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Inhibitory Effects of Heterocyclic Amine-Induced DNA Adduct Formation in Mouse Liver and Lungs by Beer

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An evaluation of the in vivo antigenotoxic potential of beer components on heterocyclic amines including 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx) and 3-amino-1-methyl-5*H*-pyrido[4.3-*b*]indole (Trp-P-2) was determined with particular focus on the target organs of tumorigenesis, and the protective mechanisms involved were investigated. Beer-solution, consisting of a freeze-dried and dissolved sample, given as drinking water, reduced the formation of MeIQx-DNA adducts in mouse liver and lungs. Beer-solution added in the diet as a mimic of food additives also significantly reduced the amount of DNA adducts present in the liver, lung, and kidney DNA of mice fed with MeIQx compared to control mice fed with MeIQx in the absence of beer-solution. The amount of adducts present in the liver of mice with single or continuous administration of Trp-P-2 was significantly reduced when beer-solution was given as part of the diet compared to control mice given Trp-P-2 without beer-solution. Protective effects were observed both with lager- and stout-type samples. In an effort to investigate the mechanisms responsible for the observed protective effects, the effects of beer-solution on metabolizing enzymes for heterocyclic amines were examined. Beer-solutions inhibited the metabolic activation of Trp-P-2 to Trp-P-2(NHOH), as demonstrated by HPLC analysis. Considering the overall suppression of the genotoxicity of MeIQx and Trp-P-2 by beer, we have shown that beer components can inhibit the metabolic activation of heterocyclic amines and subsequently suppress the observed genotoxicity. The results of this study show that beer components are protective against the genotoxic effects of heterocyclic amines on target organs associated with tumorigenesis in vivo.

KEYWORDS: Antimutagenicity, beer, DNA adduct, heterocyclic amines, metabolic activation

INTRODUCTION

Heterocyclic amines including 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx) and 3-amino-1-methyl-5*H*-pyrido[4.3-*b*]indole (Trp-P-2) have been identified as potent mutagens and carcinogens in rodents and are produced in foods during the process of cooking (*1*). Since humans are frequently exposed to heterocyclic amines, these compounds are suspected of being human carcinogens (*2*). The antimutagenicity and anticarcinogenicity of dietary components is currently receiving attention (*3*, *4*). We previously investigated the inhibitory effects of beer on the bacterial mutagenicity of preactivated heterocyclic amines (3-hydroxyamino-1-methyl-5*H*-pyrido[4.3-*b*]indole (Trp-P-2(NHOH)) and 2-hydroxyamino-6-methyldipyrido[1,2-*a*:3′,2′-*d*]imidazole (Glu-P-1(NHOH)) (*5*)), 2-chloro-4-methylthiobutanoic acid (*6*) and *N*-methyl-N′-nitro-*N*-nitrosoguanidine (MNNG) (*7*). Beer also prevented the formation of O^6-methylguanine in the DNA of *Salmonella typhimurium* YG7108 by MNNG (*5*), radiation-induced chromosome aberrations in human lymphocytes (*8*), and the genotoxicity of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) in V79 cells, as determined by the comet assay (*9*).

In this study, we evaluated the in vivo effects of beer samples in relation to MeIQx- and Trp-P-2-induced DNA adduct formation under conditions relevant to human dietary habits. The protective effects were studied in the liver and lungs, the target organs for MeIQx tumorigenesis, of mice fed with a beer-solution or by the addition of beer components to the diet, the latter being a mimic of food additives. We also investigated the effects of beer solution in relation to DNA damage in mice liver with single and continuous administration of Trp-P-2. Finally, we evaluated the effects of beer on metabolic activation in an effort to determine the protective mechanisms involved.

MATERIALS AND METHODS

Materials. Trp-P-2 (CAS 62450-07-1) and MeIQx (77500-04-0) were obtained from Wako Chemicals (Osaka, Japan). Trp-P-2(NHOH) was synthesized from Trp-P-2 according to the literature (*10*). The purity of the aforementioned hydroxyamino derivatives was >99.5%, as determined by HPLC. S9 was prepared from livers of Sprague−Dawley rats (6 weeks old) induced by phenobarbital and β-naphthoflavone (Wako Chemicals). Three different beer samples produced in Japan

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(beers A, B, and C) were examined. Beer-A was a stout beer, while beer-B and beer-C were lager beers. All were purchased in local stores in Okayama. Beer samples were freeze-dried to remove ethanol, and the solid obtained was dissolved in water to one-fifth, one-half, or an equal volume of the original sample and were designated as 'beer solution (×5)', 'beer solution (×2)', and 'beer solution (×1)', respectively.

