

Glandular stomach adenocarcinomas induced by NIAN treatment plus *H. pylori* infection were located in the pyloric region, similar to MNNG or MNU treatment plus *H. pylori* infection-induced glandular stomach adenocarcinomas in MGs.^{26,27} Meanwhile, no glandular stomach cancers were observed in the groups of *H. pylori*-infected MGs without NIAN treatment, which is consistent with previous studies,^{26,27} nor in the group treated with only NIAN. These findings indicated that *H. pylori* is a strong promoter of gastric carcinogenesis. Histological examination revealed that the tumors developed by NIAN + *H. pylori* were of well or moderately differentiated adenocarcinomas. Well or poorly differentiated adenocarcinomas and signet ring cell carcinomas were observed in *H. pylori*-infected MGs treated with MNNG or MNU.^{26,27} Further studies are required to clarify the histological variety of stomach adenocarcinomas induced by NIAN, MNNG or MNU, since the type of cancer might depend on the genotoxic action of chemical carcinogens, rather than the effects of *H. pylori* infection.²⁷ In addition, tumors were observed in skin and kidney, which were suspected to spontaneously develop. The MGs have been reported to develop spontaneous skin tumors such as sebaceous and squamous cell carcinoma.³⁴

Epidemiological studies have indicated that nitrate intake increases gastric cancer risk, and major sources are vegetables including Chinese cabbage, spinach and parsley.¹⁴ Indole-3-acetonitrile, a precursor of NIAN, is distributed widely in cruciferous vegetables including Chinese cabbage and sprouts.³⁵ Furthermore, fava beans (*Vicia faba*), which are commonly consumed in Colombia, give rise to a potent mutagen in the presence of nitrite under acidic conditions.³⁶ The nitrosatable precursor of the mutagen in fava beans and the major product of nitrosation are reported to be an indole compound, 4-chloro-6-methoxyindole and an *N*-nitroso compound, 4-chloro-2-hydroxy-*N*¹-nitroso-indolin-3-one oxime, respectively.³⁷ Other indole compounds are also reported to produce direct-acting mutagens after nitrite treatment under acidic conditions.^{38,39} In general, conversion of indole derivatives to nitrosated forms *in vitro* is known to be rapid and efficient at physiologically feasible nitrite concentrations with the low pH of the human stomach.³⁷ Thus, it is conceivable that nitrosation of indole compounds such as indole-3-acetonitrile probably occurs in human stomach. On the other hand, nitric oxide is suggested to be produced by activated macrophages in inflamed organs with *H. pylori* infection.¹⁸ Therefore, nitrosation of indole compounds could be mediated by both acid catalysis and inflammatory responses in the human stomach.^{18,20,37-40} On the basis of the conversion rate

of NIAN from indole-3-acetonitrile under physiological conditions, the dose of NIAN used in the present study appears about 500–1000 fold the expected human exposure to NIAN *via* fresh or pickled Chinese cabbage. However, humans continually consume various kinds of foods containing indole compounds and nitrate during ordinary life. Thus, it is probable that the total amount of nitroso-indole compounds would be much closer to the dose of NIAN used in the present study. Moreover, it has been reported that low doses of chemical carcinogens, such as MNNG and MNU, could induce glandular stomach cancers in rodents under inflammation conditions including NaCl treatment and *H. pylori* infection, but hardly induce glandular stomach cancer without NaCl treatment and *H. pylori* infection. Therefore, the continuous intake of indole compounds and nitrate may play an important role for gastric carcinogenesis in East Asian countries still with a high salt consumption and *H. pylori* infection rate.

Gastric cancer is tending to decline in most countries.⁴¹⁻⁴³ One of the explanations for this tendency is the reduced prevalence of *H. pylori* infection.⁴² Changes in dietary habits, mainly being lower salt consumption, could be also related to reduced gastric cancer incidence. However, the gastric cancer prevalence in East Asian countries, such as Japan and Korea, is still high.² At present, we have not succeeded in detecting NIAN in human bodies nor the exposure levels of the precursor, indole compounds for humans. Thus, it is necessary to estimate the human exposure levels to nitroso-indole compounds including NIAN, and to study further animal experiments and epidemiological analyses for clarification of contribution of nitroso-indole compounds under *H. pylori* infection in humans gastric carcinogenesis.

In conclusion, the present study demonstrated that NIAN can induce gastric cancer in *H. pylori*-infected MGs. It is noteworthy that nitrosatable precursors widely exist in foods. Thus, it is suggested that *N*-nitroso indole compounds including NIAN might contribute to the frequent development of gastric cancer in East Asian countries such as Japan and Korea in which the prevalence of *H. pylori* infection is relatively high. Further studies of interaction with other dietary elements appear warranted to promote the prevention of human gastric cancer.

Acknowledgements

The authors thank Dr. Nobuo Takasuka, Naoaki Uchiya and Yusaku Hori for their expert technical assistance. S.T. is presently the recipient of a Research Resident Fellowship from the Foundation for Promotion of Cancer Research.

