Jpn. J. Cancer Res. **90**, 1203–1206, November 1999

Difference in Target Organs in Carcinogenesis with a Heterocyclic Amine, 2-Amino-3,4-dimethylimidazo[4,5-*f*]quinoline, in Different Strains of Mice

Hirofumi Fujita,[1] Kasuke Nagano,[1] Masako Ochiai,[2] Toshikazu Ushijima,[3] Takashi Sugimura,[3] Minako Nagao[2, 3, 4] and Taijiro Matsushima[1]

[1]Japan Bioassay Research Center, 2445 Hirasawa, Hadano, Kanagawa 257-0015 and [2]Biochemistry Division and [3]Carcinogenesis Division, National Cancer Center Research Institute, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045

2-Amino-3,4-dimethylimidazo[4,5-*f*]quinoline (MeIQ) induces cancers in the forestomach and liver, but not in the colon, of CDF1 male and female mice, which are thought to be resistant to induction of colon cancer by 1,2-dimethylhydrazine. In this study, we examined the carcinogenicity of MeIQ in C57BL/6N female mice, which are susceptible to 1,2-dimethylhydrazine. This strain of mice developed carcinomas of the cecum, colon and liver, but not the forestomach, when given a diet containing 300 ppm of MeIQ. This fact indicates that the target organs of a chemical carcinogen change depending on the strain of a given animal species.

Key words: MeIQ — C57BL/6N — Colon adenocarcinoma — Strain difference

Food-borne carcinogens, such as heterocyclic amines (HCAs), are recognized to induce tumors in various organs of experimental animals when administered in the diet. However, the target organs are markedly different between F344 rats and CDF1 mice. Five of 10 mutagenic HCAs induced colon cancer in F344 rats, but none of them induced colon cancer in CDF1 mice.[1, 2] 2-Amino-3,4-dimethylimidazo[4,5-*f*]-quinoline (MeIQ), an HCA, induced colon, oral cavity, Zymbal gland and mammary gland cancers in female rats, and colon, oral cavity, Zymbal gland and skin cancers in male rats of the F344 strain,[3] while it induced forestomach and liver cancers in female mice of the CDF1 strain, and only forestomach cancers in males.[4]

However, we recently found that MeIQ induced, in addition to liver cancers, colon cancers with a significantly high incidence in females of the Big Blue mouse (BBM) which has the *lacI* transgene, with a genetic background of C57BL/6N.[5] The C57BL/6N mouse is known to be susceptible to 1,2-dimethylhydrazine (DMH)-induced colon carcinogenesis.[6] Therefore, it was considered that HCAs have the potential to induce colon cancers in this strain of mice, and the differences of target organs observed between the rat and the mouse as described above are not necessarily due to species difference. However, BBM harbors the *lacI* transgene on chromosome 4,[7] and there was a possibility that integration of the transgene had affected colon cancer susceptibility. We therefore wished to know whether HCAs induce colon cancer in mice that have not been artificially modified in genetic composition.

In this study, therefore, the colon carcinogenicity of MeIQ was examined using C57BL/6N female mice to clarify the effect of the genetic background of C57BL/6N *per se*. Differences in cancer susceptibility, including differences in target organs, are important for extrapolation of animal data to humans.

MATERIALS AND METHODS

Chemical MeIQ was purchased from Nard Institute (Osaka).

Animals Female mice of the C57BL/6NCrj strain were obtained from Charles River Japan (Atsugi) at 4 weeks old. After acclimatization at 25°C with a humidity of 55% for 3 weeks, with a diet of CE-2, CLEA (Tokyo), the animals were divided into an experimental and a control group. Each group consisted of 40 animals, and a diet containing 300 ppm of MeIQ was given to the animals of the experimental group. Animals which became moribund were killed and subjected to histological analysis. The experiment was terminated at 92 weeks.

Histology All organs were carefully examined for the presence of tumors and then fixed in neutralized formalin at 4°C, embedded in paraffin, processed, and stained with hematoxylin and eosin (H & E). Routine histological analyses were performed on the brain, bone marrow, tongue, salivary glands, esophagus, forestomach and glandular stomach, small and large intestines, ovary, uterus, lung, heart, spleen, skin, pituitary gland, Harderian glands and interscapular brown adipose tissue.

