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DNA Modification by 2-Amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine in Rats

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2-Amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) is the most abundant mutagenic heterocyclic amine by weight in cooked foods. This mutagen was found to produce DNA adducts in all ten tested organs of rats using the ^{32}P-postlabeling method. The level of DNA adducts in the pancreas, kidney and liver increased dose-dependently and feeding time-dependently up to four weeks. When diet containing 0.05% PhIP was given to rats for four weeks, levels of PhIP-DNA adducts were relatively high in the lung, pancreas and heart, being around 20 per 10^7 nucleotides, and lowest in the liver, being 2.20 per 10^7 nucleotides. Thus, PhIP showed a unique feature in the formation of DNA adducts compared to other mutagenic and carcinogenic heterocyclic amines, which produce the highest level of DNA adducts in the liver.

Key words: 2-Amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) — ^{32}P-Postlabeling method — Heterocyclic amine — DNA-adduct

Many dietary factors including mutagens/carcinogens are thought to be involved in the development of human cancer.[1)] Various kinds of heterocyclic amines, namely aminopyridoindoles, aminodipyridoimidazoles, aminoimidazoquinolines and aminoimidazoquinoxalines have been found as mutagens in cooked foods.[2-4)] Among these mutagenic heterocyclic amines, nine compounds so far tested were proven to be carcinogenic in animals.[2-4)] Recently, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) was identified as the most abundant mutagen by weight in cooked foods such as fried ground beef and fried fish,[5-8)] and the precursors were found to be creatinine, phenylalanine and glucose.[9)] PhIP showed mutagenicity not only in *Salmonella* but also in cultured mammalian cells.[10)] Furthermore, 2-hydroxamino-PhIP and 4′-PhIP sulfate were identified as a direct-acting genotoxic metabolite and a non-genotoxic major metabolite, respectively.[11, 12)] However, the potency of DNA adduct formation and carcinogenicity of this compound have not yet been reported. In this study, we investigated the DNA modification in various organs of F344 rats fed PhIP using the ^{32}P-postlabeling method. Here, we report that PhIP-DNA adducts were detected in all organs examined and the levels were relatively high in the lung, pancreas and heart but low in the liver, in contrast to the other heterocyclic amines.

PhIP•hydrochloride, purchased from Nard Institute, Osaka, was added to the basal powder diet (CE-2, CLEA Japan, Tokyo) at concentrations of 0.01, 0.02 and 0.05%. Seven-week-old F344 male rats were fed these diets *ad libitum* for two or four weeks. The animals were then killed and the major organs were excised, immediately frozen and kept at −80°C until DNA isolation. Two rats were used in each group.

DNA was isolated from the frozen tissues, and digested to deoxyribonucleoside 3′-monophosphate with micrococcal nuclease and spleen phosphodiesterase (Worthington Biochemical Co., Freehold, NJ) at 38°C for 3.5 h. Digests of DNA were ^{32}P-labeled with minor modifications of the procedure reported by Randerath *et al.*[13)] Under standard conditions, 0.17 μg (0.5 nmol) of the digest was ^{32}P-labeled with T_4 polynucleotide kinase (Pharmacia Fine Chemicals, Uppsala, Sweden) and 0.75 nmol of [γ-^{32}P]ATP (600 Ci/mmol, ICN Radiochemicals, Irvine, CA) at 37°C for 1 h.[14)] ATP-deficient conditions were used for adduct intensification; in this case digests of 5 μg of DNA were ^{32}P-labeled with carrier-free [γ-^{32}P]ATP (0.04 nmol, 6000 Ci/mmol) at 37°C for 1 h.[13, 14)]

[5′-^{32}P]Deoxyribonucleoside 3′,5′-bisphosphates thus obtained were then separated into normal and modified nucleotides by development on polyethyleneimine cellulose sheets (POLYGRAM CEL 300 PEI, Machery-Nagel, Düren, FRG) with a solvent of 1.7 *M* sodium phosphate buffer (pH 6.0). Modified nucleotides, which remained at the origin, were transferred to other PEI-

