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Carcinogenicity in Mice and Rats of Heterocyclic Amines in Cooked Foods

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Carcinogenicities of mutagenic heterocyclic amines in cooked foods have been tested in CDF_1 mice and F344 rats of both sexes. Eight heterocyclic amines—Trp-P-1, Trp-P-2, Glu-P-1, Glu-P-2, MeAαC, AαC, IQ, and MeIQ—were given to mice and/or rats at 0.02 to 0.08% in the diet continuously. In mice, all heterocyclic amines tested were demonstrated to be carcinogenic. Hepatocellular carcinomas were induced in a high incidence in all groups treated with heterocyclic amines. Hemangioendothelial sarcomas were also induced by Glu-P-1, Glu-P-2, MeAαC, and AαC. Most hemangioendothelial sarcomas were located in the interscapular brown adipose tissue. In mice given IQ, forestomach and lung tumors were also observed in a high incidence. Carcinogenicity tests on MeIQ are ongoing, and interim data by week 83 show that MeIQ also induces forestomach tumors in addition to liver tumors.

In rats, hepatocellular carcinomas were induced by Trp-P-1, Glu-P-1, Glu-P-2, and IQ. In rats given Glu-P-1, Glu-P-2, and IQ, adenocarcinomas in the small and large intestines, squamous cell carcinomas in the Zymbal gland and clitoral gland were also observed in a high incidence.

Introduction

Since charred parts of broiled fish and meat have been shown to have high mutagenicity to *Salmonella typhimurium* TA 98 and TA 100 (*1,2*), heterocyclic amines with potent mutagenicity have been isolated from pyrolysates of amino acids, proteins, and broiled fish and meat. They are 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-1) and 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-2) from tryptophan pyrolysate (*3*), 2-amino-6-methyldipyrido[1,2-*a*:3',2'-*d*]imidazole (Glu-P-1) and 2-aminodipyrido[1,2-*a*:3',2'-*d*]imidazole (Glu-P-2) from glutamic acid pyrolysate (*4*), 2-amino-3-methyl-9*H*-pyrido[2,3-*b*]indole (MeAαC) and 2-amino-9*H*-pyrido[2,3-*b*]indole (AαC) from soybean globulin pyrolysate (*5*), 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ) and 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline (MeIQ) from broiled sardine (*6,7*), and 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx) from broiled beef (*8*).

Since daily life style, especially dietary habits, are regarded as possible causative factors in the development of human cancer (*9–11*), it is important to clarify whether these mutagenic heterocyclic amines in cooked foods are carcinogenic.

We report in this paper that eight heterocyclic amines, Trp-P-1, Trp-P-2, Glu-P-1, Glu-P-2, MeAαC, AαC, IQ, and MeIQ are carcinogenic to mice and/or rats.

Materials and Methods

Synthetic Trp-P-1 acetate, Trp-P-2 acetate, MeAαC acetate, AαC acetate, IQ, and MeIQ were obtained from Nard Institute (Osaka, Japan). Glu-P-1·HCl and Glu-P-2·HCl were obtained from Katsura Chemical Co. (Tokyo, Japan). These heterocyclic amines were added to a pellet diet (CE-2; CLEA Japan, Tokyo) at 0.02 to 0.08%.

CDF_1 mice [(BALB/cAnN × DBA/2N)F_1] and F344 rats of both sexes were obtained from Charles River Japan (Atsugi, Kanagawa). Mice and rats were 6 to 7 and 8 weeks old, respectively, at the start of the experiment. Experimental groups of 40 or 42 males or females were given pellet diet containing each heterocyclic amine continuously during the experiment.

All the animals which died or became moribund were carefully autopsied. All organs were fixed in 15% neutralized formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin.

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Table 1. Incidence of liver tumors in mice given Trp-P-1 and Trp-P-2 (0.02%).

Chemical	Sex	Effective no.	No. of mice with liver tumors		Hemangioma	Total (%)
			Hepatocellular adenoma	Hepatocellular carcinoma		
Trp-P-1	M	24	1	4	0	5 (21)
	F	26	2	14	0	16 (62)
Trp-P-2	M	25	1	3	0	4 (16)
	F	24	0	22	0	22 (92)
Control	M	25	0	0	1	1 (4)
	F	24	0	0	0	0 (0)

Table 2. Incidence of liver tumors and blood vessel tumors induced in mice given Glu-P-1 and Glu-P-2 (0.05%) and MeAαC and AαC (0.08%).

