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Induction of Intestinal Adenocarcinomas by 2-Amino-1-methyl-6-phenylimidazo-[4,5-*b*]pyridine in Nagase Analbuminemic Rats

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2-Amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), the most abundant mutagenic heterocyclic amine in cooked foods, was examined for carcinogenic potential using Nagase analbuminemic rats (NARs), which are sensitive to various carcinogens. The concentration of PhIP in the diet was 0.04% at the beginning of the experiment, this being subsequently gradually reduced to 0.01% to avoid severe body weight loss. Ten of 13 treated NARs developed a total of 36 intestinal tumors within the 311-day experimental period. Among these, 22 were adenocarcinomas and 2 were adenomas of the small intestine, 4 were adenocarcinomas of the cecum and 8 were adenocarcinomas of the large intestine. The results suggest that PhIP could represent a significant risk to human populations exposed to foods containing heterocyclic amines.

Key words: Mutagen in food — Heterocyclic amine

2-Amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), isolated from fried ground beef in 1986,[1] has been shown to be the most abundant of various mutagenic heterocyclic amines present in cooked foods.[1-3] Mutagenicity of PhIP is relatively low in *Salmonella typhimurium*[4,5] but very strong in mammalian cells.[6] Esumi *et al.* recently reported that feeding PhIP in pellet diet at 0.04% induced lymphomas in CDF_1 mice.[7] However, while all 9 other heterocyclic amines so far examined were found to induce hepatocellular carcinomas,[4,5] none were observed in PhIP-treated CDF_1 mice.[7]

Recent analysis of DNA adduct formation in F344 rats after feeding 0.05% PhIP for 4 weeks,[8] revealed high levels in heart, lung and pancreas, but only a very low value for liver, in contrast to findings for other heterocyclic amines. The colon also demonstrated high levels of DNA adducts, the average value of $15.6/10^7$ nucleotides being much higher than that induced by feeding 0.05% 2-amino-6-methyldipyrido[1,2-*a*:3′,2′-*d*]-imidazole (Glu-P-1) for 4 weeks. Since feeding of 0.05% Glu-P-1 induced small and large intestinal tumors in male F344 rats at incidences of 45% and 62%, respectively, within an experimental period of 64 weeks,[9] the present study of carcinogenicity was performed.

Nagase analbuminemic mutant strain rats (NARs) devoid of serum albumin[10] are known to be more sensitive than the parental normal Sprague-Dawley strain to various carcinogens such as *N*-butyl-*N*-(4-hydroxybutyl)-nitrosamine, *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine, and azoxymethane, inducing lesions in the bladder, stomach and intestine, respectively.[11] The aim of this investigation was to determine the susceptibility of NARs to the carcinogenicity of PhIP.

Thirteen NAR males, 8 weeks old at the start of the experiment, were fed *ad libitum* on basal pellet diet (CE-2, CLEA Japan, Tokyo) containing PhIP hydrochloric acid salt (Nard Institute, Osaka). The experiment was started with a diet containing 0.04% PhIP. However, this concentration proved toxic and body weights had decreased by 25% on the 108th experimental day as compared with those of controls in our previous experiment. The concentration of PhIP in the diet was therefore reduced to 0.03%, but continuous loss of body weight was still observed, and so the concentration was reduced to 0.01% on the 144th day. This level of supplementation was maintained thereafter until termination on the 311th day. The presence of at least 90% of the added PhIP in the diet was confirmed by HPLC analysis. Rats were autopsied upon becoming moribund, the final 2 being killed at the end of the experiment. Each organ was routinely fixed in phosphate-buffered formaldehyde solution, embedded in paraffin and sectioned at 3 μm thickness, and sections were stained with hematoxylin-eosin for histological observation.

The first tumors were observed in the small and large intestine of an NAR autopsied on the 136th day. Ten of

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Table I. Carcinogenicity of PhIP in Analbuminemic Rats

Effective No.	No. of rats with tumors						
	Intestine				Zymbal glands	Pancreas	Skin
	Total	Small intestine	Cecum	Large intestine			
13	10	9	3	6	4	2	1

Table II. Sites of Intestinal Tumors Induced by PhIP

Effective No.	No. of NARs bearing intestinal tumor	No. of intestinal tumors				Average (tumors/rat)
		Small intestine	Cecum	Large intestine	Total	
13	10	24[a)]	4	8	36	2.8

a) Two were diagnosed as adenomas and the others, including the cecum and large intestinal tumors, were all adenocarcinomas.

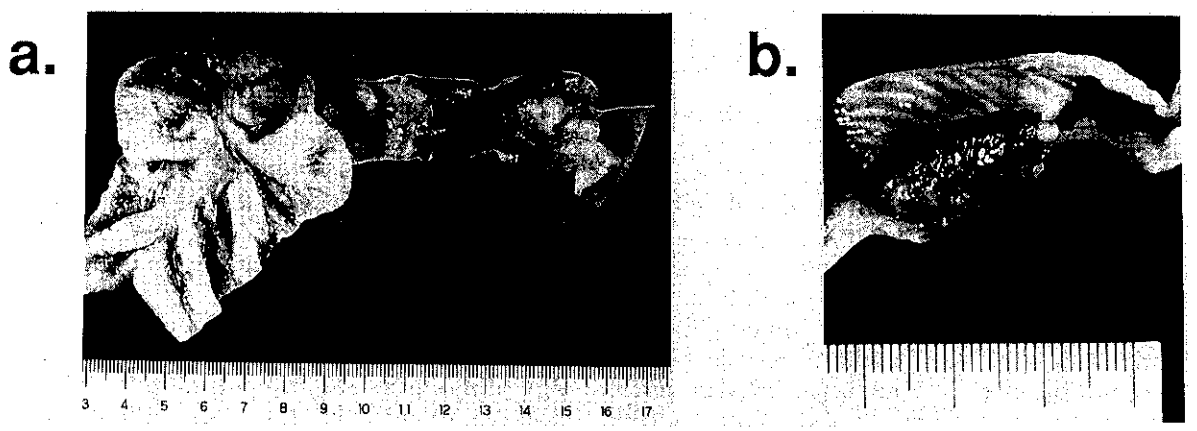

Fig. 1. Tumors of the ileum and cecum (a) and the large intestine (b) which had developed in an NAR which was autopsied at day 287.