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Low-dose carcinogenicity of 2-amino-3-methylimidazo[4,5-*f*]quinoline in rats: Evidence for the existence of no-effect levels and a mechanism involving p21^{Cip/WAF1}

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(Received June 1, 2010/Revised September 6, 2010/Accepted September 19, 2010/Accepted manuscript online September 28, 2010/Article first published online October 21, 2010)

The carcinogenicity of the low amounts of genotoxic carcinogens present in food is of pressing concern. The purpose of the present study was to determine the carcinogenicity of low doses of the dietary genotoxic carcinogen 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ) and to investigate mechanisms by which IQ exerts its carcinogenic effects. A total of 1595 male F344 rats were divided into seven groups and administered with IQ at doses of 0, 0.001, 0.01, 0.1, 1, 10 and 100 p.p.m. in the diet for 16 weeks. We found that IQ doses of 1 p.p.m. and below did not induce preneoplastic lesions in either the liver or the colon, while IQ doses of 10 and 100 p.p.m. induced preneoplastic lesions in both of these organs. These results demonstrate the presence of no-effect levels of IQ for both liver and colon carcinogenicity in rats. The finding that p21^{Cip/WAF1} was significantly induced in the liver at doses well below those required for IQ mediated carcinogenic effects suggests that induction of p21^{Cip/WAF1} is one of the mechanisms responsible for the observed no-effect of low doses of IQ. Furthermore, IQ administration caused significant induction of CYP1A2 at doses of 0.01–10 p.p.m., but administration of 100 p.p.m. IQ induced CYP1A1 rather than CYP1A2. This result indicates the importance of dosage when interpreting data on the carcinogenicity and metabolic activation of IQ. Overall, our results suggest the existence of no-effect levels for the carcinogenicity of this genotoxic compound. (*Cancer Sci* 2011; 102: 88–94)

Exposure to environmental carcinogens is one of the most significant causes of human cancers. Determination of the dose-response relationship between carcinogen exposure and induction of cancer is one of the most important areas of chemical risk assessment. Of particularly high priority is the cancer risk assessment of dietary carcinogens.

Heterocyclic amines (HCA) are well known dietary genotoxic carcinogens derived from cooked protein-rich foods such as meat and fish,^(1–3) and the carcinogenicities of 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx), 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) and 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ) have been widely investigated in various animal models. MeIQx induces cancers of the liver, zymbal gland, skin and clitoral gland in rats,⁽⁴⁾ and caners of the liver and lung, and lymphoma and leukemia in mice.⁽⁵⁾ PhIP induces colon cancers and mammary gland cancers in rats,⁽⁶⁾ and lymphomas in mice.⁽⁷⁾ IQ induces cancers of the liver, colon, mammary and zymbal glands in rats, caners of the liver, lung and forestomach in mice, and cancer of the liver in non-human primates.^(8–10) MeIQx and PhIP are classified as category

2B compounds (possibly carcinogenic to humans) and IQ is classified as a category 2A compound (probably carcinogenic to humans) by the International Agency for Research on Cancer.⁽¹¹⁾ Therefore, although the concentrations of HCA in food are low, they constitute a potential hazard, and there is concern regarding the carcinogenic effects of low doses of these HCA.

Based on the view that even minute doses of a genotoxic carcinogen has the potential to produce irreversible deleterious genetic changes in the DNA of a target organ cell and the argument that if sufficient numbers of test animals are used the carcinogenic effect of a minute dose can be demonstrated, it is generally assumed that genotoxic carcinogens exert a non-threshold carcinogenic effect. However, the carcinogenicities of most genotoxic carcinogens are determined by experimental animal carcinogenicity studies using doses that are generally orders of magnitude higher than actual human exposure levels and the dose-response curves obtained are then extrapolated to zero using a non-threshold mathematical model. This approach, however, is being challenged as advancements in the understanding of the molecular mechanisms of carcinogenesis are being made and experimental evidence showing that genotoxic carcinogens do not exert mutagenic and carcinogenic effects at low doses accumulates.^(12–19)

Previously, we demonstrated the existence of no-effect levels of MeIQx for both hepatocarcinogenicity and *in vivo* mutagenicity in various carcinogenesis models in different rat strains.^(17,20–22) It has also been shown that low doses of PhIP do not exert either initiation or promotion activities in colon carcinogenesis in the rat.^(23,24) However, little is known about the carcinogenic potential of low doses of IQ.

In addition, little is known about the mechanisms underlying the carcinogenicities of lower doses of HCA, but incorporation of mechanistic information is critical for quantitative cancer risk assessment. The purpose of the present study is to determine the relationship between administration of low doses of IQ and induction of preneoplastic lesions in the liver and colon in rats, and to investigate carcinogenic mechanisms of action of various doses of IQ by evaluating DNA-adduct formation, oxidative DNA damage and expression levels of genes involved in metabolic activation of IQ, cell proliferation and DNA damage repair in the liver.

