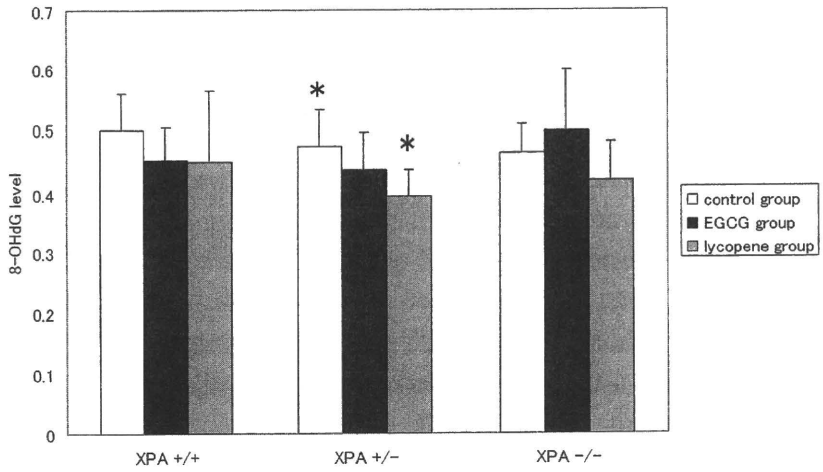

Fig. 2. Levels of 8-OHdG in non-tumorous liver tissues of mice at 8 months of age. The *XPA* +/ − mice in the lycopene group showed a significantly lower 8-OHdG level than those in the control group (*) ($p<0.05$), but no other significant differences were observed.

otype. Conversely, among the *XPA* −/− mice, the average number of HCCs per mouse was significantly higher in the lycopene group than in the control group ($p<0.05$). Contrary to our previous report, the *XPA* −/− mice did not show a higher incidence or multiplicity of liver tumors than the *XPA* +/+ and *XPA* +/− mice in the control group. With regard to tumor size, the average diameter of tumors in the *XPA* −/− mice of the lycopene group was significantly larger than that of the tumors of the *XPA* +/− and *XPA* −/− mice of the lycopene and control groups, respectively ($p<0.05$). However, no other significant differences were observed.

Formation of 8-OHdG

Figure 2 shows the levels of 8-OHdG, a biomarker of oxidative DNA damage, in non-tumorous liver tissues at 8 months of age. The *XPA* +/− mice of the lycopene group demonstrated significantly reduced 8-OHdG formation than those in the control group ($p<0.05$) ; however, no other significant differences were observed.

DISCUSSION

In addition to its powerful antioxidative activity, EGCG has been proposed to possess various other properties, including the ability to induce antiangiogenesis, apoptosis, cell cycle regulation, and antimicrobial activity[31]. EGCG has been reported to inhibit carcinogen-induced mouse duodenal[13] and skin[14] tumors and preneoplastic foci of rat liver[32]. Contrary to our results, Nishida *et al.* reported that a dose of 0.05% and 0.1% EGCG in drinking water inhibited the formation of spontaneous hepatoma in C3H/HeNCrj mice[17]. The EGCG used in their experiments was not pure and contained 10% (−)-epigallocatechin and 5% (−)-epicatechin gallate, whereas the EGCG used in our experiments was pure. Catechins other than EGCG might be the primary cause of tumor inhibition, or EGCG and other catechins might synergistically inhibit spontaneous mouse hepatotumorigenesis. In fact, Yan *et al.* reported

that polyphenon E, a mixture of green tea polyphenols, inhibited lung tumorigenesis in A/J mice although pure EGCG did not[33].

In addition to its ROS scavenging activity, lycopene has been proposed to possess various other properties, including interference of cell proliferation, inhibition of cell cycle progression, induction of gap-junctional communication, modulation of signal transduction pathways, and upregulation of carcinogen detoxification[34]. Astorg *et al.* reported that dietary lycopene inhibited diethylnitrosamine (DEN)-induced liver preneoplastic foci in rats, but it did not inhibit 2-nitropropane (2-NP)-induced liver preneoplastic foci[15] ; 2-NP is a hepatocarcinogen that has been shown to induce DNA oxidative damage[35,36]. Astorg *et al.* speculated that lycopene did not act through its antioxidant properties but through its modulating effect on the liver enzyme that activates DEN. Watanabe *et al.* reported that long-term lycopene administration did not inhibit hepatocarcinogenesis in Long-Evans Cinnamon (LEC) rats[18]. They speculated that the antioxidative activities of lycopene might be insufficient to prevent hepatocarcinogenesis in LEC rats. Also, in the present study, lycopene administration did not inhibit spontaneous liver tumorigenesis in the C3H/HeN mice. Conversely, among the *XPA* −/− mice, the average number of HCCs per mouse was significantly higher and the average diameter of tumors was significantly larger in the lycopene group than in the control group. This result might suggest the possibility that lycopene caused earlier occurrence of tumors or lycopene promoted tumor proliferation in *XPA* −/− mice.

An important question that requires attention is why EGCG and lycopene did not inhibit spontaneous liver tumorigenesis in C3H/HeN mice in the present study, although these compounds have been previously reported to inhibit the formation of various carcinogen-induced tumors[13-16]. The concentration of EGCG and lycopene in the present study was comparable to that used in previous studies in which these compounds inhibited chemically induced tumorigenesis. In addition, EGCG and lycopene were administered for a long period in the present study. With regard to the 8-OHdG levels in liver tissues, the administration of EGCG and lycopene had limited effects in the present study. This result implies that EGCG and lycopene showed only limited antioxidant activity in liver tissues. Thus, EGCG and lycopene may inhibit tumorigenesis not through their antioxidant property but through their other properties. The mechanism of spontaneous liver tumorigenesis of C3H mice might be different from that of various chemically induced animal tumor models.

