(M, C)
	Otonecine	(M, C)
2.	*Flavonoids*	
	Quercetin	(M, C)
	Kaemferol	(M, C)
	Galangin	(M)
	Fisetine	(M)
	Rutin	(M)
	Astragalin	(M)
	Morin	(M)
	7,8 Benzoflavone	
3.	*Bracken fern*	
	Pteroside	
	Pterosin	
	Pterolactam	
	Tannin	
	Shikimic acid	
	Ptaquiloside	(M, C)
	Quercetin	
	Aquilide A	(M) (Van der Hoeven, 1983)
4.	*Alkylbenzene derivatives*	
	Safrol	(weak C, metabolites are M)
	Estragole	(weak, C, M)
	Methyl engenol	(weak C)
	Isosafrol	(weak C)
	Beta-asarone	(weak C)
5.	*Betel nut*	(M, C)
	Areca derived nitrosamines:	
	N-nitrosoguvacoline	(NG)
	N-nitrosoguvacine	(NGC)
	N-(nitrosomethylamino)propionitrile (NMAP)	
	N-nitroso(3-oxopropyl)methylamine (NOPMA)	
	Tobacco specific nitrosamines (NNN, NNK)	(Prokopczyk *et al.* 1987)
6.	*Hydrazines*	
	N-methyl-N-formylhydrazine (M, C)	
	Agaritine(4-hydroxymethyl-phenylhydrazine) (M, C)	
	Acetaldehyde-methyl-formyl-hydrazone (M, C)	
	N-methyl-hydrazine (M, C)	
7.	*Cycad azoxyglycosides (cacasin)*	
	Cycasin (C only in conventional animals)	
	MAM (methylazoxymethanol)	(M, C)
	MAM-acetate	(M, C)
8.	*Linear furocumarins*	
	Psoralens (light-activated carcinogens)	
9.	*Quinones and their phenol precursors*	
	Anthraquinon	(M)
	Chrysazin (synth. Anthraquinon)	(M, C)
	Dihydroxylated phenols	
	catechol (skin-promoter)	
	Trihydroxylated compounds	
	pyrogallol (clastogenic)	
	gallic acid (clastogenic)	
10.	*Vicia faba*	
	Toxins: vicine	
	convicine	
	divicine	
	Indols: 4-methoxyindol (NOC precursor)	
	nitrosochloroindo (strong mutagen)	
	(Sugimura *et al.*, 1986; Correa, 1987)	
11.	*Alkyl isothiocyanate*	
	Clastogenic (in hamster cell)	
	Carcinogenic (in rat)	

M: mutagenic
C: carcinogenic

It seems to be evident that ptaquiloside is the only carcinogenic principles of bracken fern. It was also demonstrated that acut bracken-poisoning in cattle can be produced with ptaquiloside. Van der Hoeven *et al.* [20] isolated from bracken fern a new mutagenic compound which has the same planar structure as ptaquiloside and named it Aquilide A.

Numerous *alkylbenzene* (alkyl, propenyl) *derivatives* occur in oils of a wide variety of plants [21]. Among these naturally occurring compounds safrol, estragol, methyl eugenol, isosafrol and beta-asarone have weak or moderate hepatocarcinogenic activity in mice and rats. These agents occur in mixed human diet at very low levels (e.g. in black pepper). Human intake of black pepper is over 2 mg/kg/day.

Betel nut has been considered as one of the important causative factors in the high incidence of human oral cancer in many Asian countries [22].

In animal experiment mice skin was painted with an extract of a typical betel (tobacco quid), and squamous cell carcinomas and papillomas occurred in the painted area [23]. Several authors suggested that tobacco contains materials which are not carcinogenic themselves, althought can enhance the carcinogenic effect of substances present in betel nut. Indian scientists suggested [24] that the habit of chewing betel, in association with a pungent diet, malnutrition, vitamin A deficiency as well as poor oral hygiene, leads to the development of cancer in the oral cavity.

