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Induction of Aberrant Crypt Foci in the Large Intestine of F344 Rats by Oral Administration of 2-Amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine

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Carcinogenicity of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) to rat colon was investigated using the appearance of colonic aberrant crypt (AC), a preneoplastic lesion, as a marker. The number of AC foci per colon at experimental week 4 was 1.3±0.8; almost half the level of AC foci induced by 2-amino-6-methyldipyrido[1,2-*a*:3′,2′-*d*]imidazole (Glu-P-1), which is a known colon carcinogen. No ACs were observed in rats of the control group. A repeat experiment showed that induction of AC foci by PhIP administration was reproducible and a significant increase in the number of AC foci, 3.0±0.0, was observed after 12 weeks of PhIP administration. The majority of ACs induced by PhIP were localized in the distal part of the colon. The distribution was similar to those induced by Glu-P-1 and 1,2-dimethylhydrazine. Those data suggested that PhIP is possibly carcinogenic to rat colon.

Key words: PhIP — Heterocyclic amine — Colon carcinogen — Aberrant crypt

Many mutagenic compounds have been found in cooked foods and most of them are heterocyclic amines.[1] Many of them were demonstrated in long-term studies to cause tumors in various organs of rodents, such as the liver, small and large intestines, Zymbal's gland, skin and clitoral gland. Particularly high incidences of malignant tumors were observed in the liver, large intestine and Zymbal's gland.[2]

2-Amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) is one of the heterocyclic amines isolated from fried ground beef[3] and is considered to be an important heterocyclic amine because of its high content in various cooked foods. PhIP has been shown to induce various kinds of genetic damage in both *in vitro* and *in vivo* mammalian systems.[4] Although PhIP was shown to induce lymphomas in mice,[5] ^{32}P-postlabeling analysis of DNA adduct formation of PhIP in rats showed high levels of adduct formation in the heart, pancreas and colon.[6] Therefore, it is of special interest to know if PhIP is carcinogenic to these organs, especially to the pancreas and colon.

The aberrant crypt (AC) of colonic mucosa is found specifically in response to colon carcinogens in early stages of carcinogenesis[7] and they are considered to be a preneoplastic lesion of the colon. In the present study, the formation of colonic ACs was evaluated in F344 rats given PhIP for a short term to investigate whether PhIP is carcinogenic to rat colon or not.

PhIP was obtained from Nard Institute (Osaka), 2-amino-6-methyldipyrido[1,2-*a*:3′,2′-*d*]imidazole (Glu-P-1) from Katsura Chemical Co. (Tokyo), and 1,2-dimethylhydrazine (DMH) from Aldrich Chemical Co. Inc. (Milwaukee, WI). Male F344 rats were purchased from Charles River Japan, Inc. (Kanagawa), and used at 6 weeks of age. Animals were housed in wire cages in an air-conditioned room with a 12 h light/dark cycle. PhIP and Glu-P-1 were given in the powder basal diet (CE-2, CLEA Japan, Tokyo) at a concentration of 0.05% in both cases. DMH was administered to rats at a dose of 20 mg/kg body weight by subcutaneous injection twice a week for 2 weeks. Animals in all groups were killed at weeks 2 and 4 in the 1st experiment and weeks 4, 8, 12 and 16 in the second experiment. The large intestines were removed, slit open from the anus to the cecum along the longitudinal median axis, and fixed flat between filter papers in 10% buffered formalin. Following the procedure described by Bird,[8] the large intestines were stained with 0.2% methylene blue for 30 to 60 min in order to examine the AC. The number and distribution of ACs were examined under a light microscope.

A representative AC induced by PhIP is shown in Fig. 1. Quantitative results of AC induction in experiment 1 are shown in Table I. The numbers of AC foci induced by PhIP were 0.3±0.4 and 1.3±0.8 at 2 weeks

Abbreviations: PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; DMH, 1,2-dimethylhydrazine; Glu-P-1, 2-amino-6-methyldipyrido[1,2-*a*:3′,2′-*d*]imidazole; AC, aberrant crypt.

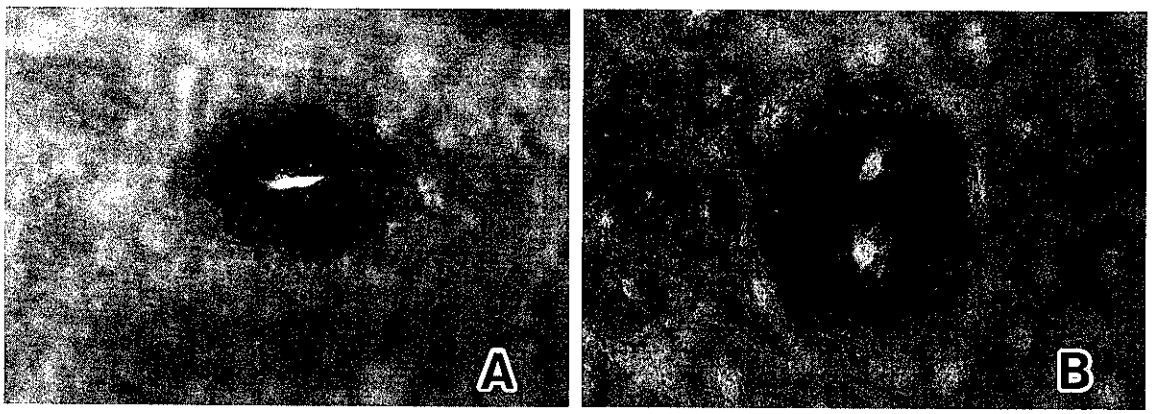

Fig. 1. Aberrant crypt foci in the colon of F344 rats treated with DMH (A) and PhIP (B) (×120).