In our previous study, *XPA* −/− mice showed a higher susceptibility to spontaneous liver tumorigenesis than *XPA* +/+ and *XPA* +/− mice[23]. Thus, the results of the present study were contradictory to those of our previous study. The two studies were performed in different laboratories (the previous study was performed at Animal Center of Department of Medicine, University of Tokyo ; the present study was performed at Animal Center of Teikyo University School of Medicine). We speculate that the different experimental conditions caused the discrepancy. In particular, the amount of contaminating genotoxic agents might be important. It was suggested that *XPA*-deficient mice could not be used as a sensitive animal model for the conditions used in the present study.

In conclusion, EGCG and lycopene exerted no obvious inhibitory effects on spontaneous hepatotumorigenesis in C3H/HeN mice, although these compounds have been reported

to inhibit the formation of various carcinogen-induced tumors. The mechanisms underlying the inhibitory effects of these compounds on carcinogen-induced tumorigenesis should be investigated in the future.

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CHAPTER 8

THRESHOLDS FOR GENOTOXIC CARCINOGENS: EVIDENCE FROM MECHANISM-BASED CARCINOGENICITY STUDIES

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8.1. OVERVIEW

In this chapter, the results of a medium-term rat carcinogenicity bioassay for rapid *in vivo* detection of carcinogenic potential are presented to examine the carcinogenicity of low doses of five genotoxic carcinogens: 2-amino-3,8-dimethylimidazo [4,5-*f*] quinoxaline (MeIQx), a heptocarcinogen contained in seared fish and meat; *N*-nitrosodiethylamine (DEN) and *N*-nitrosodimethylamine (DMN), heptocarcinogens synthesized in the stomach through the reaction of secondary amines and nitrites; 2-amino-1-methyl-6-phenylimidazo[4,5-*b*] pyridine (PhIP), a colon carcinogen contained in seared meat and fish; and potassium bromate, a kidney carcinogen that is a contaminate of tap water and also used as a food additive in some countries. DNA damage, gene mutation, and surrogate endpoints for carcinogenicity were examined: Carcinogenic endpoints were glutathione *S*-transferase placental form (GST-P) positive foci in the liver, a well-known preneoplastic lesion marker in rat hepatocarcinogenesis, and altered crypt foci (ACF), a well-known surrogate marker of preneoplastic lesions in the colon. Low doses of MeIQx induced formation of DNA-MeIQx adducts; somewhat higher doses caused elevation of 8-hydroxy-2′-deoxyquanosine (8-OHdG) levels; at still higher doses, gene mutations occurred; and the very highest dose of MeIQx induced formation of GST-P positive foci. Similarly, only the highest doses of DEN and DMN caused an increase in the number of GST-P positive foci in the liver; the lower doses had no effect. Similar results were obtained with the colon carcinogen PhIP. PhIP–DNA adduct formation was

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observed after treatment with low doses, while only high doses were found to induce ACF. Finally, in experiments with potassium bromate, 8-OHdG formation, GC-to-TA transversions, and gene mutations in the rat kidney were observed only after administration of the highest doses of $KBrO_3$; histopathological changes related to carcinogenicity were not observed at any dose used. These data support the existence of thresholds for the genotoxic carcinogens examined in this chapter.

8.2. INTRODUCTION

Compounds known to be carcinogenic to humans have primarily been identified by epidemiological methods—for instance, cancer development in factory workers (occupational cancer) (see Chapter 15). Epidemiological data, however, are usually not suitable to establish risk from exposure to different levels of human carcinogens. Also, epidemiological data are available only after exposed humans develop cancers. Carcinogen risk assessment aspires to identify and assess risk from exposure to carcinogens prior to extensive human exposure. Identification and assessment of most carcinogens is done using two-year carcinogenicity tests performed in rodents, particularly rats and mice (see Chapter 14). To assess risk in humans, carcinogenic response curves obtained from these tests are used. Importantly, the carcinogenicity of low doses of carcinogenic compounds is generally extrapolated from the carcinogenicity data obtained using high doses; to obtain statistically acceptable data, carcinogens are generally used in rodent carcinogenicity tests at high doses, including the maximum tolerated dose. The principal method of assessing risk posed to humans by exposure to genotoxic carcinogens uses nonthreshold approaches to model experimental data: The curves generated by nonthreshold approach modeling are S-shaped or linear low-dose straight lines that reach zero (see Chapter 24). This "nonthreshold concept" of genotoxic carcinogenicity reflects the idea that a single event caused by a genotoxic carcinogen can have a positive influence on cancer development in humans. However, the physiology of living organisms suggests that, in practical terms, thresholds can exist, even for genotoxic carcinogens. Most chemical carcinogens must be metabolized within the cell to their active forms, known as the ultimate carcinogen, before they are able to exert their carcinogenic activity. The ultimate carcinogen formed from most genotoxic compounds binds covalently to DNA, forming an adduct. These adducts can interfere with normal DNA metabolism, leading to DNA mutations and carcinogenicity. However, these DNA adducts are efficiently repaired by the cell. Still, for any particular adduct there is the possibility of misrepair or replication of damaged DNA resulting in fixation of a mutation into the cell's genome. Therefore, there is a finite risk of mutation arising from a single adduct. Next, at the level of DNA mutation, carcinogen–DNA adduct formation is essentially random in the euchromatic DNA (DNA that is not highly condensed); consequently, only a minute fraction of the mutations arising from these adducts will actually occur in a gene and have an affect on the cell, and only a very small fraction of these will be carcinogenic. In practical terms then, only a minute fraction of adducts actually give rise to DNA mutations and only a minute fraction of these mutations will affect the cell. As the number of adducts increases, however, the possibility of mutations occurring increases and mutated cells eventually begin

