

Lack of Carcinogenicity of 2-Amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx) in Cynomolgus Monkeys

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The carcinogenic potential of 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx) was evaluated in cynomolgus monkeys. The animals received MeIQx, beginning at the age of one year, at doses of 10 or 20 mg/kg body weight by gavage five times a week for 84 months and were autopsied 8 months thereafter. Although sporadic development of aberrant crypt foci in the colon and glutathione *S*-transferase π -positive foci in the liver as well as hyperplastic changes of the lymphatic tissue in the lung and gastro-intestinal tract were observed in several monkeys, this was not treatment-related. No neoplastic or preneoplastic lesions were found in other organs. Serum chemistry data and organ weights were also within the normal ranges. From these data, it is concluded that MeIQx is not carcinogenic in the cynomolgus monkey under the conditions examined. This lack of carcinogenicity is probably related to the poor activation of MeIQx due to the lack of constitutive expression of CYP1A2 as well as an inability of other cytochrome P450s to catalyze *N*-hydroxylation of MeIQx in the cynomolgus monkey.

Key words: Heterocyclic amines — MeIQx — Cynomolgus monkey — Carcinogenicity

Mutagenic heterocyclic amines (HCAs) are produced when protein-rich foods such as meat and fish are heated.^{1,2)} Of more than 20 compounds which have been identified in this family, the concentration in cooked foods of 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx) is generally the largest next to 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), so that daily consumption may be as high as several μg per person.^{2,3)}

MeIQx is a genotoxic rodent carcinogen, inducing liver tumors, lung tumors, lymphomas and leukemia in CDF₁ mice following oral administration in the diet at 0.06% for 84 weeks,⁴⁾ and liver, Zymbal gland, clitoral gland and skin neoplasms in F344 rats receiving 0.04% for 61 weeks.⁵⁾ Another HCA, 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ), has been proved to be carcinogenic in the liver, forestomach and lung of mice⁶⁾ and in the Zymbal glands, colon, small intestine, liver, skin, oral cavity, clitoral gland, and mammary glands in rats.^{7,8)} To evaluate the potential of such chemicals as human carcinogens in a species with a close phylogenetic relationship to man, several chemicals have been studied in nonhuman primates.⁹⁾ When IQ was administered by gavage five times a week at

doses of 10 and 20 mg/kg body weight to cynomolgus monkeys, this HCA was found to induce hepatocellular carcinoma at high incidences with average latent periods of 60 and 43 months, respectively.¹⁰⁻¹²⁾ The present report documents findings from an 8-year carcinogenicity study in MeIQx-treated cynomolgus monkeys initiated in October 1988.¹²⁾

MATERIALS AND METHODS

Experiment MeIQx was purchased from the Nard Institute, Osaka and kept refrigerated in a desiccator until use. The purity was higher than 99.7%. The carcinogen was suspended daily with hydroxypropyl cellulose and administered to cynomolgus monkeys (*Macaca fascicularis*), born in a closed colony in the Covance Laboratories America (previously Corning Hazleton Laboratories, Inc.), Vienna, VA, USA, mother-reared, and assigned to the study as they reached approximately 1 year of age. Three males and 6 females were given MeIQx at a dose of 10 mg/kg body weight and 6 males and 4 females were given 20 mg/kg, by nasogastric intubation five times a week for a total of 84 months. The animals were killed 8 months after cessation of MeIQx administration. Single monkeys of each sex were maintained as controls. All were housed in wall-mounted, individual stainless steel cages equipped with an automatic watering device in an air-conditioned

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room with a 12 h light/12 h dark cycle. Purina High Protein Monkey Chow #5045 was offered twice daily, and the animals also received a vitamin sandwich and a portion of apple daily.

Observations All monkeys were observed daily and body weights were measured weekly. Evaluation by liver palpation and clinical chemistry and hematology tests were also performed every three months. Laparoscopic examination of the liver was carried out every 6–12 months. At the termination, the monkeys were killed with an overdose of sodium pentobarbital. All the organs were carefully examined for macroscopic lesions, weighed and fixed in 4% buffered formalin. The entire colons were spread, pinned on cork boards and stained with 0.2% methylene blue for 30 to 60 min after formalin fixation, in order to count aberrant crypt foci under a stereoscopic microscope. For histopathological examination, hematoxylin-eosin-stained sections were made from specimens of the heart, lung, trachea, esophagus, stomach, small intestine, colon, liver, gallbladder, pancreas, kidney, urinary bladder, spleen, thymus, thyroid, adrenal glands, mammary glands, brain,

nasal cavity, vertebra and reproductive organs. Liver sections were additionally immunostained for glutathione *S*-transferase (GST) placental form with a polyclonal antibody specific for the human homologue, GST- π (generous gift from the late Dr. K. Sato, Second Department of Biochemistry, Hirosaki University School of Medicine) and Vectastain ABC kits (Vector Laboratories, Burlingame, CA).