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Materials and Methods

Chemical and diets. IQ was purchased from Nard Institute Ltd (Osaka, Japan) with a purity of 99.9%. Basal diets (powdered MF; Oriental Yeast Co., Tokyo, Japan) and the diets containing IQ were prepared once a month by Oriental Yeast Co.

Animals. A total of 1595 male F344 rats were supplied by Charles River Japan, Inc. (Hino, Shiga, Japan) and were used at 21 days of age. Animals were housed in polycarbonate cages (five per cage) in experimental animal rooms with a targeted temperature of $22 \pm 3^\circ\text{C}$, relative humidity of $55 \pm 5\%$ and a 12-h light/dark cycle. Diet and tap water were available *ad libitum* throughout the study.

Experimental design. The animal experiment protocols were approved by the Institutional Animal Care and Use Committee of Osaka City University Medical School. Rats were randomized into seven groups, 245 rats in each of groups 1–6 and 125 rats in group 7. Since the levels of IQ in cooked foods are lower than those of MeIQx and PhIP,⁽¹¹⁾ IQ dosage and treatment duration in this study were the same as the previous low dose carcinogenicity studies with MeIQx and PhIP.^(18,24) Animals were fed diets containing IQ as follows: 0 (group 1, control), 0.001 (group 2), 0.01 (group 3), 0.1 (group 4), 1 (group 5), 10 (group 6) and 100 p.p.m. (group 7) for 16 weeks. Fresh diet was supplied to the animals twice weekly. Bodyweights, food consumption and water intake were measured weekly.

Five rats in each group were killed at week 4 under ether anesthesia. At death, livers were snap frozen in liquid nitrogen and stored at -80°C for examination of IQ-DNA adducts and 8-hydroxy-2'-deoxyguanosine (8-OHdG) formation in the DNA. The remaining rats were killed at the end of week 16 under ether anesthesia for examination of the development of glutathione S-transferase placental form (GST-P) positive foci, which is a well-established preneoplastic lesion in the rat liver,^(25,26) and aberrant crypt foci (ACF), which is a surrogate marker for preneoplastic lesions in the rat colon.^(24,27,28) At death, livers were excised, weighed and then three slices each from the left lateral, medial and right lateral lobes were cut and placed in 10% phosphate-buffered formalin. The remaining liver tissues were snap frozen in liquid nitrogen and stored at -80°C for mRNA expression analysis. Following fixation, liver tissues were embedded in paraffin and processed for histopathological examination.

Examination of GST-P positive foci in the liver. Anti-rat GST-P polyclonal antibody (Medical and Biological Laboratories Co., Ltd, Nagoya, Japan) at a dilution of 1:1000 was used for immunohistochemical staining of GST-P. The GST-P-positive hepatocellular foci composed of two or more cells were counted under a light microscope.^(17,18,20,22) Total areas of livers were measured using a color image processor IPAP (Sumica Technos, Osaka, Japan) and the number of GST-P-positive foci per square centimeter of liver tissue was calculated.

IQ-DNA adduct and 8-OHdG formation in livers. IQ-DNA adducts were measured by the ^{32}P -postlabeling method as described previously.^(29,30) Levels of 8-OHdG formation in liver DNA were determined by high-performance liquid chromatography with electrochemical detection as previously described.⁽³¹⁾

TaqMan real-time quantitative PCR. The mRNA expression levels of genes involved in IQ metabolism (CYP1A1, CYP1A2 and CYP1B1), DNA damage repair (8-oxoguanine DNA glycosylase [Ogg1], growth arrest and DNA damage-inducible protein 45 [GADD45], AP endonuclease-1 [APE-1], MSH2 and MSH3) and cell cycle regulation (p53 and p21^{Cip/WAF1} and proliferating cell nuclear antigen [PCNA]) were evaluated in the livers by TaqMan real-time quantitative PCR as described previously.⁽³¹⁾ Sequence-specific primers and probes (Taqman Gene Expression Assay) were purchased from Applied Biosystems, Inc., Carlsbad, CA, USA. Beta-2-microglobulin (B2M) was used as an internal control.

Examination of ACF in colon. Formation of ACF was examined as described previously.⁽²⁴⁾ Although ACF consisting of four or more crypts are considered to be better predictors of colon tumor outcome in rats,⁽³²⁾ to ensure that all doses of IQ that have the potential to induce colon carcinogenesis were accounted for, doses of IQ that caused an increase of any size of ACF were considered to have the potential to induce colon carcinogenesis in the present study.⁽²⁴⁾

Statistical analysis. All mean values are reported as mean \pm SD. Statistical analyses were performed using the Stat-light program (Yukms Co., Ltd, Tokyo, Japan). Homogeneity of variance was tested by the Bartlett test. Differences in mean values between the control and IQ-treated groups were evaluated by the 2-tailed Dunnett test when variance was homogeneous and the 2-tailed Steel test when variance was heterogeneous.^(22,31) *P* values <0.05 were considered significant.