Chemical	Sex	Effective no.	No. of mice with liver tumors				No. of mice with blood vessel tumors		
			Hepatocellular adenoma	Hepatocellular carcinoma	Others	Total (%)	Hemangio-endothelioma	Hemangio-endothelial sarcoma	Total (%)
Glu-P-1	M	34	4	0	0	4 (12)	4	27	30 (88)
	F	38	13	24	3	37 (97)	3	28	31 (82)
Glu-P-2	M	37	5	4	1	10 (27)	3	25	27 (73)
	F	36	6	30	0	36 (100)	3	19	20 (56)
MeAαC	M	37	12	9	0	21 (57)	0	35	35 (95)
	F	33	13	15	0	28 (85)	0	28	28 (85)
AαC	M	38	6	9	0	15 (39)	2	17	20 (53)
	F	34	3	30	0	33 (97)	0	6	6 (18)
Control	M	39	0	0	0	0 (0)	0	0	0 (0)
	F	40	0	0	0	0 (0)	0	0	0 (0)

Table 3. Incidence of liver tumors in mice given IQ (0.03%).

Chemical	Sex	Effective no.	No. of mice with liver tumors			
			Hepatocellular adenoma	Hepatocellular carcinoma	Hemangio-endothelioma	Total (%)
IQ	M	39	8	8	0	16 (41)
	F	36	5	22	0	27 (75)
Control	M	33	2	0	1	3 (9)
	F	38	0	0	3	3 (8)

Results

Carcinogenicity of Heterocyclic Amines in Mice

Trp-P-1 and Trp-P-2. A high incidence of liver tumors was induced between experimental days 402 and 621 in mice given Trp-P-1 and Trp-P-2 at a concentration of 0.02% (Table 1) (*12*). In both groups fed these compounds, females were more susceptible to development of liver tumors than males. Histologically these liver tumors were hepatocellular carcinomas or hepatocellular adenomas. Metastases of liver tumors to the lung were observed in two female mice given Trp-P-2.

Glu-P-1, Glu-P-2, MeAαC, and AαC. As summarized in Table 2, Glu-P-1, Glu-P-2, MeAαC, and AαC induced blood vessel tumors as well as liver tumors in high incidence when given to mice at concentrations of 0.05 or 0.08% (*13*). Most blood vessel tumors were hemangioendothelial sarcomas, and a few were hemangioendotheliomas; 70 to 90% of blood vessel tumors were located in the interscapular brown adipose tissue in all experimental groups except the group treated with AαC, and a few were observed in the pleural cavity, abdominal cavity, and axilla. About half of the blood vessel tumors induced by AαC were in the abdominal cavity.

Most liver tumors were hepatocellular carcinomas and hepatocellular adenomas and a few were cholangiocellular carcinomas. Incidence of liver tumors was significantly higher in females than in males with all compounds.

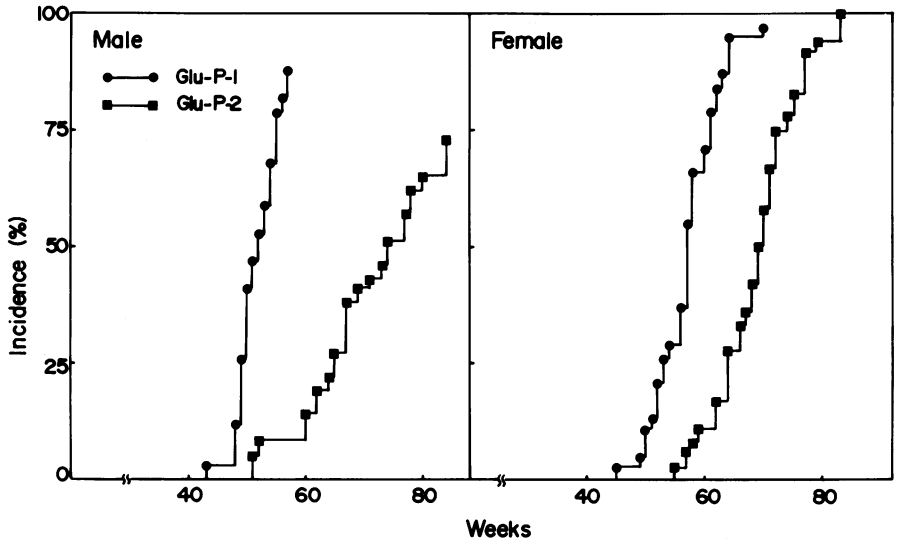

FIGURE 1. Cumulative incidence of liver and blood vessel tumors induced by Glu-P-1 and Glu-P-2 in mice.