RESULTS

During the entire experiment, no unusual clinical symptoms were displayed by any of the animals. The final average body and organ weights are summarized in Table I. All individual body weights and organ weights (data not shown) were within the normal ranges. The average clinical chemistry values at termination were also normal (Table II). Macroscopically, one female monkey in the group fed MeIQx at a dose of 20 mg/kg had an approximately 7 mm diameter yellowish nodule-like lesion and another had multiple small cysts (1–2 mm diameter) in the

Table I. Final Body and Organ Weights (g)

	MeIQx 20 mg/kg b.w.		MeIQx 10 mg/kg b.w.		Control	
	Male	Female	Male	Female	Male	Female
No. of animals	6	4	3	6	1	1
Body	6500±518	4800±949	6233±833	3983±578	5600	3400
Brain	73.3±3.1	64.9±5.4	74.9±5.2	63.9±5.3	72.8	62.1
Spleen	4.8±0.8	5.1±2.0	3.9±0.8	3.8±1.4	4.9	3.7
Lung	26.0±3.5	18.8±1.7	29.5±6.1	17.5±2.0	23.1	12.2
Heart	23.6±3.6	15.9±5.1	22.2±1.2	14.0±2.5	15	10.9
Liver	93.0±5.5	87.3±13.8	86.7±13.1	71.8±9.9	79	57
Adrenal glands	0.54±0.14	0.55±0.10	0.69±0.25	0.48±0.07	0.87	0.53
Kidney	19.1±1.8	15.1±3.1	17.5±3.0	14.7±1.7	15.9	12.8
Thymus	1.60±1.10	3.08±1.45	1.09±0.72	1.97±0.91	0.60	2.06

Values are averages±SD.

Table II. Clinical Chemistry Values at the End Point

	MeIQx 20 mg/kg b.w.		MeIQx 10 mg/kg b.w.		Control	
	Male	Female	Male	Female	Male	Female
No. of animals	6	4	3	6	1	1
GOT	46.2±12.3	45.3±4.9	57.3±6.4	42.0±3.2	70	39
GPT	27.8±9.9	62.8±39.6	47.7±11.2	60.5±22.7	16	23
LDH	511.3±146.3	569.8±173.1	689.7±163.6	540.8±127.2	1277	812
ALP	168.5±31.4	159.0±31.1	208.7±91.9	205.3±71.8	134	206
T. BIL	0.28±0.12	0.23±0.05	0.27±0.06	0.20±0.00	0.3	0.1

GOT, glutamate oxaloacetate transaminase; GPT, glutamate pyruvate transaminase; LDH, lactate dehydrogenase; ALP, alkaline phosphatase; T.BIL, total bilirubin.

Values are averages±SD.

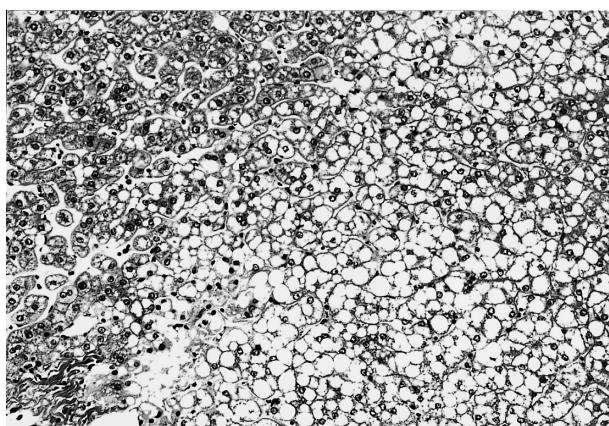


Fig. 1. Fat storage focus (right) found in the liver of a female monkey fed MeIQx at the dose of 20 mg/kg. Clear vacuoles are observed as fat droplets (hematoxylin-eosin staining).

liver. Microscopically, these were a fat storage focus that contained some hepatocytes with a few PAS-positive glycogen granules in their narrow cytoplasm, without compression of the surrounding tissue (Fig. 1), and bile duct cysts, respectively. No other lesions were noted on macroscopic examination.