to appear (Kuraoka 2008). This is especially relevant as the number of adducts becomes greater than the capacity of the cell to repair this damage. Most damaged or mutated cells will die due to metabolic dysfunctions or be eliminated by irreversible senescence or apoptosis, but it is possible that some will survive and be viable. In the two-stage chemical carcinogenesis model, this sequence of events is thought to occur during the initiation stage. Cell proliferation enhances the ability of initiated cells to form preneoplastic lesions and to develop into tumors, benign and then malignant. Evidence indicates that, before developing into tumors, most preneoplastic lesions disappear spontaneously, presumably due, at least in part, to elimination by the immune system. The development from initiated cells into tumors is the promotion stage of the two-stage chemical carcinogenesis model. Therefore, in a finite population, if physiological functions such as DNA repair, induction of senescence or apoptosis, and immune surveillance are effective, there will be levels of exposure to genotoxic carcinogens below which induction of carcinogenesis is effectively zero (see Chapter 26).

For detection of carcinogenicity, the standard method is long-term carcinogenicity testing in two rodent species, such as mice and rats (≥50 animals/sex/group), with at least three dose levels, and in-life study termination at 18 months for mice and 24 months for rats (OECD 1981). However, such tests are extremely time-consuming, laborious, and expensive. This is particularly true when examining the effects of low doses of suspected carcinogens since many more animals are required to reliably determine whether the low doses used are in fact able to induce an increase in tumor formation. In practical terms, it is currently impossible to examine the carcinogenicity of all suspect compounds using long-term rodent assays. Therefore, recently, an alternative method to long-term carcinogenicity testing in which preneoplastic lesions are accepted as endpoint markers for the assessment of carcinogenicity has been proposed (Tsuda et al. 2003). Results are obtained from this *in vivo* medium-term bioassay system of carcinogens in a matter of weeks rather than, as with long-term testing, many months.

The presence or absence of a threshold will determine the reliability of carcinogenic risk assessment when extrapolated from high-dose rodent testing. Therefore, it is essential to verify scientifically whether the nonthreshold concept is valid. Herein, we provide data from low-dose carcinogenicity studies for genotoxic carcinogens using a medium-term bioassay for carcinogens. In addition to determining no-effect doses for carcinogenicity, we also examined markers that cells typically acquire as they move through the initiation and promotion stages of carcinogenesis. Analysis of all the data strongly support the existence of thresholds for the carcinogenetic effects of the five genotoxic carcinogens examined.

8.3. LOW-DOSE CARCINOGENICITY OF 2-AMINO-3,8-DIMETHYLIMIDAZO[4,5-*f*]QUINOXALINE (MeIQx) IN THE RAT LIVER

MeIQx is a heterocyclic amine contained in fried meat and fish. MeIQx at doses of 100–400 ppm in the diet is carcinogenic in the rat liver (Kato et al. 1988). To investigate the effect of exposure to low doses of MeIQx, 1145 21-day-old male F344

rats were divided into seven groups and administered MeIQx in the diet at doses of 0, 0.001, 0.01, 0.1, 1, 10 ppm (low-dose groups) and 100 ppm (high-dose group) for 4–32 weeks (Fukushima et al. 2002). The data on the induction of the GST-P positive foci after treatment with various doses of MeIQx for 16 weeks is presented in Table 8.1 (GST-P positive foci is a preneoplastic lesion in rat hepatocarcinogenesis and the endpoint marker in the rat liver medium-term carcinogenicity bioassay). The numbers of GST-P positive foci were not significantly elevated in the 0.001–10 ppm MeIQx groups, but a statistically significant increase was detected in the 100 ppm group. The same results were observed when the treatments with MeIQx were continued for 32 weeks (Figure 8.1).

MeIQx is metabolized in liver cells to an ultimate carcinogen capable of covalently binding DNA. In contrast to GST-P foci induction, the formation of

TABLE 8.1. Induction of GST-P positive Foci in the Liver of Rats Treated with MeIQx for 16 Weeks

Group	MeIQx Dose (ppm)	Number of Rats	Size Distribution of GST-P Positive Foci (No./cm^2)			Total
			2–4 Cells	5–10 Cells	≥11 Cells	
1	0	150	0.12 ± 0.17	0.05 ± 0.17	0.02 ± 0.09	0.18 ± 0.35
2	0.001	150	0.12 ± 0.18	0.02 ± 0.06	0.01 ± 0.05	0.15 ± 0.19
3	0.01	150	0.13 ± 0.21	0.03 ± 0.07	0.01 ± 0.05	0.16 ± 0.24
4	0.1	150	0.14 ± 0.20	0.04 ± 0.08	0.02 ± 0.10	0.19 ± 0.25
5	1	150	0.16 ± 0.20	0.04 ± 0.08	0.02 ± 0.07	0.21 ± 0.24
6	10	50	0.35 ± 0.33	0.10 ± 0.12	0.01 ± 0.05	0.47 ± 0.35
7	100	50	13.86 ± 5.11[a]	8.85 ± 3.23[a]	6.51 ± 4.06[a]	29.2 ± 10.99[a]

[a] $p > 0.01$ (vs. group 1).