Animal experimental data suggest the possibility that betel quid ingredients have some carcinogenic or tumor promoting activities in the liver as well as the upper digestive tract. Regardless of the intensive studies on the carcinogenicity of betel quid, active principles clearly carcinogenic have not yet been isolated from betel nuts or other ingredients of the quid [25].

It is well known that the betel nut contains several pyridine alkaloids such as arecoline, guvacoline, arecaidine and guvacine [22].

It has been justified that from betel constituents in the presence of salivary nitrite and other factors N-nitroso compounds could be formed. Arecoline gave rise to N-nitrosamines such as 3-(methylnitrosamino)-propionitrile (MNPN) and 3-(methylnitrosamino)-propioaldehyde [26-28].

The former is potent animal carcinogen. Very recently stated that the saliva of chewers contains the areca derived N-nitrosoguvacoline, and other tobacco specific nitrosamines when tobacco is added to the quid [29].

Most *hydrazines* that have been tested are carcinogens and mutagens, and large amount of carcinogenic hydrazines are present in edible mushrooms. The widely eaten *Gyromitra esculenta* contains 11 hydrazines, three of which are known carcinogens. One of these, N-methyl-N-formylhydrazine, causes lung and blood vessels tumors in mice. The most common mushroom, *Agaricus bisporus* contains *agaritine*, derivative of the mutagenic 4-hydroxymethylphenyl-hydrazine. Some agaritine is metabolized

by the mushroom to a diazonium derivative which is a very potent carcinogen. *Gyromytrin* can be converted at low pH to methylhydrazine which is an indirect mutagen in Ames test and a potent colon carcinogen in mice [21, 30, 31].

The nuts and roots of the *cycad plant* is a source of food for some native groups in Guam, Kenya, and the Miyak Islands of Japan. Studies carried out showed that cycad nuts are carcinogenic on oral administration to rats, and demonstrated that one of their component *cycasin* is a potent carcinogen for the liver, kidney and intestine of rats. Cycasin is readily hydrolyzed by bacterial enzymes (beta-glucosidases) in the gastrointestinal tract to *methylascoxymethanol* (MAM), which is further metabolized to the potent alkylating agent methyldiazonium hydroxide [32]. Several studies support the conclusion that the carcinogenic component of cycad meal is cycasin and that MAM is the proximate carcinogen of cycasin.

MAM and MAM-acetate lead to decrease in DNA, RNA and protein synthesis, and methylate both DNA and RNA *in vitro* and *in vivo*. It seems to be important that the carcinogenicity of cycasin depends on the release of MAM, catalyzed by enzymes of the microflora of the gut. Thus, cycasin is carcinogenic in conventional animal but not in germ free rodents, while MAM is tumorigenic in both [33].

Linear *furocumarins* such as *psoralen derivatives* are potent high-activated carcinogens and mutagens and are widespread in barley, parsnips, figs and celery. Psoralens, when activated in sunlight *damage* DNA and induce tanning more rapidly than the ultraviolet component of sunlight which is also a carcinogen [21].

Quinones and their *phenol* precursors [34] are widespread in human diet. Mutagenic anthraquinone derivatives are found in plants such as rhubarb and mould toxins.

Dihydroxylated phenols such as catechol, resorcinol, caffeic acid, and trihydroxylated phenols such as pyrogallol and gallic acid have chromosome demaging potential in CHO cells and are mutagenic in Ames test. Catechol, for example, is a tumor promoter of animal carcinogenesis [35], and an inducer of DNA *damage*.

Fava bean *(Vicia faba)* is a common food. Contains toxins vicine, convicine, and divicine. The latter is a hydrolyzed form of vicine. But fava bean also contains indols such as 4-chloro-6-methoxyindol which can be converted to a strong mutagen compound in the presence of nitrite [36]. Coworkers of the MIT using epidemiological data from Colombia that showed a high intake of fava bean by high-risk population identified a nitrosochloroindol, a very potent mutagen after nitrosating fava beans [37]. Its carcinogenic potential has not been evaluated. It whould be very relevant in the search for *in situ* synthesis of N-nitroso carcinogens in humans.