Results

General observation. All animals survived to the end of study without any apparent abnormal pathological features. The final average body and liver weights and IQ intake are summarized in Table 1. The final bodyweight of the 100 p.p.m. group was significantly lower than that of the 0 p.p.m. group. Absolute and relative liver weights were significantly decreased in the 0.1 and 1 p.p.m. groups and were significantly increased in the 100 p.p.m. group compared with the 0 p.p.m. group. There were no significant differences in either food or water consumption among groups (data not shown). The intake of IQ was proportional to the administered doses (Table 1). No tumors were found in any organs including the liver and colon in any of the groups.

Induction of GST-P-positive foci in the livers. No histopathological changes were observed in any of the IQ-treated groups.

Table 1. Body and organ weights, and IQ intake

Group	IQ (p.p.m.)	No. rats	Bodyweight (g)	Liver		Average IQ intake	
				Absolute weight (g)	Relative weight (%)	Daily intake (mg/kg b.w.)	Total (mg/kg b.w.)
1	0	240	331 \pm 23	9.3 \pm 1.7	2.8 \pm 0.4	0	
2	0.001	240	332 \pm 17	9.1 \pm 1.4	2.8 \pm 0.4	0.0001	0.008
3	0.01	240	331 \pm 19	9.0 \pm 1.5	2.8 \pm 0.4	0.0007	0.08
4	0.1	240	331 \pm 22	8.5 \pm 1.2*	2.6 \pm 0.3*	0.008	0.9
5	1	240	331 \pm 17	8.5 \pm 1.2*	2.6 \pm 0.3*	0.08	8.7
6	10	240	330 \pm 18	9.0 \pm 1.3	2.7 \pm 0.4	0.76	85.1
7	100	120	319 \pm 19*	10.0 \pm 1.6*	3.2 \pm 0.4*	7.83	877.5

*Significantly different from group 1. IQ, 2-amino-3-methylimidazo[4,5-f]quinoline.

The number and size of GST-P-positive foci in rat livers at week 16 is summarized in Table 2. The total numbers of GST-P-positive foci per unit area in the livers in the groups administered 0.001–1 p.p.m. IQ did not differ from the control value (0 p.p.m. group), and no significant increases were observed in any size range of GST-P-positive foci in these groups. Significant increases in the total numbers of GST-P-positive foci per unit area in the liver were observed in the 10 and 100 p.p.m. groups compared with the control. The numbers of GST-P-positive foci composed of 2–4 cells and 5–10 cells in the 10 p.p.m. group and GST-P-positive foci of all sizes in the 100 p.p.m. group were significantly increased.

Formation of IQ-DNA adduct and 8-OHdG in liver DNA. Representative autoradiograms of IQ-DNA adducts in livers are shown in Figure 1. The levels of IQ-DNA adducts in the livers of the 0 and 0.001 p.p.m. IQ-treated groups were under the detectable limit at week 4 (Table 3). IQ-DNA adducts were detectable in the livers of rats administered 0.01 p.p.m. IQ, and adduct formation increased in a dose-dependent manner in groups administered higher doses of IQ. No significant differences in 8-OHdG levels were observed in the liver DNA between any of the groups administered IQ and the control group (Table 3).

Gene expression changes in the liver. Relative mRNA expression of IQ metabolizing genes CYP1A1 and CYP1A2, cell cycle genes PCNA and p21^{Cip/WAF1}, p53, and DNA repair genes APE-1 and GADD45 in the livers at week 16 is shown in Figure 2. CYP1A1 was significantly increased in the livers of rats treated with 100 p.p.m. IQ, but not in the lower doses of IQ. CYP1A2, on the other hand, was significantly increased in the 0.01–10 p.p.m. groups, but no significant change was observed in the 100 p.p.m. group. There was no significant difference in the CYP1B1 expression level among groups (data not shown).

A significant increase in PCNA was observed in the 100 p.p.m. group, but not in the groups administered lower doses of IQ, while the negative cell cycle regulator p21^{Cip/WAF1} was significantly induced in the 0.01 p.p.m. group and maximally induced in the 100 p.p.m. group. The expression level of p21^{Cip/WAF1} in the 100 p.p.m. group was significantly higher than in the 10 p.p.m. and lower dose groups. There were no significant changes in p53 expression levels in the IQ-treated groups.

APE-1 was significantly induced in the 10 and 100 p.p.m. groups and GADD45 was significantly induced in the 100 p.p.m. group. IQ had no effect on the expression of Ogg-1, MSH2 or MSH3 (data not shown).

Induction of ACF in the colon. The number and size of ACF in rat colons at week 16 is summarized in Table 4. In the 10 p.p.m. group, the number of ACF composed of one crypt was significantly increased compared with the control. In the 100 p.p.m. group, significant increases were observed in the

numbers of all sizes of ACF. In contrast, in the groups administered 0.001–1 p.p.m. IQ, neither the number of any size ACF nor the total number of ACF differed from the control.