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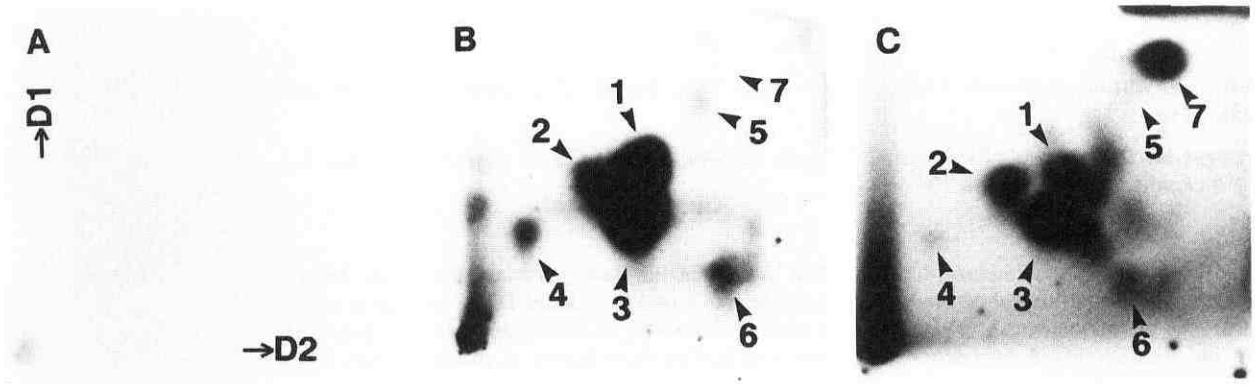

Fig. 1. Autoradiograms of PhIP-DNA adducts in the pancreas of rats fed 0.05% PhIP in the diet or control diet for four weeks. (A) control (adduct-intensification conditions) (B) 0.05% PhIP (adduct-intensification conditions) and (C) 0.05% PhIP (standard conditions). Films were exposed at −80°C for 24 h (A) or 4 h (B and C). Individual spots of DNA adducts are numbered.

cellulose sheets and developed with the following solvent systems: 3.6 *M* lithium formate/6.8 *M* urea buffer (pH 3.5) from the bottom to the top (D1), 1.0 *M* lithium chloride/0.5 *M* Tris-HCl/8.5 *M* urea buffer (pH 8.0) at right angles to the previous development (D2) and 1.7 *M* sodium phosphate buffer (pH 6.0) with a 3.5 cm paper wick in the same direction as D2. Adducts were detected by autoradiography on Kodak XAR-5 film with intensifying screens (DuPont Lightning Plus) at −80°C, and their levels were estimated from the radioactivity of each adduct spot and of the total nucleotides. Intensification factors for individual PhIP-DNA adducts were calculated by dividing the adduct level in the pancreas of rats fed 0.05% PhIP for four weeks under adduct-intensification (ATP-deficient) conditions by that under standard conditions.

Fig. 1 shows autoradiograms of PhIP-DNA adducts under both the standard and adduct-intensification conditions from the pancreas of rats, which had been fed 0.05% PhIP for four weeks. The number of spots of DNA adducts commonly observed under both conditions was seven; the intensification factors were estimated to be 256, 104, 318, 128, 19, 34 and 1.7, for adduct numbers 1 to 7. A similar DNA-adduct pattern was also obtained in the other nine organs examined although the testis only produced six of the spots with adduct-intensification. Under these conditions, undigested dinucleotides may be present and it is possible that a single DNA adduct produces several spots. Further investigation to determine the number of DNA adducts and their structure is necessary. No spots were observed in the pancreas of rats given control diet.