In immunohistochemical analysis, spotty distribution of GST- π positive hepatocytes (Fig. 2A) was observed in two controls, in 3 (one male and two females) of 9 monkeys that received 10 mg/kg and in 2 (two females) of 10 monkeys that received 20 mg/kg. Clear diffuse induction of cytosolic GST- π in the hepatocytes around the central veins (Fig. 2B) was observed in 2 (one male and one female) and 8 (six males and two females) monkeys at doses of 10 and 20 mg/kg MeIQx, respectively. The intensity of expression was less in untreated animals. Typical GST placental form (GST- π , human; GST-p, rats)-positive liver cell foci, which are frequently observed in the liver of rats treated with various carcinogens,¹³⁻¹⁵ were not seen, but partial positive staining was seen in the fat storage focus (Fig. 2C).

Sporadic aberrant crypt foci in the colon were seen in each group of monkeys (Table III). In the male control, 2 foci composed of 16 crypts and 8 crypts were encountered in the transverse colon. In animals given 10 mg/kg MeIQx, a focus of 46 crypts in the transverse colon of a male and foci of 2 and 4 crypts (Fig. 3) in the descending colons of two females were observed. In the animals given 20 mg/kg of MeIQx, lesions composed of 6 crypts in the transverse colon in a male and 12 crypts in the transverse colon in a female were found.

Neither neoplastic nor preneoplastic lesions were found in any other organs. Hyperplastic changes of lymphatic tissue in the stomach (Fig. 4A), small and large intestines

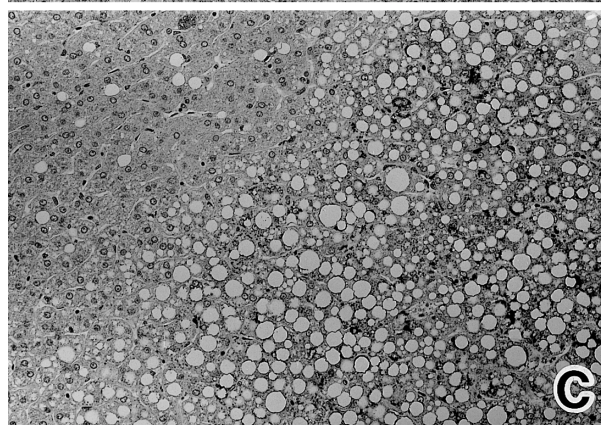
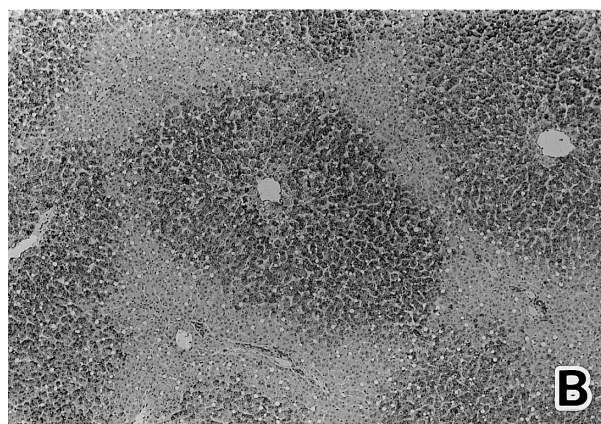
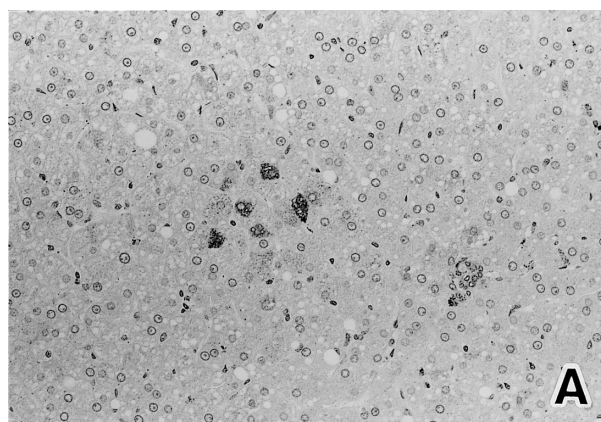