Discussion

Dose-response relationships for genotoxic carcinogens have been a topic of intense scientific and public debate. High doses of the genotoxic dietary carcinogen IQ have been demonstrated to induce liver and colon cancers in rats (300 p.p.m. in diet)⁽⁸⁾ and liver cancers in nonhuman primates (10 mg/kg b.w./day).⁽¹⁰⁾ However, as the concentrations of IQ in food are generally extremely low,⁽¹¹⁾ there is uncertainty regarding the carcinogenicity of the doses of IQ to which humans are exposed. The present study shows that IQ at doses of 1 p.p.m. (0.08 mg/kg body weight [b.w.]/day) and lower did not induce either GST-P-positive foci in the liver or ACF in the colon. Only in the groups administered higher doses of IQ, 10 p.p.m. (0.76 mg/kg b.w./day) and 100 p.p.m. (7.83 mg/kg b.w./day), were increases in GST-P-positive foci and ACF observed.

GST-P-positive foci and ACF are well-established preneoplastic lesions of the liver and colon, respectively, in rats. These lesions have been accepted as useful end-point markers in the assessment of carcinogenic effects of environmentally relevant concentrations of carcinogens as they can extend the range of observable effect levels.^(24,26) Therefore, the results of the present study suggest the presence of no-effect levels of IQ for both liver and colon carcinogenicity in rats and indicate that the dose-response relationship for carcinogenicity of low dose IQ is nonlinear.

Several threshold mechanisms for genotoxic carcinogens have been suggested, including induction of detoxification processes, cell cycle delay, DNA repair, apoptosis and the suppression of neoplastically transformed cells by the immune system.^(12,13,15,33) However, little *in vivo* evidence is available. To explore mechanisms underlying the carcinogenicity of low doses of IQ, we examined the relative mRNA expression of a panel of genes involved in cell proliferation, cell cycle regulation, DNA repair and IQ metabolic activation. We found that the cell proliferation marker PCNA was significantly increased only at a dose of 100 p.p.m., a dose that is carcinogenic. The cell cycle negative regulator p21^{Cip/WAF1}, on the other hand, was significantly induced at a dose of 0.01 p.p.m., a dose well below that which induced the formation of preneoplastic lesions. Furthermore, the finding that the levels of p21^{Cip/WAF1} in the groups administered 10 p.p.m. and less were much lower than that of the group administered 100 p.p.m. implies that hepatocytes have adequate capacity to cope with the type of damage that is repaired by the p21^{Cip/WAF1} pathway when exposed to low doses of IQ, but that the repair capacity of these hepatocytes, even in the presence of high p21^{Cip/WAF1} expression, can be overwhelmed when the cell is subjected to very high doses of IQ. It is reasonable to suggest

Table 2. Development of GST-P-positive foci in the livers of rats administered IQ for 16 weeks

Group	IQ (p.p.m.)	No. rats	Size of GST-P positive foci				Total
			2–4	5–10	11–20	≥21	
1	0	240	0.09 ± 0.25	0.03 ± 0.11	0.02 ± 0.11	0.00 ± 0.02	0.15 ± 0.31
2	0.001	240	0.10 ± 0.24	0.04 ± 0.15	0.01 ± 0.07	0	0.16 ± 0.31
3	0.01	240	0.15 ± 0.47	0.07 ± 0.41	0.02 ± 0.22	0.02 ± 0.03	0.26 ± 1.30
4	0.1	240	0.10 ± 0.28	0.04 ± 0.15	0.01 ± 0.07	0.01 ± 0.08	0.15 ± 0.35
5	1	240	0.10 ± 0.25	0.04 ± 0.16	0.01 ± 0.06	0	0.14 ± 0.33
6	10	240	0.51 ± 0.65	0.19 ± 0.36*	0.02 ± 0.10	0.01 ± 0.11	0.74 ± 0.88*
7	100	120	26.23 ± 18.24*	23.81 ± 16.23*	19.25 ± 11.70*	18.74 ± 11.81*	88.03 ± 50.41*

*Significantly different from group 1. GST-P, glutathione S-transferase placental form positive foci; IQ, 2-amino-3-methylimidazo[4,5-f]quinoline.

Fig. 1. Autoradiograms of 2-amino-3-methylimidazo[4,5-f]quinoline (IQ)-DNA adducts the livers of 0 (a), 0.001 (b) and 100 (c) p.p.m. IQ-treated groups at week 4. Arrowheads indicate IQ-DNA adduct. The imaging plates were exposed for 3 h (a) and 24 h (b and c).