Allyl isothiocyanate, a major ingredient in oil of mustard and horse-radish and has been shown to cause chromosome aberrations in hamster cells and to be carcinogen in rats [21].

It seems that there are many tumor initiators in our environment. Meanwhile, the two (or multi-) step carcinogenesis has been widely accepted by experimental and clinical oncologists as well as epidemiologists. The crucial step in the development of human cancer may be the second stage, that of promotion, not the first, tumor initiation stage. *Phorbol esters* isolated from croton oil have been most intensively studied as tumor promoters. They may have been a cause of cancer in China and esophageal cancer in Curacao [37]. For a long time, the phorbol ester TPA (12-O-tetradecanoyl-phorbol-13-acetate) has been used in experimental cancer research as typical tumor promoter [37-39].

In Sugimura's laboratory, a survey was successfully made to detect other tumor promoters that were as effective as TPA (Fig. 1). As a result *dehydroteleocidin B, teleocidin* and *lyngbyatoxin A* were found to induce ODC and exert various biological activities *in vitro* [40].

Teleocidin is a product of streptomyces and dihydroteleocidin B, is a catalytically dehydrogenated derivative of telelocidin B. Lyngbyatoxin A is produced by the blue-green alga lyngbya majuscula.

Teleocidin and lyngbyatoxin A are indol alkaloids [41].

Later *aplysiatoxin* and *debromoaplysiatoxin* were also isolated from an other variety of the blue-green alga. They have strong promoter effect on carcinogenesis [18, 42].

Since indol alkaloids and polyacetates are structurally unrelated to TPA, it was of interest to determine and compare their mechanism of action. They found that TPA, teleocidin and lyngbyatoxin A act via the same receptor system on the cell membrane. In the case of polyacetate derivatives, it seemed that debromination greatly enhanced the effect on the membrane [43].

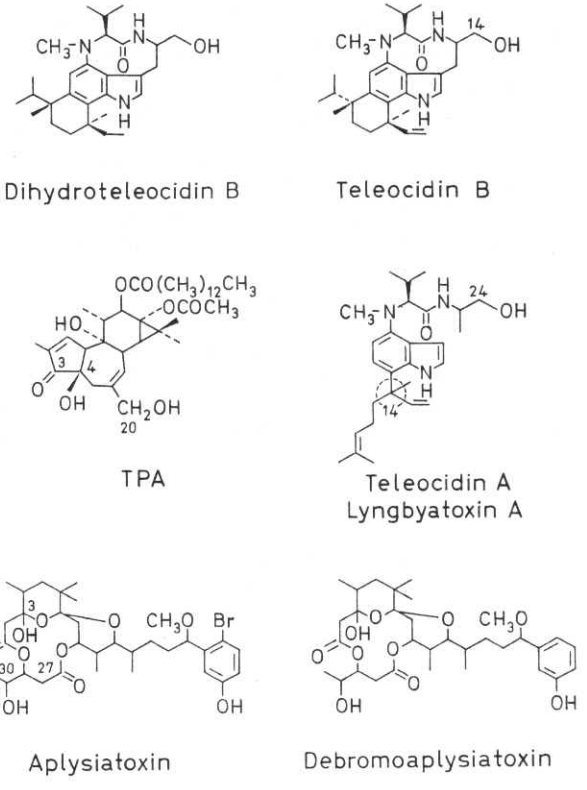

Fig. 1. - Structures of new classes of tumor promoters.

Heterocyclic amines and related compounds

After the overlapping of mutagens and carcinogens was established mainly from data obtained by using Ames test, it became possible to detect the presence of probable carcinogens in food by detecting mutagenic activity of food. The formation of mutagens upon broiling dried fish and ground meat was first noticed by Japanese authors.