Fig. 2. Immunohistochemical staining for GST- π . A: Spotty positive cells in the liver of a female monkey fed MeIQx at the dose of 10 mg/kg. GST- π -positive cells were also seen in the bile duct. B: Diffuse distribution of GST- π -positive cells around the central vein area seen in a 10 mg/kg MeIQx-treated male monkey. C: Most of the hepatocytes in the fat storage focus (right) weakly express GST- π in their cytosol.

and lung (Fig. 4B) were noted in several monkeys but without any apparent relation to the treatment (Table IV). Increase in mesenchymal cells in the kidney was observed

Table III. Data for Colonic Aberrant Crypt Foci (ACF)

Treatment	Dose (mg/kg)	Sex	No. of monkeys	No. of ACF-bearing monkeys	Size of ACF (No. of crypts)
MeIQx	20	Male	6	1	6
		Female	4	1	12
MeIQx	10	Male	3	1	46
		Female	6	2	2, 4
Control		Male	1	1	16
		Female	1	0	—

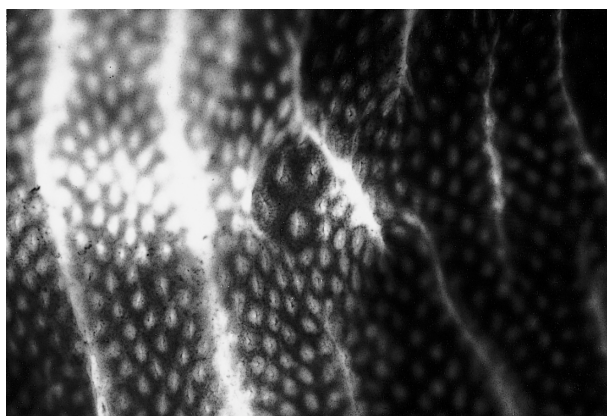


Fig. 3. Aberrant crypt focus in the colon of a 10 mg/kg MeIQx-treated monkey. The focus is composed of 4 enlarged crypts with elongated slit-like orifices (methylene blue staining).

in the two out of six males and one out of four females that received 20 mg/kg of MeIQx (Table IV).

DISCUSSION

In previous studies of long-term administration of IQ to cynomolgus monkeys, obvious carcinogenicity was seen in the liver.¹⁰⁻¹² However, in the present investigation of MeIQx in cynomolgus monkeys, no carcinogenicity was apparent in any organ, including the liver. If MeIQx is not carcinogenic in the cynomolgus monkey, the following factors may be pertinent. First, species differences in the capacity for metabolic activation of certain HCAs have been seen among the rat, cynomolgus monkey and man.¹⁶⁻¹⁸ MeIQx, like other HCAs, requires metabolism via *N*-oxidation for conversion into reactive species with genotoxic activity. This is reported to be catalyzed primarily by hepatic CYP1A2.¹⁹ In the cynomolgus monkey, expression of CYP3A4 and/or CYP2C9/10 which preferentially activate IQ, but not MeIQx, is dominant in the hepatic microsomes, whereas the levels of CYP1A1 and 1A2