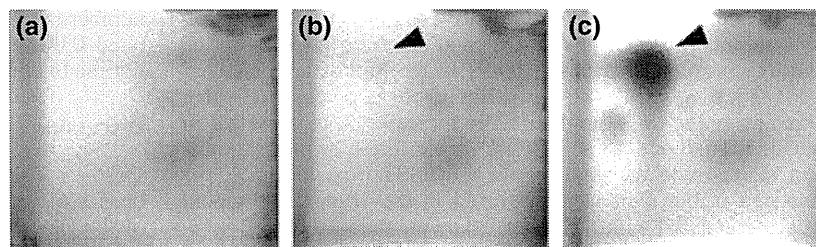


Table 3. IQ-DNA adduct and 8-OHdG formation in liver DNA

Group	IQ (p.p.m.)	No. rats	Adduct level ($\times 10^{-7}$ ntd)	8-OHdG ($\times 10^{-5}$ dG)
1	0	5	UDL	0.23 \pm 0.07
2	0.001	5	UDL	0.25 \pm 0.05
3	0.01	5	0.045 \pm 0.02	0.24 \pm 0.07
4	0.1	5	0.1 \pm 0.004	0.32 \pm 0.10
5	1	5	1.7 \pm 0.07	0.24 \pm 0.08
6	10	5	12.7 \pm 0.07	0.22 \pm 0.07
7	100	5	107.0 \pm 0.07	0.23 \pm 0.08

IQ, 2-amino-3-methylimidazo[4,5-f]quinoline; ntd, nucleotide; 8-OHdG: 8-hydroxy-2'-deoxyguanosine; UDL, under the detectable limit.

that suppression of cell cycle progression by p21^{Cip/WAF1} followed by DNA repair is at least one of the mechanisms responsible for the observed no-effect of low doses of IQ in rats in the present model.

It is known that the vast majority of DNA damage is repaired by base excision repair (BER), nucleotide excision repair (NER) and mismatch repair (MMR).⁽³⁴⁾ APE-1 plays an essential role in the BER repair process by cleaving the phosphodiester backbone.⁽³⁵⁾ The activities of two different heterodimeric complexes, MSH2-MSH3 and MSH2-MSH6, belonging to the MMR system are mainly responsible for the post-replicative repair of mismatches.⁽³⁶⁾ We found that IQ significantly increased the expression levels of APE-1 but not MSH2 and MSH3 at doses of 10 and 100 p.p.m. in the liver. It has also been reported that IQ has no effect on expression of ERCC1, which is a key molecule in the NER process.⁽³⁷⁾ These findings suggest that BER rather than MMR or NER responds to IQ-induced DNA damage.

GADD45 is involved in a variety of growth regulatory mechanisms, including DNA repair, growth arrest and apoptosis.⁽³⁸⁾ It is induced by genotoxic and certain other cell stresses by p53-dependent and independent pathways.^(39,40) GADD45 expression was significantly induced in the 100 p.p.m. group. The fact that significant induction of APE-1 and GADD45 was observed only at the highest doses of 10 and/or 100 p.p.m. indicate the IQ-induced DNA damage response is dose-dependent. Moreover, the fact that in the groups with low doses expression of APE-1 and GADD45 were not affected and that there was a significant but moderate induction of p21^{Cip/WAF1} imply that normal physiological levels of these genes are sufficient to repair the DNA damage caused by low doses of IQ. However, the expression levels of these genes are all increased by higher carcinogenic doses of IQ. A reasonable explanation of the no-effect of low doses of IQ and the carcinogenicity of high doses of IQ is that carcinogenicity is the consequence of a disruption in the balance between DNA damage and repair and between abnormal cell proliferation and apoptosis or cell cycle regulation.

Our results show that p53 gene expression is not induced by administration of IQ. Furthermore, p53-deficient mice do not show higher susceptibility to IQ-induced liver carcinogenesis

than wild type mice.⁽⁴¹⁾ These results suggest that p53 does not have a significant impact on the carcinogenicity of IQ.

DNA adduct formation by metabolic activation of IQ is believed to play an important role in the carcinogenicity of IQ.⁽⁴²⁾ Formation of IQ-DNA adducts in the liver showed a linear dose-dependency and proved to be one of the most sensitive end-points for the detection of exposure to IQ. Adduct formation was detectable in groups administered far lower doses of IQ compared with detection of other end-points such as cell proliferation and preneoplastic lesion induction. That IQ-DNA adduct formation was not detected in the 0.001 p.p.m. group was most likely due to the detection limit of the assay. It should be noted that DNA adduct is a premutagenic lesion and not necessarily correlated to the frequencies of mutation and cancer induced by genotoxic compounds. For example, it is known that IQ forms DNA adducts in the kidneys and stomach of both rats and monkeys, but does not induce tumors in these organs.^(43,44) Our present findings of a linear dose-response of IQ-DNA adduct formation and a nonlinear carcinogenic dose-response to IQ administration support the idea that IQ-DNA adducts do not necessarily lead to mutation and formation of cancerous lesions. Our results are also in line with previous results on HCA including MeIQx^(1,18,45) and PhIP.⁽²⁴⁾ These results can be explained, at least in part, by the actions of gene products such as p21^{Cip/WAF1}, GADD45 and APE-1 and the other repair genes for DNA damage. Moreover, in the case of MeIQx, it has been suggested that formation of DNA adducts alone might not be sufficient to produce cancers and that the MeIQx-induced genetic alterations in the liver are enhanced by liver regeneration induced by high doses of MeIQx itself.⁽¹⁾ Therefore, while IQ-DNA adduct formation is important in IQ carcinogenicity, high levels of adduct formation are likely required and other factors such as cell proliferation can affect the balance between DNA damage and repair and lead to fixation of DNA mutations into the cell's genome.