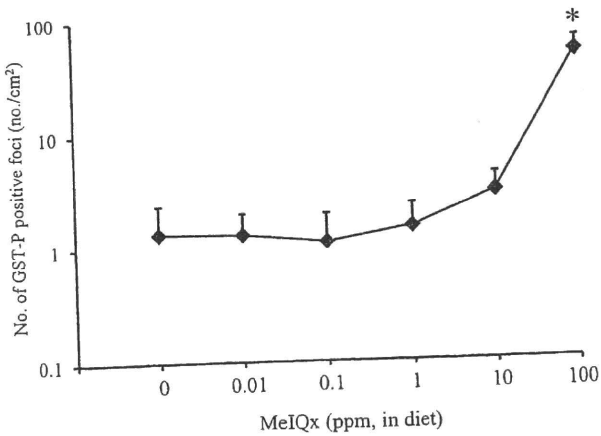

Figure 8.1. GST-P positive foci in the livers of F344 rats treated with MeIQx at various doses for 32 weeks. Asterisk (*) indicates $p < 0.01$ versus 0 ppm group.

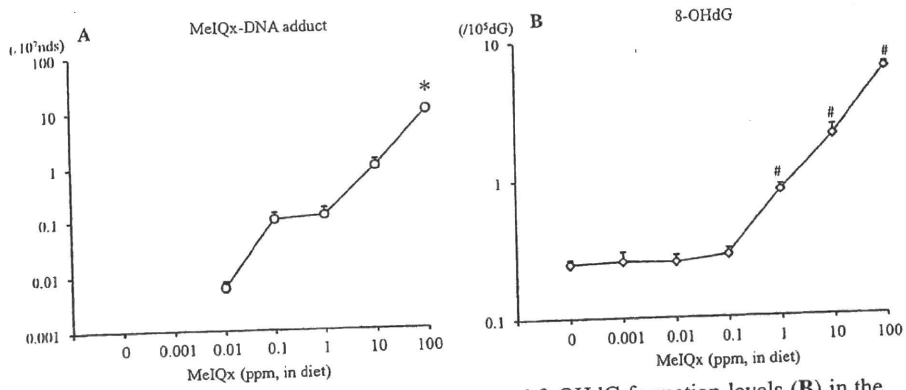

Figure 8.2. MeIQx–DNA adduct formation (**A**) and 8-OHdG formation levels (**B**) in the liver of F344 rats treated with MeIQx at various doses for 4 weeks. Asterisk (*) $p < 0.01$ versus 0.01 ppm group; number symble (#) indicates $p < 0.01$ versus 0 ppm group.

MeIQx–DNA adducts at week 4 was induced by administration of 0.01 ppm and higher doses of MeIQx, and induction was dose-dependent and statistically significant in the 100 ppm dose group (Figure 8.2); adduct formations in the 0 and 0.001 ppm MeIQx groups were below the limit of detection. Similar results were obtained after 16 weeks of MeIQx administration.

DNA is subject to constant oxidative damage from endogenous oxidants. 8-Hydroxy-2′-deoxyguanosine (8-OHdG) is a marker for oxidative DNA damage, and 8-OHdG levels rise as a cell becomes more metabolically active. 8-OHdG levels at week 4 were unaffected by treatment with 0.001, 0.01, or 0.1 ppm MeIQx, but became statistically significantly elevated after treatment with MeIQx at doses of 1, 10, and 100 ppm (Figure 8.2). Similar results were obtained after 16 weeks of MeIQx administration.

Finally, mutation of the H-*ras* gene, whose role in rat hepatocarcinogenesis is still unclear, was statistically significantly increased in the liver of rats treated with MeIQx for 2 weeks at 10 and 100, but did not differ at 0.001, 0.01. 0.1 and 1 ppm compared to the control value (unpublished data).

We also examined mutation of the *lacI* gene and induction of GST-P positive foci in the livers of Big Blue® rats (Hoshi et al. 2004). Forty male Big Blue® rats were divided into 7 groups and administered MeIQx in the diet at doses of 0, 0.001, 0.01, 0.1, 1, 10, and 100 ppm for 16 weeks. A statistically significant elevation of *lacI* gene mutation level was detected in the 10 and 100 ppm groups (Figure 8.3). On the other hand, formation of GST-P positive foci was statistically significantly induced by administration of 100 ppm but not 10 ppm or less MeIQx (Figure 8.3).

The results obtained from the experiments described above demonstrate the existence of a no-effect level (the highest dose of MeIQx at which there is no effect) for MeIQx mutagenicity and carcinogenicity. Since there is a no-effect level of MeIQx for gene mutagenicity, the initiation activity of MeIQx was examined in a two-stage carcinogenesis model using phenobarbital as a promoter of hepatocarcinogenesis (Fukushima et al. 2003). A total of 850 21-day-old male F344 rats were

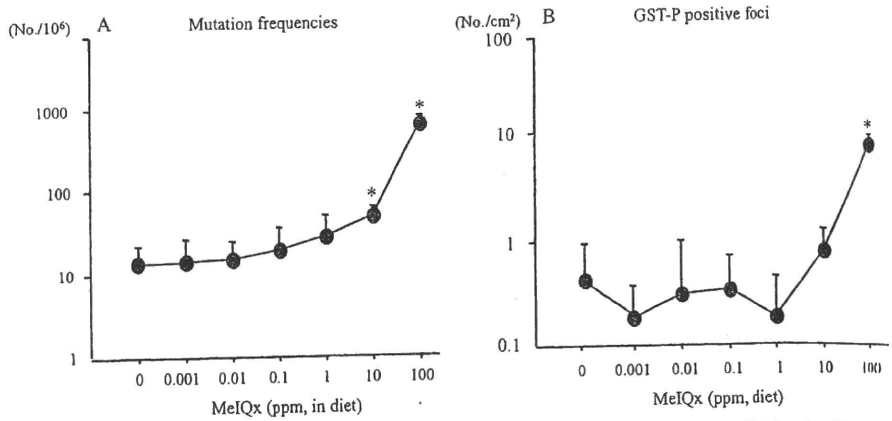

Figure 8.3. Lac I gene mutation frequencies (**A**) and GST-P positive foci (**B**) in the liver of Big Blue® rats treated with MeIQx at various doses for 16 weeks. Asterisk (*) indicates $p < 0.001$ versus 0 ppm group.