13 NARs eventually developed intestinal tumors, 9, 3 and 6 NARs demonstrating tumors in the small intestine, cecum and large intestine, respectively (Table I). Neoplastic lesions were also observed in the Zymbal glands, pancreas and skin.

The numbers of intestinal tumors of different types which developed in the 10 NARs are summarized in Table II. Some NARs developed multiple intestinal tumors. For example, one NAR autopsied at day 287 had 7 intestinal tumors, 3 in the ileum, 2 in the cecum and 2 in the large intestine (Fig. 1). The average number of intestinal tumors per rat was 2.8. Small intestinal tumors were abundant, 9 of 24 such lesions being observed in the ileo-cecal region. Four and 8 carcinomas developed in the cecum and large intestine, respectively (Table II). Most of the intestinal tumors were diagnosed as well-differentiated adenocarcinomas (Fig. 2), only two small lesions of a total of 36 being diagnosed as adenomas.

The first Zymbal gland tumor was noticed on day 150. A total of four NARs developed Zymbal gland tumors, 2 rats demonstrated tumors in the pancreas and one rat had a skin tumor (Table I). One Zymbal gland tumor was diagnosed as a sebaceous adenoma, one as a squamous cell papilloma and two as squamous cell carcinomas. One pancreas tumor was diagnosed as an acinar cell adenoma and the other as an acinar cell carcinoma. The one skin tumor was a squamous cell papilloma. It is noteworthy that no tumors were observed in the liver.

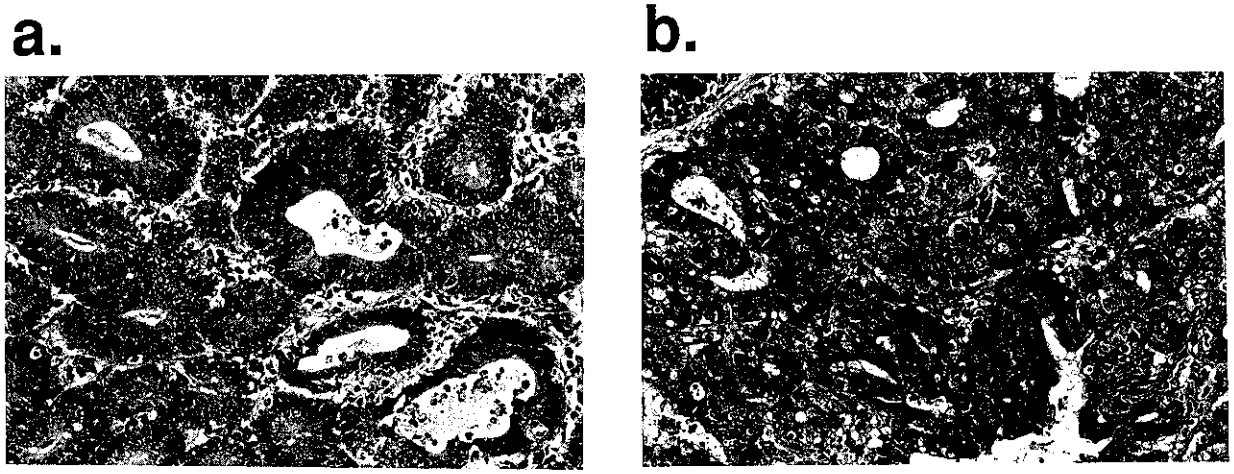

Fig. 2. Photomicrographs of well-differentiated adenocarcinomas of the small (a) and large intestine (b) of the NAR shown in Fig. 1.

In a previous experiment conducted by Hosaka *et al.*,[12] no tumors were detected in the small intestine, cecum or large intestine of NARs fed basal diet for at least 2 years. The intestinal tumors observed in this study therefore be concluded to have been induced by feeding of the diet containing PhIP. Furthermore, since Hosaka *et al.*,[12] also reported that spontaneous tumor development was not observed in the Zymbal glands, pancreas or skin of NARs, the lesions apparent in these organs were presumably also induced.

We previously found that analbuminemic congenic Fischer 344 rats developed intestinal tumors more rapidly than normal F344 rats after feeding of 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ).[13] Albumin binds many chemicals, and the tissue distribution of chemicals in various organs would therefore be expected to be different between analbuminemic and normal rats. It was reported that amounts of bile acids in the bile and their composition differ between NARs and SD rats.[14] It is possible that the promoting activity of bile may vary in analbuminemic and normal animals. NARs are hyperlipidemic and this trait has been proved to be associated with the lack of serum albumin.[10]

Since only quantitative and not qualitative differences have so far been detected between analbuminemic and wild-type rats in carcinogenicity studies, on the basis of the present results we predict intestinal tumor-inducing activity of PhIP in normal animals. The potential significance of these findings for human populations consuming PhIP-contaminated foodstuffs clearly warrants further investigation.

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