These observations urged them to identify mutagens during cooking. These compounds could probably be produced from creatinine, aldehydes, and Maillard reaction products. First they [44-46] identified from broiled dried sardines and beef IQ, MeIQ, MeIQx and later 4,8DiMeIQx and 7,8DiMeIQx. All compounds have a common *2-amino imidazol* structure (Fig. 2). The former two are quinolin congeners, while the others are quinoxalin congeners [47, 48, 42].

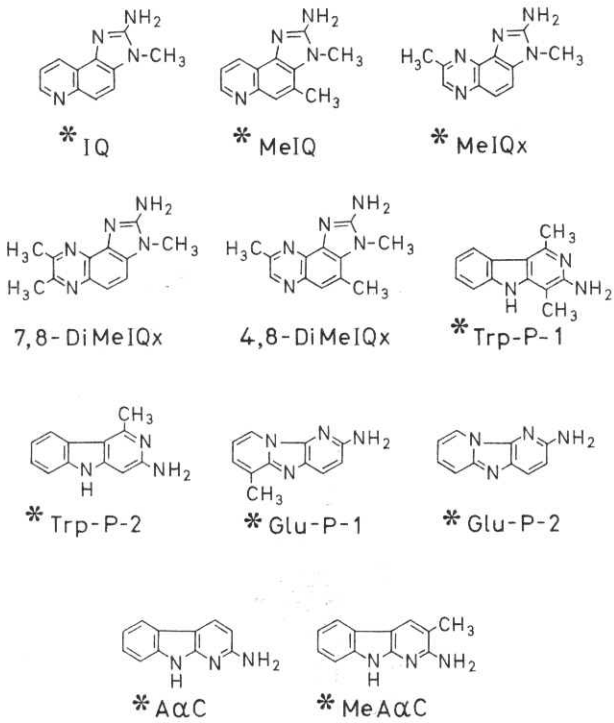

Fig. 2. - Structures of mutagenic heterocyclic amines.

IQ, MeIQ and MeIQx bear a structural similarity to the potent colon, breast and prostate carcinogen DMAB (3,2'-dimethyl-4-aminobiphenyl).

Other type of heterocyclic amines were isolated from pyrolisate of amino acids and proteins. They are Trp-P-1 and Trp-P-2 from tryptophan pyrolisate, and Glu-P-1 and Glu-P-2 from glutamic acid pyrolysate. *Amino-α-carboline* (AαC) and *methyl-amino-α–carboline* (MeAαC) from a pyrolysate of soybean globulin (Fig. 2). They have a common 2-amino-pyridine structure [48-50].

Compounds in the third group (Har, NorHaR and Lys-P-1) are heterocyclic amino compounds. These heterocyclic amines are highly mutagenic (Table 2) toward *S. typhimurium* TA98 with S9 mix [51, 52].

The pyrolysis products induced diphteria toxin and quabain resistance, chromosomal aberration, SCE and *in vitro* transformation in mammalian cells. They also induced 8-azaguanin resistance, chromosomal damage and SCE in human cells [2].

In carcinogenicity studies IQ induced predominantly hepatomas, forestomach carcinomas and lung tumors in mice (Table 3). MeIQ induced squamous cell carcinomas in the forestomach with liver metastases, and hepatocellular carcinomas in mice. MeIQx proved to be non carcinogenic.

Table 2. - *Mutagenic activities of heterocyclic amines toward* S. typhimurium *with S9 mix*

	Mutagenic activity (revertants/μg) TA98	TA100
IQ	433,000	7,000
MeIQ	661,000	30,000
MeIQx	145,000	14,000
4,8-DiMeIQx	183,000	8,000
7,8-DiMeIQx	163,000	9,900
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
AaC	300	20
MeAaC	200	120

Table 3. - *Carcinogenicity of IQ, MoIQ and MeIQx in CDF$_1$ mice*

Compound	Sex	Mice examined (no.)	Liver	Forestomach	Intestine	Lung
IQ	Male	39	16 *(a)*	16	0	27
(0.03%)	Female	36	27	11	0	15
MeIQ	Male	38	7	35	15	12
(0.04%)	Female	39	27	34	6	7
MeIQx	Male	11	1	1	0	1
(0.06%)	Female	15	5	0	1	2
None	Male	33	3	1	0	7
	Female	38	3	0	0	7