divided into seven groups and administered MeIQx at doses of 0, 0.001, 0.01, 0.1, 1, 10, and 100 ppm for 4 weeks. This was followed by administration of 500 ppm phenobarbital in the diet. The numbers of GST-P positive foci were not elevated in the 0.001–1 ppm MeIQx groups, but statistically significant increases in GST-P positive foci formation were detected in the 10 and 100 ppm MeIQx groups. These results indicate the existence of a no-effect level for MeIQx initiation activity and are consistent with the existence of a no-effect level for MeIQx mutagenicity.

Little is known about differences in the low dose–response relationship of genotoxic carcinogens among different strains of rat. Therefore, we examined MeIQx hepatocarcinogenicity using GST-P positive foci in both F344 and BN strains, with a total of 180 in each group. The background level of GST-P positive foci in the nontreated F344 rats was statistically significantly lower than that of BN rats, and the numbers of GST-P positive foci in the livers of MeIQx-treated F344 rats were statistically significantly lower in nearly all treatment groups compared with the corresponding BN strain groups (Wei et al. 2006). However, the results of MeIQx induction of GST-P positive foci in these two strains was the same: Lower doses of MeIQx, 0.1–10 ppm, had no statistically significant effect on the number of GST-P positive foci compared to the corresponding controls, while a statistically significant increase was detected at 100 ppm in both strains compared to the respective control groups (Table 8.2).

Finally, we examined the carcinogenicity MeIQx in damaged livers (Kang et al. 2006). A total of 280 male F344 rats were divided into 14 groups. Liver damage was induced in 7 of these groups by administration of 0.03% thioacetamide (TAA), a well-known hepatotoxin, in their drinking water for 12 weeks. After cessation of TAA treatment, the rats received 0, 0.001, 0.01, 0.1, 1, 10, and 100 ppm MeIQx in the diet for 16 weeks. In both TAA-treated and untreated groups, the lower doses of MeIQx had no effect on the number of GST-P positive foci, but a statistically

TABLE 8.2. Development of GST-P Positive Foci in the Livers of BN and F344 Rats Treated with Various Doses of MeIQx

Group	MeIQx (ppm)	Number of Rats	Size Distribution of GST-P Positive Foci (No./cm^2)			Total
			2–4 Cells	5–10 Cells	≥11 Cells	
BN Rat						
1	0	30	0.16 ± 0.21	0.06 ± 0.12	0.02 ± 0.10	0.24 ± 0.29
2	0.1	30	0.14 ± 0.23	0.03 ± 0.08	0.03 ± 0.19	0.19 ± 0.30
3	1	30	0.12 ± 0.21	0.04 ± 0.09	0.04 ± 0.14	0.20 ± 0.33
4	5	30	0.23 ± 0.33	0.11 ± 0.19	0.02 ± 0.09	0.36 ± 0.49
5	10	30	1.17 ± 0.98	0.42 ± 0.57	0.06 ± 0.14	1.64 ± 1.43
6	100	30	13.26 ± 7.07[a]	7.37 ± 4.78[a]	4.25 ± 3.88[a]	24.88 ± 14.67[a]
F344 Rat						
7	0	30	0.01 ± 0.05[c]	0[c]	0	0.01 ± 0.05[c]
8	0.1	30	0.03 ± 0.08[c]	0	0.01 ± 0.05	0.04 ± 0.10[c]
9	1	30	0.07 ± 0.15	0[c]	0	0.07 ± 0.15
10	5	30	0.08 ± 0.16[c]	0.01 ± 0.05[c]	0	0.08 ± 0.18[c]
11	10	30	0.29 ± 0.49[c]	0.04 ± 0.12[c]	0[c]	0.33 ± 0.59[c]
12	100	30	3.60 ± 2.22[b,c]	1.83 ± 1.33[b,c]	0.99 ± 1.01[b,c]	6.41 ± 4.04[b,c]

[a] $p < 0.01$ (vs. group1).

[b] $p < 0.01$ (vs. group7).

[c] $p < 0.01$ (vs. corresponding BN rat group).

significant increase was observed in the 100 ppm MeIQx groups (Figure 8.4). Using the method of maximum likelihood to model this data, the numbers of GST-P positive foci, with and without TAA treatment, fitted the hockey stick regression model; that is, no statistically significant differences in foci number were observed in the 0–10 ppm MeIQx groups, whereas a statistically significant increase in foci number was observed in the 100 ppm MeIQx group. In contrast, a linear dose-dependent increase of MeIQx–DNA adduct formation was evident from 0.1 to 100 ppm; adduct formation in the 0.001 and 0.01 ppm MeIQx groups were below the limit of detection (Figure 8.4). The formation of MeIQx-DNA adducts was virtually identical in undamaged and damaged livers. These results are consistent with the previous results and support the existence of a no-effect level for MeIQx hepatocarcinogenicity, even on a background of liver damage.