It has been demonstrated *in vitro* that IQ is more efficiently metabolized and activated by CYP1A2 than by CYP1A1 or CYP1B1.⁽⁴⁶⁾ However, limited *in vivo* data are available. In a study by McPherson *et al.*⁽⁴⁷⁾, no significant induction in mRNA expression level or activity of either CYP1A1 or CYP1A2 were reported in the livers of rats receiving 300 p.p.m. IQ in the diet for 52 weeks, but these enzymes were significantly increased after daily administration of 20 mg/kg b.w. IQ by oral gavage for 3 days; in the average adult rat, a dose of 300 p.p.m. IQ in the diet is approximately equivalent to administration of 20 mg/kg b.w. IQ by oral gavage. The results of the present study revealed that IQ significantly induced CYP1A2 expression at doses from 0.01 to 10 p.p.m., but CYP1A2 was not induced in the 100 p.p.m. group. The lack of effect of 100 p.p.m. IQ on CYP1A2 expression is consistent with the results in rats receiving 300 p.p.m. IQ in the diet for 52 weeks.⁽⁴⁷⁾ Significant increases in CYP1A1 expression in the 100 p.p.m. group provide an alternative mechanism that can compensate for decreased CYP1A2 activity. However, as noted above, in apparent contrast to our results, in the study by McPherson *et al.*,⁽⁴⁷⁾ administration of 300 p.p.m. IQ over the course of 52 weeks did