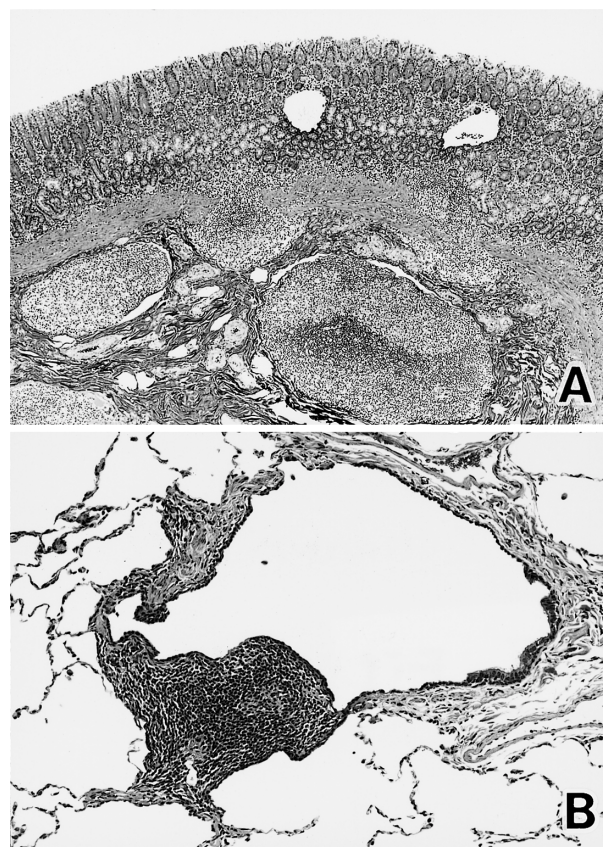


Fig. 4. Hyperplastic changes of the lymphatic tissue. A: Hyperplastic lymph nodes seen in stomach submucosa in a 20 mg/kg MeIQx-treated female (hematoxylin-eosin staining). B: Hyperplastic lymphatic tissue observed in the lung of a 20 mg/kg MeIQx-treated female (hematoxylin-eosin staining).

(responsible for activation of MeIQx) are very low or not detectable.²⁰⁻²² Snyderwine *et al.*²² reported that in cynomolgus monkeys, only 5.7% of dosed IQ was excreted into the urine unmetabolized and *N*-demethylated-IQ, which is weakly mutagenic, accounted for nearly 40% of

Table IV. Microscopic Findings for the MeIQx-treated Monkeys

Treatment	Dose (mg/kg)	Sex	No. of monkeys	Hyperplastic changes of lymphatic tissues				Increase of mesenchymal cells
				Stomach	Small intestine	Large intestine	Lung	Kidney
MeIQx	20	Male	6	4	3	0	1	2
		Female	4	4	4	2	2	1
MeIQx	10	Male	3	1	1	1	1	0
		Female	6	4	4	5	2	0
Control		Male	1	1	0	0	1	0
		Female	1	1	1	0	1	0

all urinary metabolites. On the other hand, 24% of the MeIQx was excreted unchanged in the urine and all the metabolites were detoxified forms, including the N^1 -glucuronide conjugate of MeIQx. Indeed, it was reported that intense IQ-DNA adduct formation, but no detectable level of MeIQx-DNA adducts, was observed in a number of organs of cynomolgus monkeys treated with these HCAs, using the ^{32}P -postlabeling method.²³⁾ A second consideration is that the doses and period of the experiment might not have been long enough to induce tumors. Thirdly, inactivation or detoxification of the chemical might have been important. Diffuse induction of GST- π was seen in the livers of most animals that received 20 mg/kg of MeIQx. Sporadic GST- π -positive hepatocytes were detected even in the controls, but they were much fewer than in MeIQx-treated monkeys. Thus, the induction of GST- π in the treated animals might have resulted in elevated detoxification of MeIQx. Proliferating oval cells, prominent in IQ-treated monkeys,²⁴⁾ were not observed in this experiment, so the liver toxicity of MeIQx was presumably also weaker than that of IQ.

In humans, 1.8–4.9% of oral MeIQx is excreted unmetabolized in urine after ingestion of a cooked meat meal²⁵⁾ and MeIQx-DNA adducts, most of them being dG-C8-MeIQx, have been detected in the colon and kidney.^{26, 27)} The human hepatic microsomal fraction activates MeIQx

as well as IQ and PhIP, through expression of CYP1A2 *in vitro*,²⁰⁾ and foodstuffs such as pan-fried meat increase CYP1A2 activity, as measured by the ratio of urinary caffeine metabolites, in healthy volunteers.²⁸⁾ Administration of furafylline, an inhibitor of CYP1A2, increased the levels of unchanged MeIQx and PhIP in human urine.²⁹⁾ Furthermore, healthy individuals with high CYP1A2 activity excrete lower levels of total unconjugated MeIQx in the urine when adjusted for the amount of meat eaten.³⁰⁾ CYP1A2 activity can be affected by life style factors such as diet or smoking.³¹⁾ Thus, a low susceptibility of cynomolgus monkeys to MeIQx might support the concept that inter-individual variation in CYP1A2 activity is relevant to cancers associated with exposure to HCAs.

In conclusion, a 7-year oral administration of MeIQx did not induce any neoplastic lesions in the cynomolgus monkey, which lacks expression of CYP1A2.

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