A summary of the results obtained in our experiments is presented in Figure 8.5. The formation of DNA–MeIQx adducts was observed at very low doses of MeIQx. Due to limitations in detection of these adducts, we were unable to determine whether a threshold dose of MeIQx was required for MeIQx–DNA adduct formation. Increasing the dose of MeIQx next resulted in an elevation of 8-OHdG formation, then gene mutation and the appearance of initiation activity, and, finally, at the highest dose used, an increase in the endpoint marker for carcinogenicity (GST-P positive foci). Notably, these data demonstrate that increased doses

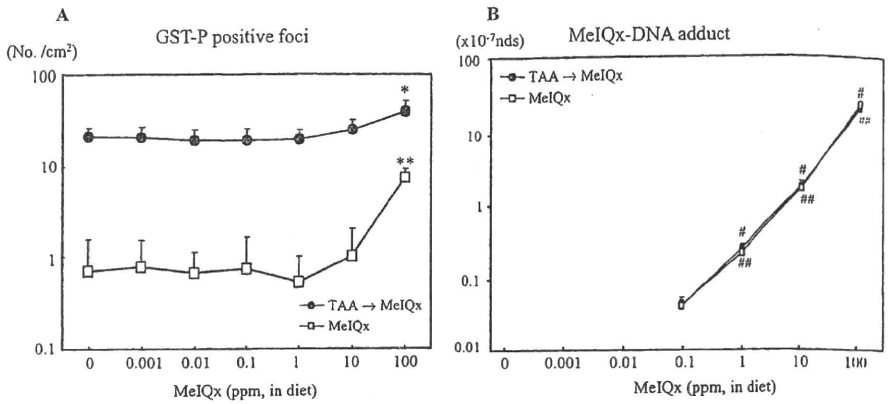

Figure 8.4. GST-P positive foci (**A**) and formations of MeIQx-DNA adduct (**B**) in the liver of F334 rats treated with MeIQx with or without thioacetamide. Asterisk (*) indicates $p < 0.01$ versus TAA intiation alone group; double asterisk (**) indicates $p < 0.01$ versus nontreatment group; Number symbol (#) indicates $p < 0.01$ versus 0.1 ppm MeIQx without TAA intiation; double number symbol (##) indicates $p < 0.01$ versus 0.1 ppm MeIQx with TAA intiation.

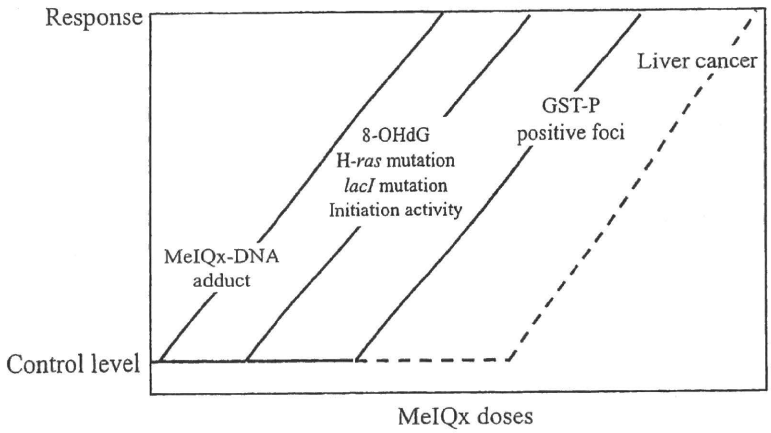

Figure 8.5. Risk of liver cancer: Reaction curves for carcinogenesis markers are dependent on the dose of MeIQx.

of MeIQx were required as MeIQx-mediated effects moved from simple adduct formation to cellular metabolic changes (possibly due in part to increased DNA repair) to gene mutation and cancer initiation to carcinogenesis. These results argue strongly for the existence of a threshold, at least a practical threshold, for MeIQx hepatocarcinogenicity in the rat. In support of this conclusion, our 2-year carcinogenicity test of MeIQx in rats showed no hepatocarcinogenicity at low doses (Murai et al. 2008).

8.4. LOW-DOSE HEPATOCARCINOGENICITY OF *N*-NITROSO COMPOUNDS

N-nitroso compounds such as diethylnitroamine (DEN) and dimethylnitrosamine (DMN) are synthesized in the stomach through the reaction of secondary amines and nitrites in the diet. They are also found as contaminants of a variety of manufactured food products.

Peto et al. (1991) investigated the carcinogenicity of DEN using 2040 male and 2040 female Colworth rats. DEN at doses of 0.033–16.896 ppm was administered to the rats in their drinking water, induction of liver tumors was found to be dependent on the applied dose of DEN, and at the lower doses a linear dose–tumor incidence relationship was observed (Peto et al. 1991). Therefore, it was concluded that DEN had no threshold for its carcinogenicity in the rat liver.

We have reexamined the carcinogenic influence of low doses of DEN (Fukushima et al. 2002). Approximately 2000 21-day-old male F344 rats were administered DEN at doses ranging from 0.0001 to 10 ppm in their drinking water for 16 weeks. No increase in the number of GST-P positive foci was found at DEN doses of 0.0001–0.01 ppm; however, the number of GST-P positive foci was statistically significantly elevated at 0.1 and 1 ppm DEN. In the 10 ppm group, the numbers of GST-P positive foci were so numerous that quanitation was not possible. Therefore, we conclude that there is a no-effect level for DEN hepatocarcinogenicity in the rat.

Low-dose carcinogenicity experiments were also performed with DMN (Fukushima et al. 2005). The carcinogen was applied to 540 21-day-old F344 rats at doses ranging from 0.001 to 10 ppm in their drinking water for 16 weeks. No induction of GST-P positive foci was found at doses of 0.001 to 0.1 ppm; however, statistically significant increases in the number of GST-P positive foci were observed at 1 and 10 ppm. Therefore, similarly to DEN, we concluded that there is a no-effect level for DMN hepatocarcinogenicity in the rat.