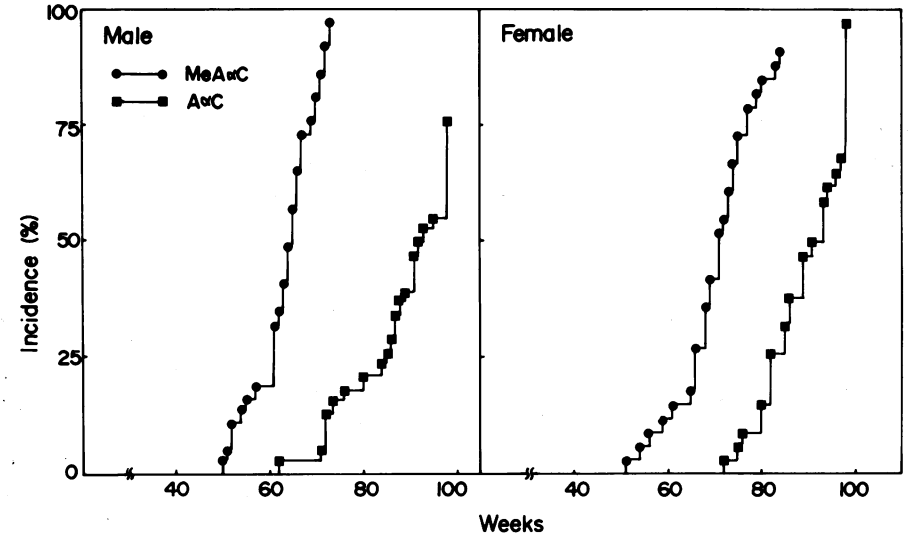

FIGURE 2. Cumulative incidence of liver and blood vessel tumors induced by MeAαC and AαC in mice.

Table 4. Incidence of forestomach and lung tumors in mice given IQ (0.03%).

			No. of mice with forestomach tumors			No. of mice with lung tumors		
	Sex	Effective no.	Papilloma	Squamous cell carcinoma	Total (%)	Adenoma	Adenocarcinoma	Total (%)
IQ	M	39	11	5	16 (41)	13	14	27 (69)
	F	36	8	3	11 (31)	7	8	15 (42)
None	M	33	1	0	1 (3)	4	3	7 (21)
	F	38	0	0	0 (0)	3	4	7 (18)

Table 5. Incidence of liver and forestomach tumors in mice given MeIQ (0.04%) by week 83 of the experiment.

Sex	Initial no. of mice	No. of mice examined	No. of mice with liver tumor			No. of mice with forestomach tumor	
			Hepatocellular adenoma	Hepatocellular carcinoma	Cholangio-carcinoma	Papilloma	Squamous cell carcinoma[a]
M	40	16	0	0	1	2	10 (6)
F	40	22	3	6	0	5	11 (4)

[a] Numbers in parentheses are those of mice with metastases of squamous cell carcinoma to the liver.

Cumulative incidence of liver and blood vessel tumors are shown in Figures 1 and 2. Development of liver and blood vessel tumors was 10 to 20 weeks later in groups treated with Glu-P-2 than in the groups treated with Glu-P-1 in both sexes. Tumor development was about 20 weeks later for liver and blood vessel tumors in mice given AαC than in mice of both sexes given MeAαC. Thus the carcinogenic potency of Glu-P-1 was slightly higher than that of Glu-P-2, and MeAαC showed slightly more potent carcinogenicity than AαC.

IQ. When mice were given IQ at a concentration of 0.03%, tumors were observed in three organs, the liver, forestomach, and lungs (Tables 3 and 4) (*14*). Liver tumors were macroscopically multiple yellowish white tumors and histologically hepatocellular carcinomas or hepatocellular adenomas. Forestomach tumors were squamous cell carcinomas or papillomas. Most squamous cell carcinomas were of a well-differentiated type which showed keratin pearl formation. One squamous cell carcinoma in the forestomach metastisized to the liver and lung. Lung tumors were adenocarcinomas or adenomas. The incidence of lung tumors in the groups treated with IQ was significantly higher than in control groups in both sexes.

MeIQ. A carcinogenicity test on MeIQ is ongoing. Interim data by week 83 of the experiment are shown in Table 5 (*15*). A high incidence of squamous cell carcinomas in the forestomach was observed in mice given MeIQ at a concentration of 0.04%. Ten out of twenty-one squamous cell carcinomas metastisized to the liver. Hepatocellular carcinomas and hepatocellular adenomas were also found in some female mice given MeIQ.

Carcinogenicity of Heterocyclic Amines in Rats

Trp-P-1. Male and female rats were given Trp-P-1 at a concentration of 0.015% and 0.02%, respectively. As summarized in Table 6, the target organ was the liver. Most liver tumors were histologically hepatocellular carcinomas. Although there was no significant difference compared to controls, tumors were also found in the small and large intestines.