The levels of PhIP-DNA adducts in the ten organs are summarized in Table I. Among the organs tested total adduct levels were relatively high in the lung, pancreas and heart, being 23.37, 20.71 and 24.61 adducts per 10^7

Table I. DNA Adduct Levels in Various Organs of Rats Fed PhIP

Adduct No.	PhIP-DNA adducts per 10^7 nucleotides[a]									
	Liver	Kidney	Lung	Pancreas	Stomach	Colon	Spleen	Heart	Testis	Brain
1	0.32	1.02	1.76	2.62	1.79	1.61	1.13	2.54	0.33	0.57
2	0.25	0.63	1.42	1.58	0.93	1.05	0.59	1.61	0.17	0.58
3	0.38	2.00	3.26	3.87	2.37	2.31	2.28	4.09	0.57	0.76
4	0.08	0.43	0.48	0.93	1.60	0.77	0.75	1.22	0.22	0.33
5	0.07	0.32	0.79	0.70	1.11	0.46	0.40	0.76	ND[b]	0.25
6	0.21	0.76	1.95	2.21	0.91	1.52	1.93	2.05	0.35	0.66
7	0.89	4.86	13.71	8.80	4.05	7.84	6.15	12.34	1.76	4.40
Total	2.20	10.02	23.37	20.71	12.76	15.56	13.23	24.61	3.40	7.55

a) The level of DNA adducts is expressed as mean value of duplicate analyses with two rats.
b) ND: not detected.

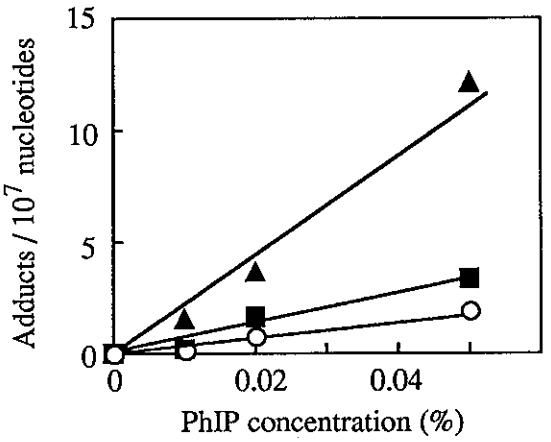

Fig. 2. Dose dependency of PhIP-DNA adduct levels in various organs of rats fed PhIP for two weeks. (○) Liver, (■) kidney and (▲) pancreas.

nucleotides respectively. In the kidney, stomach, colon and spleen, they ranged from 10.02 to 15.56 adducts per 10^7 nucleotides. Unexpectedly, the total adduct level in the liver was lowest, being 2.20 adducts per 10^7 nucleotides and about 10-fold less than those of the lung, pancreas and heart.

The effect of the dose of PhIP on formation of adducts in the liver, kidney and pancreas was examined next. DNA samples in the three organs from rats fed diets containing 0.01, 0.02 and 0.05% PhIP for two weeks showed dose-dependent adduct formation (Fig. 2). Furthermore, feeding of 0.05% PhIP for two and four weeks resulted in time-dependent formation of the DNA adduct in the organs including liver, kidney and pancreas. The total levels in each organ after the four-week treatment were about 1.5 to 2 times those after two-week treatment.

As mentioned above, the PhIP-DNA adduct level was lower in the liver than other organs such as pancreas, kidney and colon. Contrary to this, other heterocyclic amines yielded higher levels of adducts in the liver than in the other organs. For example, four-week feeding of 0.04% 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx) in male F344 rats gave 107.2 adducts in the liver, 32.22 in the pancreas, 41.70 in the kidney and 3.74 in the colon per 10^7 nucleotides (K. Yamashita *et al.*, unpublished results). In the case of 2-amino-6-methyldipyrido[1,2-*a*:3′,2′-*d*]imidazole (Glu-P-1), total DNA-adduct levels were 22.60 in the liver, 6.01 in the pancreas, 7.30 in the kidney and 3.10 in the colon per 10^7 nucleotides after administration of 0.05% of the chemical in the diet for four weeks in male F344 rats (unpublished results). The apparent primary target organ of nine carcinogenic heterocyclic amines including MeIQx and Glu-P-1 has been demonstrated to be the liver in rats and/or mice in long-term feeding experiments.[2-4] These results correlated very well with the observation of a high level of DNA-adduct formation in the liver of animals.[14, 15] From the above data, it is suggested that PhIP may show a different organ specificity compared to other carcinogenic heterocyclic amines.

PhIP levels in cooked meat and fish were more than ten-fold higher than those of other heterocyclic amines.[5, 6] It is, therefore, very important to study the contribution of PhIP to the development of human cancer.

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