8.5. LOW-DOSE CARCINOGENICITY OF 2-AMINO-1-METHYL-6-PHENYLIMIDAZO[5,6-*B*]PYRIDINE (PhIP) IN THE RAT COLON

The heterocyclic amine PhIP is a carcinogen contained in seared meat and fish, and it exerts its carcinogenicity in the rat colon. We investigated the carcinogenicity of PhIP in the rat colon when applied at doses of 0.001–400 ppm (Fukushima et al. 2004). A total of 1759 6-week-old F344 male rats were administered PhIP in their diet for 16 weeks. The development of aberrant cell foci (ACF), the surrogate marker of preneoplastic lesions in the colon, was not altered by PhIP administration at 0.001–10 ppm; however, at doses of 50–400 ppm, statistically significant increases in ACF were observed (Figure 8.6).

Like MeIQx, DEN, and DMN, PhIP is a genotoxic compound and is metabolized in cells to an ultimate carcinogen capable of covalently binding DNA.

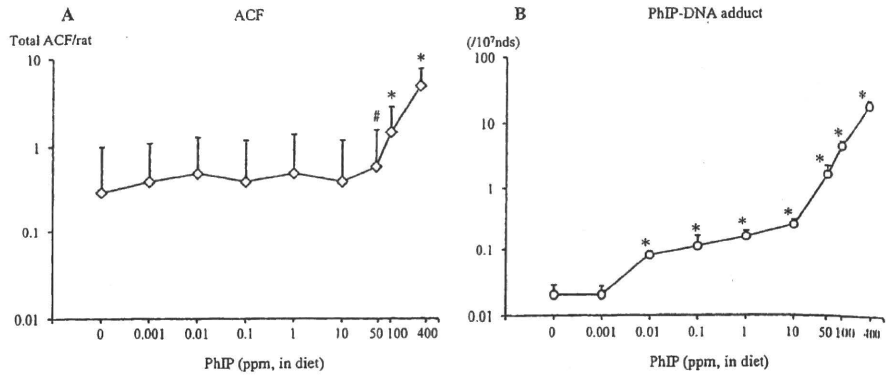

Figure 8.6. Aberrant crypt foci (**A**) and formations PhIP–DNA adduct (**B**) in the colons of F344 rats treated with PhIP at various doses for 16 weeks. Number symbol (#) indicates $p < 0.05$ versus 0 ppm group. Asterisk (*) indicates $p < 0.01$ versus 0 ppm group. Note that PhIP–DNA adduct levels were also statistically significantly increased in the same manner at week 4.

Statistically significant increases in the formation of PhIP–DNA adduct levels were found in the groups treated with 0.01 ppm and higher doses of PhIP at 16 weeks (Figure 8.6). Thus, similarly to MeIQx, DNA adduct formation is observed after administration of low doses of PhIP while doses required to induce ACF are much higher (approximately 50,000 times higher) than that needed for PhIP–DNA adduct formation. These results argue for a no-effect level and a threshold dose for PhIP colon carcinogenicity in the rat.

Finally, we assessed the effect of low doses of PhIP in the progression of colon tumors (Doi et al. 2005). A total of 192 6-week-old male F344 rats were subcutaneously injected twice with the colon carcinogen azoxymethane (AOM) with a 1-week interval, and then the animals were continuously fed PhIP at doses ranging from 0.001 to 200 ppm for 16 weeks. Lower doses (0.001–10 ppm) of PhIP had no significant effect on AOM-initiated colon carcinogenesis; higher doses (50–200 ppm) of PhIP caused a statistically significantly enhancement of AOM-initiated colon carcinogenesis (Table 8.3). Results obtained from this initiation–promotion model show a no-effect level of 10 ppm for PhIP promotion of colon carcinogenesis and again argue for a threshold dose for PhIP colon carcinogenicity in the rat.

8.6. LOW-DOSE CARCINOGENICITY OF POTASSIUM BROMATE, $KBRO_3$ IN THE RAT KIDNEY

Potassium bromate is a rodent renal carcinogen which can be found as a contaminant of tap water and which is used as a dough conditioner and food additive in some countries. It is a genotoxic carcinogen that is reduced in renal proximal tubular cells to yield bromine oxides and radicals, which are the ultimate carcinogens that specifically cause guanine oxidation, leading to renal mutagenesis and carcinogenesis.

TABLE 8.3. Induction of Tumors in the Colon of F344 Rats Treated with Azoxymethane Followed by PhIP

Histologic Findings	Incidences (%)							
	0 ppm (n = 16)	0.001 ppm (n = 16)	0.01 ppm (n = 16)	0.1 ppm (n = 16)	1 ppm (n = 16)	10 ppm (n = 16)	50 ppm (n = 16)	200 ppm (n = 14)
Adenoma	2 (12.5)	3 (18.8)	1 (6.3)	5 (31.3)	3 (18.8)	2 (12.5)	14 (87.5)[b]	14 (100)[d]
Adenocarcinoma	8 (50)	7 (43.8)	10 (62.5)	5 (31.3)	9 (56.3)	8 (50)	14 (87.5)	14 (100)[c]
Total[a]	9 (56.3)	10 (62.5)	10 (62.5)	8 (50)	11 (68.8)	8 (50)	16 (100)[c]	14 (100)[c]

[a]Total of adenoma and adenocarcinoma.

[b]$p < 0.005$ (vs. 0 ppm).

[c]$p < 0.05$ (vs. 0 ppm).

[d]$p < 0.0001$ (vs. 0 ppm).