[4] To whom correspondence should be addressed at the Biochemistry Division, National Cancer Center Research Institute.
E-mail: mnagao@ncc.go.jp

Statistical analyses were performed using the χ^2 test.

RESULTS

The body weight in the experimental group given the MeIQ-containing diet was about 80% of the control during most of the experimental period. At 43 weeks, a mouse in the experimental group died of lymphoma. Survival curves of the experimental and control groups are shown in Fig. 1. All animals in the experimental group were killed by 85 weeks. All the mice, except two in the experimental group, developed tumors in some organ(s). Cancer incidences in the major target organs are summarized in Table I. Seventeen of 40 mice developed adenocarcinomas in the large intestine. Among those, five were in the colon, 10 in the cecum and two in the colon and cecum. Histologically, five of the colon cancers were tubular adenocarcinomas and the remaining two were mucinous adenocarcinomas (Fig. 2).

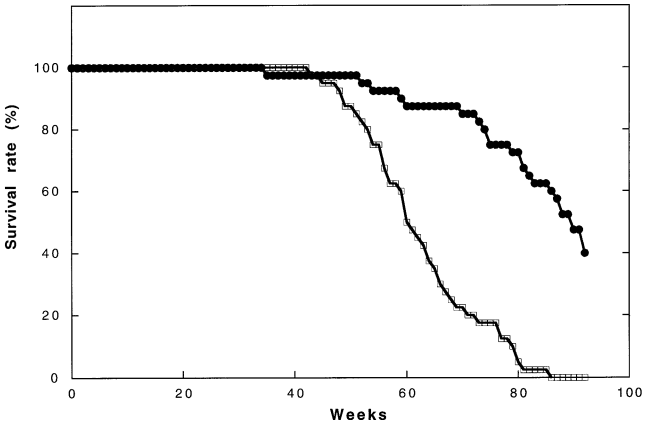

Fig. 1. Survival rates of female C57BL/6N mice administered basal diet or diet containing 300 ppm of MeIQ. ● control, □ MeIQ.

Fibrosarcomas were detected in the liver of 68% of the MeIQ-group mice, though the incidence was also high in the control group (23%), as reported previously.[8] Hepatocellular adenomas and carcinomas were observed only in the MeIQ group, with incidences of 20% and 25%, respectively. All of the hepatocellular carcinomas were of the well differentiated type. Lymphomas were more frequently observed in the control group than in the experimental group. This lower incidence in the experimental group might be due to the shorter survival in the experimental group.

One stomach adenocarcinoma and two gall bladder adenomas were observed in the experimental group, and four uterus fibrosarcomas in the control group.

Table I. Comparison of MeIQ Carcinogenicity among C57BL/6N, CDF1 and BBM Female Mice

	Effective No.	Number of animals with tumor (% incidence)									
		Colon		Cecum		Forestomach		Small intestine	Liver		
		AD[a]	AC	AD	AC	PA	SCC	AD	HCA	HCC	FS
C57BL/6N[b]											
MeIQ	40	0 (0)[i]	7 (18)[e, g, i]	1 (3)	12 (30)[f, h, j]	0 (0)[h, i]	1 (3)[h]	0 (0)[i]	8 (20)[f, g]	10 (25)[f, h]	27 (68)[f, h, j]
Control	39	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	9 (23)
CDF1[c]											
MeIQ	38	0 (0)	0 (0)	0 (0)	0 (0)	9 (24)	24 (63)	0 (0)	11 (29)	16 (42)	0 (0)
Control	40	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
BBM[d]											
MeIQ	19	2 (11)	8 (42)	1 (5)	13 (68)	3 (16)	0 (0)	2 (11)	10 (53)	16 (84)	0 (0)
Control	19	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (11)

a) AD, adenoma; AC, adenocarcinoma; PA, papilloma; SCC, squamous cell carcinoma; HCA, hepatocellular adenoma; HCC, hepatocellular carcinoma; FS; fibrosarcoma.
b) This study.
c) CDF1 mice (3). The diet (CE-2) containing 400 ppm of MeIQ was given to the experimental group for 91 weeks.
d) BBM with genetic background of C57BL/6N (5). The diet (CE-2) containing 300 ppm of MeIQ was given to the experimental group for 92 weeks.
e) Significantly different from the control group of C57BL/6N with a P value of <0.05.
f) Significantly different from the control group of C57BL/6N with a P value of <0.005.
g) Significantly different from the experimental group of CDF1 with a P value of <0.05.
h) Significantly different from the experimental group of CDF1 with a P value of <0.005.
i) Significantly different from the experimental group of BBM with a P value of <0.05.
j) Significantly different from the experimental group of BBM with a P value of <0.005.