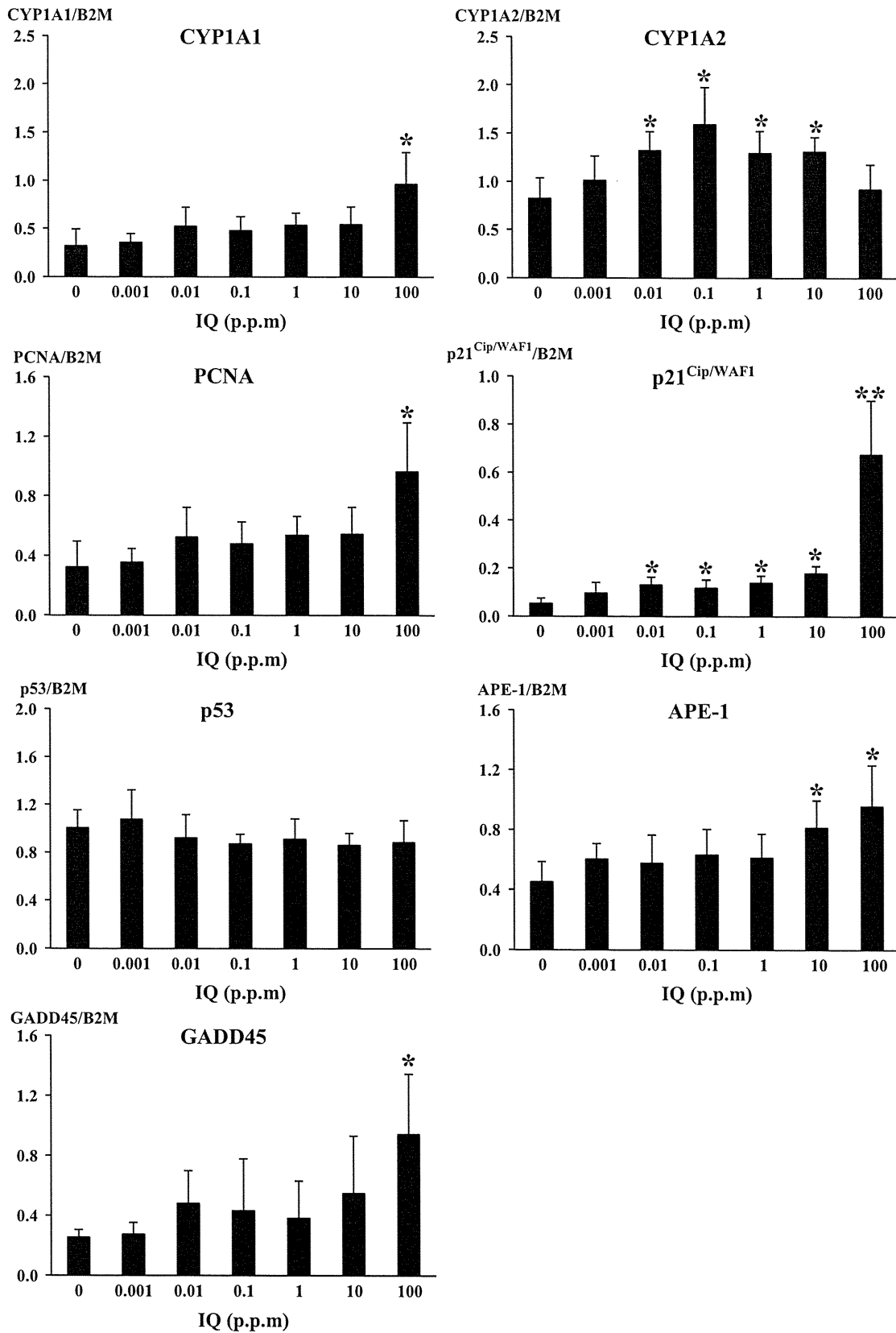


Fig. 2. Relative mRNA expression in the livers of rats at week 16. *Significantly different from 0 p.p.m. **Significantly different from all other groups. APE-1, AP endonuclease-1; B2M, beta-2-microglobulin; GADD45, growth arrest and DNA damage-inducible protein 45; PCNA, proliferating cell nuclear antigen.

Table 4. Development of ACF in the colons of rats administered IQ for 16 weeks

Group	IQ (p.p.m.)	No. rats	Size of ACF				Total
			1	2	3	≥4	
1	0	240	0.08 ± 0.28	0.12 ± 0.32	0.06 ± 0.25	0.08 ± 0.29	0.33 ± 0.64
2	0.001	240	0.12 ± 0.36	0.08 ± 0.29	0.10 ± 0.32	0.09 ± 0.30	0.39 ± 0.69
3	0.01	240	0.15 ± 0.41	0.15 ± 0.42	0.06 ± 0.24	0.06 ± 0.24	0.43 ± 0.77
4	0.1	240	0.11 ± 0.33	0.11 ± 0.35	0.06 ± 0.25	0.08 ± 0.27	0.36 ± 0.63
5	1	240	0.15 ± 0.45	0.10 ± 0.30	0.10 ± 0.33	0.05 ± 0.23	0.41 ± 0.80
6	10	240	0.19 ± 0.48*	0.16 ± 0.41	0.07 ± 0.25	0.09 ± 0.40	0.50 ± 0.86
7	100	120	1.48 ± 1.46*	1.29 ± 1.51*	0.70 ± 0.93*	0.72 ± 1.01*	4.19 ± 3.34*

*Significantly different from group 1. ACF, aberrant crypt foci; IQ, 2-amino-3-methylimidazo[4,5-f]quinoline.

not induce CYP1A1. Therefore, it is reasonable to postulate that the dose-relationship between IQ and induction of CYP1A1 is not a simple dose-response. CYP1B1 does not appear to be involved in the metabolism of IQ at doses up to 100 p.p.m. in rats. The findings described above demonstrate the importance of taking into account dosage, duration and route of exposure in interpretation of the data on metabolic activation of IQ. Further studies on the dose-response relationships between chronic IQ exposure and the protein expression levels and activities of detoxifying enzymes, especially at doses relevant to human exposure, would provide further insight into the role of metabolic activation in IQ carcinogenicity.

Oxidative DNA damage does not appear to play a role in IQ-induced carcinogenesis. In the present study, no significant changes in 8-OHdG levels or Ogg1 expression levels in the livers of IQ-treated rats were observed. Our results are consistent with the recent findings in IQ-treated Big Blue rats that oxidative stress was not responsible for the initiation of IQ-induced carcinogenesis in the liver and colon.⁽³⁷⁾ In this respect, IQ is different from MeIQx, in which oxidative DNA damage plays an important role in liver carcinogenesis.⁽⁴⁸⁾

In summary, the present study provides the first experimental data on the carcinogenicity of low doses of IQ in both the liver and colon of the test animal and compares the effect of IQ at the

cellular level with its carcinogenic effect. Our findings support the idea that there is a practical threshold that should be considered when evaluating the risk of genotoxic carcinogens. To this end, further accumulation of data, especially mechanistic data, should be promoted to facilitate not only an understanding of the carcinogenic effects of low doses of genotoxic carcinogens but also to establish an accurate means of quantitative risk assessment.

Acknowledgments

The authors would like to acknowledge the encouragement of Dr N. Ito (Emeritus Professor, Nagoya City University Medical School, Nagoya, Japan) and Dr T. Kitagawa (Emeritus Director, the Cancer Institute of Japanese Foundation for Cancer Research, Tokyo). This research was supported by a grant from the Japan Science and Technology Corporation, included in the Project of Core Research for Evolutional Science and Technology (CREST) and a Grant-in-Aid for Specially Promoted Research from the Ministry of Education, Science, Sports, Culture and Technology of Japan.

Disclosure Statement

The authors have no conflict of interest.

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