Glu-P-1 and Glu-P-2. When rats were given Glu-P-1 and Glu-P-2 at a concentration of 0.05%, a high incidence of tumors were induced in the liver, small and large intestines, Zymbal gland, and clitoral gland (*16*) (Table 7). Most liver tumors were hepatocellular carcinomas. Multiple intestinal tumors often developed in the small and large intestines, and the most preferential sites of their development were terminal ileum and ascending colon. Histologically, most tumors were diagnosed as adenocarcinomas. Tumors in the Zymbal gland

Table 6. Incidence of tumors in rats given Trp-P-1.

Chemical	Sex	Effective no.	No. of rats with tumors (%)		
			Liver (hepatocellular carcinoma)	Intestine (adenocarcinoma)	
				Small	Large
Trp-P-1					
0.015%	M	40	30 (75)	1 (3)	2 (5)
0.02%	F	40	37 (93)	1 (3)	0
Control	M	50	1 (2)	0	0
	F	50	0	0	0

Table 7. Incidence of tumors in rats given Glu-P-1 and Glu-P-2 (0.05%).

Chemical	Sex	Initial no.	No. of rats with tumors (%)				
			Liver (hepatocellular carcinoma)	Intestine (adenocarcinoma)		Zymbal gland (squamous cell carcinoma)	Clitoral gland (squamous cell carcinoma)
				Small	Large		
Glu-P-1	M	42	35 (83)	26 (62)	19 (45)	18 (43)	—
	F	42	24 (57)	10 (24)	7 (17)	18 (43)	5 (12)
Glu-P-2	M	42	11 (26)	14 (33)	6 (14)	1 (2)	—
	F	42	2 (5)	8 (19)	8 (19)	7 (17)	11 (26)
Control	M	50	2 (4)	0	0	0	—
	F	50	0	0	0	0	0

Table 8. Incidence of tumors in rats given IQ (0.03%).

Chemical	Sex	Effective no.	No. of rats with tumors (%)						
			Liver (hepatocellular carcinoma)	Intestine (adenocarcinoma)		Zymbal gland (squamous cell carcinoma)	Clitoral gland (squamous cell carcinoma)	Skin (squamous cell carcinoma)	Oral cavity (squamous cell carcinoma)
				Small	Large				
IQ	M	40	27 (68)	12 (30)	25 (63)	36 (90)	—	17 (43)	2 (5)
	F	40	18 (45)	1 (3)	9 (23)	27 (68)	20 (50)	3 (8)	1 (3)
Control	M	50	1 (2)	0	0	0	—	0	0
	F	50	0	0	0	0	0	0	0

and clitoral gland were histologically squamous cell carcinomas. The incidence of tumors in the liver, intestine and Zymbal gland were higher in groups treated with Glu-P-1 than in groups treated with Glu-P-2 in both sexes.

IQ. The incidence of tumors in rats given IQ at a concentration of 0.03% is shown in Table 8. IQ showed similar carcinogenic effects to those of Glu-P-1 and Glu-P-2 in rats (*17*). Hepatocellular carcinomas, adenocarcinomas in the small and large intestines, and squamous cell carcinomas in the Zymbal gland and clitoral gland were observed in rats given IQ at high incidence. Squamous cell carcinomas were also observed in the skin and oral cavity.

Discussion

All the mutagenic heterocyclic amines so far subjected to carcinogenicity tests in mice and rats have shown positive results, indicating the validity of short-term mutation assay by *Salmonella typhimurium* TA 98 and TA 100 as the screening for carcinogens.

Heterocyclic amines showed carcinogenicities in various organs and most of them were carcinogenic to two or more organs. In the experiments on rats, especially Glu-P-1, Glu-P-2, and IQ showed multipotent carcinogenic effects, inducing tumors in the liver, small and large intestines, Zymbal gland, and clitoral gland.

The target organs for each heterocyclic amine were different in each species. Differences in the metabolic activation or inactivation of heterocyclic amines or different fates of DNA adducts produced by the ultimate forms of heterocyclic amines in each tissue might explain these phenomena.

Since heterocyclic amines are actually contained in various cooked foods (*7,18–23*) and cigarette smoke condensate (*20,24*), there is a possibility that these heterocyclic amines play some role as initiators in carcinogenesis in human cancer development. For the assessment of the risk of heterocyclic amines to humans, the amounts of heterocyclic amines in various cooked foods should be further investigated. The carcinogenicities of heterocyclic amines at lower doses should also be investigated to assess the carcinogenic potencies and dose responses more precisely.

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