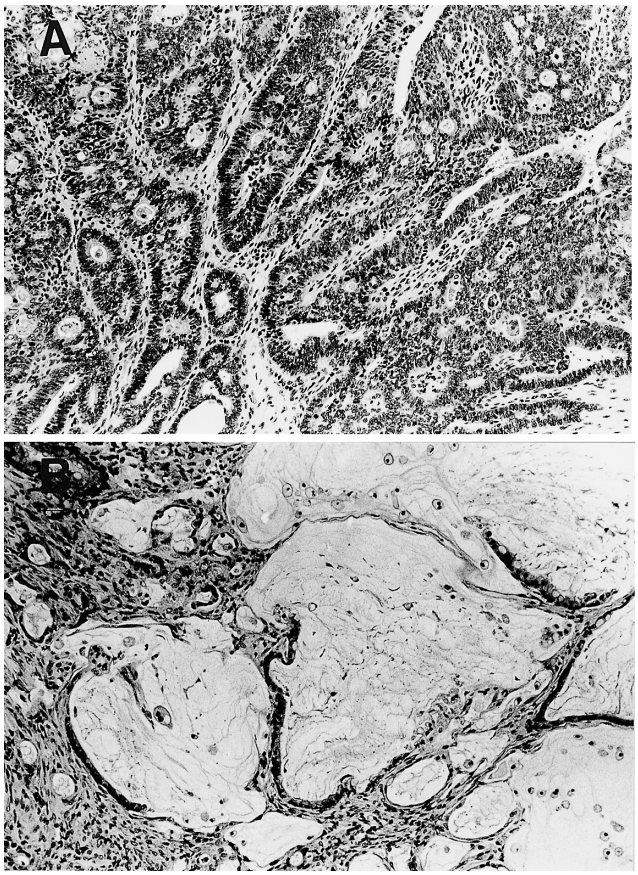

Fig. 2. Histology of the colon adenocarcinoma induced by MeIQ in female C57BL/6N mice. A. Tubular adenocarcinoma. H & E stain, ×40. B. Mucinous adenocarcinoma. H & E stain, ×40.

DISCUSSION

This study clearly demonstrated that 300 ppm of MeIQ in the diet induced adenocarcinomas of the colon and cecum, and hepatocellular carcinomas in female C57BL/6N mice. The target organs of MeIQ in female C57BL/6N were clearly different from those of the male and female CDF1, which developed forestomach squamous cell carcinomas and hepatocellular carcinomas. However, the target organs of the former were the same as those in the BBM female mouse (Table I).

After treatment with MeIQ, the sum of cancer incidences of various organs in the two strains of mice, C57BL/6N and CDF1, were almost the same, under similar experimental conditions, including the experimental period.[3] As for the CDF1 mouse, the target organs did not change on administration of the 100 ppm MeIQ diet or 400 ppm MeIQ diet; in both cases, hepatocellular carcinomas and forestomach carcinomas developed, although the incidence increased dose-dependently. It is concluded that CDF1 is highly susceptible to developing forestomach squamous cell carcinomas and C57BL/6N to developing large intestinal adenocarcinomas after MeIQ insult. At present, it is not clear what mechanisms are involved in this difference in target organs. However, C57BL/6N is also sensitive to DMH-induced colon carcinogenesis.[6] C57BL/6J, another substrain, developed a large number of intestinal polyps after introduction of the *Min* allele (*Apc* gene mutation),[9] owing to low or no expression of the phospholipase A2 secretory type (PLA2g2a).[10] In the C57BL/6N strain, the *Pla2g2a* gene is also not expressed, in contrast to the high expression levels in CDF1 and its parental strains, BALB/c and DBA (unpublished results, Ochiai, M. *et al.*) Lack of expression of *Pla2g2a* may play some role in the MeIQ-induced colon carcinogenesis in C57BL/6N.