(a) number of mice with tumors

Trp-P-1 and Trp-P-2 induced hepatocarcinomas, predominantly in female mice (Table 4). Tumors of groups treated with Glu-P-1, Glu-P-2 Me-AαC (methyl-amino-alfa-carboline) and AαC are summarized on the same table (Table 4). Distribution of rat tumors after IQ treatment are summarized on Table 5. Tumors in F344 rats after Trp-P-1, Glu-P-1, Glu-P-2 treatment are shown on Table 6.

Recently Ishikawa *et al.* [53] observed the activation of Ha-*ras* oncogene in a rat hepatoma which was induced by IQ feeding. They also found and activation of *raf* oncogene in another rat liver tumor induced by IQ. In an Glu-P-2 induced adenocarcinoma in the small intestine, the activation of N-*ras* oncogen was detected.

Mutagens other than heterocyclic compounds formed during cooking

Kinouchi *et al.* [54] and Ohnishi *et al.* [55] observed the formation of *1-nitropyrene* and *dinitropyrenes* in grilled

Table 4. - *Carcinogenicity of heterocyclic amines isolated from pyrolysates of amino acids and protein in CDF$_1$ mice*

Compound	Sex	Effective (no.)	Liver	Blood vessel
Trp-P-1	Male	24	5 *(a)*	0
(0.02%)	Female	26	16	0
Irp-P-2	Male	25	3	0
(0.02%)	Female	24	22	0
Glu-P-1	Male	34	4	30
(0.05%)	Female	38	37	31
Glu-P-2	Male	37	10	27
(0.05%)	Female	36	36	20
AαC	Male	38	15	20
(0.08%)	Female	34	33	6
MeAαC	Male	37	21	35
(0.08%)	Female	33	28	28
None	Male	39	0	0
	Female	40	0	0

(a) number of mice with tumors

Table 5. - *Tumors in F344 rats fed 0.03% IQ*

Compound	Sex	Effective (no.)	Liver	Intestine		Zymbal gland	Clitoral gland	Skin	Oral cavity
				Small	Large				
IQ	Male	40	27 *(a)*	12	25	36	-	17	2
	Female	40	18	1	9	27	20	3	1
None	Male	50	1	0	0	0	-	0	0
	Female	50	0	0	0	0	0	0	0

(a) number of rats with tumors

Table 6. - *Carcinogenicity of Trp-P-1, Glu-P-1 and Glu-P-2 in F344 rats*

Compound	Sex	Effective (no.)	Liver	Intestine		Zymbal gland	Clitoral gland
				Small	Large		
Trp-P-1							
(0.015%)	Male	40	30 *(a)*	1	2	0	-
(0.02%)	Female	40	37	1	0	0	0
Glu-P-1	Male	42	35	26	19	18	-
(0.05%)	Female	42	24	10	7	18	5
Glu-P-2	Male	42	11	14	6	1	-
(0.05%)	Female	42	2	8	8	7	11
None	Male	50	2	0	0	0	-
	Female	50	0	0	0	0	0

(a) number of rats with tumors

chicken. Nitropyrens are the most potent mutagens detected widely in the environmental pollutants. Their widespread occurrence is not surprising because NPs are readily formed by exposure of pyrene to NO_2. In addition, if pyrene formed in incomplete combustion of fats in food is exposed to NO_2 in burning urban gas, mutagenic nitro derivatives would readily induced. NPs are carcinogenic.

The same Japanese group [56] have detected 1-NP in grilled food such as corn, mackerel, pork, beef and bacon, but not in white chicken whose fat content is low. Roasting of coffee beans yields mutagenic activity. Some portion of the mutagenicity in coffee can be accounted for by methylglyoxal. The co-present of hydrogen peroxide and methylglyoxal showed enhanced mutagenicity [57].

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