Table I. Appearance of Aberrant Crypts in Rat Colon

Treatment	Period (weeks)	No. of rats bearing AC	No. of foci/ colon	No. of AC/ focus
Experiment 1				
PhIP	2	1/4	0.3±0.4	2.00
	4	4/4	1.3±0.8	1.2±0.2
Glu-P-1	2	3/4	1.3±1.1	1.0±0.0
	4	4/4	2.8±2.1	1.0±0.1
DMH	2	4/4	15.0±8.6[a]	1.0±0.0
	4	4/4	158.5±46.1[b]	1.7±0.1
Control	2	0/4	0	0
	4	0/4	0	0
Experiment 2				
PhIP	4	3/3	1.3±0.6	1.3±0.9
	8	2/3	0.7±0.6	2.0±1.5
	12	3/3	3.0±0[c]	2.3±2.9
	16	3/3	11.3±7.0	2.5±3.0
Control	4	0/3	0	0
	8	1/3	0.3±0.6	1.0±0
	12	0/3	0	0
	16	1/3	0.3±0.6	2.0±0

Values are mean±SD.
a, b, c) Significantly different from the respective controls at $P<0.05$, 0.01, 0.001.

and 4 weeks after the start of administration, respectively. Those numbers are almost half of those induced by Glu-P-1, 1.3±1.1 and 2.8±2.1, respectively. DMH induced tremendous numbers of ACs under the same experimental conditions. No ACs were observed in the control group. Although the number of AC foci induced by PhIP in experiment 1 was not statistically significantly higher than that of the control, it seemed to be potentially significant because spontaneous formation of ACs in the control group was quite rare. In order to confirm the reproducibility of the induction of AC and to investigate the effects of long-term exposure to PhIP, the second experiment was conducted. The results are summarized in Table I. Reproducibility of the induction of AC by PhIP was clearly confirmed. Moreover, time-dependent increase of the number of AC foci and time-dependent increase in the number of crypts per focus were also noticed. The distribution of ACs along the large intestine was carefully examined. The ACs induced by PhIP were observed in the distal colon in early stages, while some ACs appeared in the proximal part of the colon in experiment 2 (Table II). The majority of ACs induced by DMH in the large intestine were located in the distal colon, excepting the rectum. We arbitrarily defined the rectum as 2 cm on the proximal side of the large intestine from the anus. Only limited numbers of ACs were observed in the rectum (Table II). The distribution of ACs in F344 rats was found to be different from that in Sprague-Dawley rats, in which half of them are located in the rectum and the rest in the midcolon.[9] Glu-P-1 is a colonic carcinogen in rats.[10] Induction of AC by Glu-P-1 has been reported in Sprague-Dawley rats.[9] We confirmed in the present study that Glu-P-1 induced AC in F344 rats, in which colon carcinogenesis by Glu-P-1 is established.

PhIP was found to induce ACs in the colon of the rats in the present study, and large amounts of DNA adduct formation have been observed in the colon of PhIP-treated F344 rats.[6] Therefore, it is very likely that PhIP is carcinogenic to the colon in rats. Quite recently, it was found to induce colon tumors in Nagase analbuminemic rats (Ochiai *et al.*, unpublished data). The induction of colonic ACs by PhIP was once reported to have been

Table II. The Distribution of Aberrant Crypt Foci in the Large Intestine of F344 Rats

Treatment	Period (weeks)	No. of foci	Colon		Rectum	Cecum
			proximal	distal		
Experiment 1						
PhIP	2	1	0	1	0	0
	4	5	0	5	0	0
Glu-P-1	2	5	0	5	0	0
	4	11	2	9	0	0
DMH	2	60	0	54	6	0
	4	634	0	610	24	0
Control	2	0	0	0	0	0
	4	0	0	0	0	0
Experiment 2						
PhIP	4	4	1	3	0	0
	8	2	1	1	0	0
	12	9	3	6	0	0
	16	34	8	22	3	1
Control	4	0	0	0	0	0
	8	1	0	1	0	0
	12	0	0	0	0	0
	16	1	0	1	0	0

examined in CF1 mice. The results were quite marginal.[9] PhIP was found to induce lymphoma and tumors of the small intestine in CDF1 mice but no tumors were observed in the large intestine. Like many other heterocyclic amines, PhIP might be a colonic carcinogen in rats, but not in mice.

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