There were some differences in incidences of cancers between female mice of C57BL/6N and BBM strains (Table I). These differences may be due to the transgene integrated into the genome, or genetic changes caused during maintenance in the breeding facilities (Charles River Japan for C57BL/6N and Stratagene (La Jolla, CA) for BBM).

This study clearly demonstrated that the target organs of MeIQ-induced cancer development are markedly different between CDF1 mice and C57BL/6N mice, and such differences in target organs may also arise in many other strains of mice. Thus, our data show that target organs in chemical carcinogenesis depend upon the strains of mice. The possibility therefore arises that the same environmental carcinogen may induce cancers in different organs of different human populations, because humans are genetically heterogeneous. Further, the marked difference in target organs of HCAs-induced cancers previously observed between CDF1 mice and F344 rats might not be simply due to species difference.

It is critically important to clarify the mechanisms that are involved in the differences in target organs for carcinogenesis among different strains and among different species, for extrapolation of animal data to humans.

ACKNOWLEDGMENTS

This study was supported in part by a Grant-in-Aid for Cancer Research from the Ministry of Health and Welfare, Japan.

(Received June 25, 1999/Revised August 17, 1999/Accepted August 20, 1999)

REFERENCES

1) Ohgaki, H., Takayama, S. and Sugimura, T. Carcinogenicities of heterocyclic amines in cooked food. *Mutat. Res.*, **259**, 399–410 (1991).
2) Nagao, M. and Sugimura, T. Carcinogenic factors in food with relevance to colon cancer development. *Mutat. Res.*, **290**, 43–51 (1993).
3) Kato, T., Migita, H., Ohgaki, H., Sato, S., Takayama, S. and Sugimura, T. Induction of tumors in the Zymbal gland, oral cavity, colon, skin and mammary gland of F344 rats by a mutagenic compound, 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline. *Carcinogenesis*, **10**, 601–603 (1989).
4) Ohgaki, H., Hasegawa, H., Suenaga, M., Kato, T., Sato, S., Takayama, S. and Sugimura, T. Induction of hepatocellular carcinoma and highly metastatic squamous cell carcinoma in the forestomach of mice by feeding 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline. *Carcinogenesis*, **7**, 1889–1893 (1986).
5) Nagao, M., Fujita, H., Ochiai, M., Wakabayashi, K., Sofuni, T., Matsushima, T., Sugimura, T. and Ushijima, T. No direct correlation between mutant frequencies and cancer incidence by MeIQ in various organs of Big Blue mice. *Mutat. Res.*, **400**, 251–257 (1998).
6) Diwan, B. A. and Blackman, K. E. Differential susceptibility of 3 sublines of C57BL/6 mice to the induction of colorectal tumors by 1,2-dimethylhydrazine. *Cancer Lett.*, **9**, 111–115 (1980).
7) Dycaico, M. J., Provost, G. S., Kretz, P. L., Ransom, S. L., Moores, J. C. and Short, J. M. The use of shuttle vectors for mutation analysis in transgenic mice and rats. *Mutat. Res.*, **307**, 461–478 (1994).
8) Nakamura, K., Kuramoto, K., Shibasaki, K., Shumiya, S. and Ohtubo, K. Age-related incidence of spontaneous tumors in SPF C57BL/6 and BDF1 mice. *Exp. Anim.*, **41**, 279–285 (1992).
9) Dietrich, W. F., Lander, E. S., Smith, J. S., Moser, A. R., Gould, K. A., Luongo, C., Borenstein, N. and Dove, W. Genetic identification of *Mom-1*, a major modifier locus affecting *Min*-induced intestinal neoplasia in the mouse. *Cell*, **75**, 631–639 (1993).
10) MacPhee, M., Chepenik, K. P., Liddle, R. A., Nelson, K. K., Siracusa, L. D. and Buchberg, A. M. The secretory phospholipase A2 gene is a candidate for the *Mom1* locus, a major modifier of *Apc Min*-induced intestinal neoplasia. *Cell*, **81**, 957–966 (1995).