Thus, the genotoxic mechanism of potassium bromate is different from that of MeIQx, DEN, DMN, and PhIP. The studies described to this point indicate that the genotoxic compounds MeIQx, DEN, DMN, and PhIP have no-effect levels for induction of various carcinogenesis markers and strongly suggest that a threshold dose exists for induction of carcinogenesis by these compounds. In the following experiments, we investigated the relationship between potassium bromate dose and induction of gene mutation, one of the markers of carcinogenesis. A total of 40 male Big Blue® rats were divided into 8 groups and administered potassium bromate in their drinking water at doses of 0, 0.02, 0.2, 2, 8, 30, 125, and 500 ppm for 16 weeks (Yamaguchi et al. 2008). No significant induction of *lacI* gene mutation was observed in the 0.02–125 ppm groups, but a statistically significant increase in *lacI* gene mutation was observed in the 500 ppm group (Figure 8.7). Similarly, statistically significantly elevated 8-OHdG levels and GC to TA transversions, a mutation known to occur as a result of 8-OHdG adduct formation, also occurred only at a potassium bromate dose of 500 ppm (Figure 8.7). No preneoplastic or neoplastic lesions were detected in the kidney in these experiments. Therefore, we concluded that there is a no-effect level for potassium bromate-induced 8-OHdG formation and mutagenicity in the rat kidney.

Finally, the renal carcinogenicity of potassium bromate was examined using a two-stage carcinogenesis model. A total of 240 male Wistar rats were treated with *N*-ethyl-*N*-hydroxyethylnitrosamine for the initiation of kidney carcinogenesis and were thereafter administered potassium bromate at doses of 0, 0.02, 0.2, 2, 8, 30, 125, and 500 ppm in their drinking water for 16 weeks (Wei et al. 2009): Due to

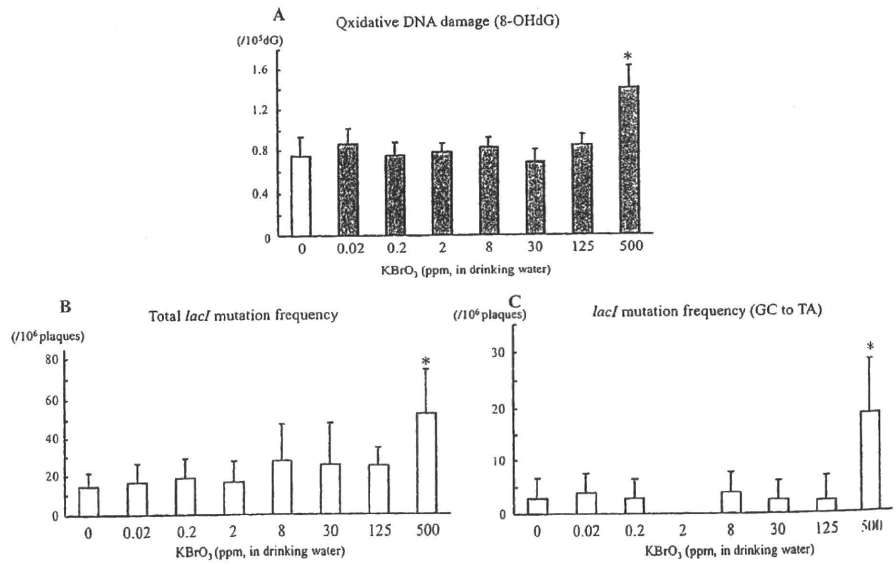

Figure 8.7. 8-OHdG formation levels (**A**) and LacI gene mutation frequencies (**B**, **C**) in the kidney of Big Blue rats treated with $KBrO_3$ for 16 weeks. (*) $p < 0.05$ versus 0 ppm group.

toxicity, the highest dose, 500 ppm, was reduced to 250 ppm from week 12. Enhancement of a preneoplastic lesion, an atypical tubular hyperplasia, and enhancement of tumorigenesis in the kidney was observed only in the highest dosed group.

The results of these two sets of experiments support the conclusion that there is a no-effect level and threshold dose for potassium bromate renal carcinogenicity in the rat.

8.7. CONCLUSION

For the genotoxic carcinogens examined, the no-effect doses for initiation markers (i.e., DNA adduct formation, 8-OHdG formation, and gene mutation) were much lower than the no-effect doses for promotion marker (i.e., GST-P positive foci and ACF); and, generally, induction of promotion markers occurred at doses of carcinogen which did not induce carcinogenesis. These results strongly suggest that processes such as DNA repair, irreversible senescence, apoptosis, and immune system function operate to inhibit the effects of genotoxic carcinogens and that the inhibition is significant. Therefore, we conclude that there are thresholds, at least practical thresholds, for the carcinogens examined in this study.

The genotoxic carcinogens examined in this study can be classified into two types from the viewpoint of mechanism (Hengstler et al. 2003). In one type, the carcinogen is metabolized by the cell to an ultimate carcinogen, which binds covalently to the DNA to form DNA adducts. In the second type, the compound is metabolized by the cell to an ultimate carcinogen, which causes oxidative damage to the DNA. The first type of genotoxic carcinogen encompasses heterocyclic amines (e.g., MeIQx and PhIP) and *N*-nitrosocompounds (e.g., DEN and DMN). The second type of genotoxic carcinogen is represented by potassium bromate. Notably, the first type of genotoxic carcinogen induces formation of DNA adducts at low doses but higher doses are required for gene mutation, while the second type of genotoxic carcinogen causes DNA damage and gene mutation at equivalent doses. This undoubtedly reflects the different mechanisms by which these two types of genotoxic compounds cause DNA damage. Nevertheless, both types of genotoxic compounds clearly have no-effect doses for initiation, which are lower than the no-effect doses for promotion and carcinogenicity. It is probable, therefore, that other (perhaps most or even all) genotoxic carcinogens also have this pattern of no-effect dose and, consequently, do have thresholds for carcinogenicity.

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