Detection of DNA Adducts in Mice. C57BL/6N mice (male, 6 weeks old) were obtained from Charles River Japan (Atsugi, Japan) and housed as one or two mice in a cage with access to food and water ad libitum. For the investigation of the influence of beer on MeIQx-induced DNA adduct formation, experiment 1 consisted of providing beer-A solution to mice in lieu of drinking water for 5 days and a diet carrying MeIQx was given for three subsequent days. Four groups of mice received a diet mixed with MeIQx (0.005%) (group 1), MeIQx in the diet and beer-A solution (×1) in water (group 2), MeIQx in the diet and beer-A solution (×2) in water (group 3), or a control diet (group 4). The calorie content of the diet was adjusted using maltose. On day 6, mice were sacrificed by cervical dislocation, tissues were excised, washed with ice-cold KCl (0.15 M), frozen in liquid nitrogen, and stored at −80 °C until use. The amount of heterocyclic amine−DNA adducts in the tissue DNA of treated mice was determined by a modified adduct-intensification analysis using the ^{32}P-postlabeling method (*11*). Experiment 2 consisted of mice being fed with a paste that consisted of mixing beer-A solution and MeIQx (0.005%) with an equal weight of control diet (powder). For 2 days, mice received a control diet mixed with water (groups 1 and 4), a diet mixed with beer-A solution (×1) (group 2), or a diet mixed with beer-A solution (×5) (group 3). For the subsequent 3 days, mice were given a diet mixed with MeIQx (group 1), beer-A solution (×1) and MeIQx (group 2), beer-A solution (×5) and MeIQx (group 3), or a control diet (group 4). On day 6, mice were sacrificed. Subsequent procedures were similar to those outlined for experiment 1.

The effects of beer on Trp-P-2-induced DNA adduct formation were also investigated. Experiment 3 consisted of giving mice (groups 1−3) Trp-P-2 (30 mg/kg) by gastric intubation in a single dose dissolved in sterile water on day 3. For controls animals not receiving Trp-P-2 (group 4), solvent (water) was given by intubation. A diet mixed with water (groups 1 and 4), a diet mixed with beer-A (original, not freeze-dried) (group 2), or a diet mixed with beer-A solution (×5) (group-3) was given to mice for 3 days, and on day 4, mice were sacrificed. Experiment 4 consisted of mice being fed with a paste for 5 days that consisted of mixing Trp-P-2 and beer-A solution with an equal weight of control diet (powder). The total amount of Trp-P-2 given was 30 mg/kg. A diet mixed with water (group 1 and 4), a diet mixed with beer-A solution (×1) (group 2), or a diet mixed with beer-A solution (×5) (group-3) was given to the mice for 2 days. Following this, a diet mixed with Trp-P-2 (group 1), mixed with Trp-P-2 and beer-A solution (×1) (group 2), mixed with Trp-p-2 and beer-A solution (×5) (group 3), or mixed with water only (group 4) was given for 5 days. On day 8, mice were sacrificed. Experiment 5 consisted of mice being given beer-B solution. The concentration of Trp-P-2 in the diet was 0.005%. A diet mixed with beer-B solution was given for 2 days, and this was then changed to a diet carrying Trp-P-2 with beer solution for 3 days. On day 5, mice were sacrificed. Subsequent procedures were similar to those outlined for experiment 2.

Effects of Beer on the Metabolic Activation of Trp-P-2. Metabolic activation of Trp-P-2 to Trp-P-2(NHOH) was estimated as follows (*12*). Solutions (0.6 mL total volume for each) containing 20 nmol of Trp-P-2, beer solution (0−0.1 mL equivalent of original beer), and 0.5 mL of S9 mix (including 20 mL of S9) were prepared. Following incubation of the solution at 37 °C for 20 min, ice-cold acetone (0.6 mL) was added and the mixture was allowed to stand in an ice-bath for 10 min. The mixture was centrifuged at 3000 rpm at 4 °C for 10 min. The supernatant was taken, and to this was added 0.02 mL of 0.5 M citric acid. The mixture was evaporated under reduced pressure, and the volume of the residual solution was made to be 0.1 mL using cold water. A portion of the solution was then analyzed by HPLC, and the net amounts of Trp-P-2(NHOH) formed and Trp-P-2 remaining in the sample were estimated on the basis of the A_{266} peak areas of authentic specimens. The analyses was performed in quadruplicate. HPLC analysis was conducted on a Waters 600 system, equipped with a programmable multiwavelength detector (Model 490), using a reversed phase column (Inertsil ODS, 4.6 mm × 250 mm (GL Science, Tokyo, Japan)).

Table 1. Effects of Beer Solution in the Drinking Water on the DNA-Adduct Formation in Mice Fed with MeIQx (Experiment 1)

group	beer solution added in the drinking water	MeIQx in the diet(%)	adducts/10^7 nucleotide	no. of mice
		Liver		
1	water	0.005	124.3 ± 53.2	8
2	beer-A solution (×1)	0.005	73.3 ± 32.6[a]	8
3	beer-A solution (×2)	0.005	60.5 ± 21.5[b]	8
		Lungs		
1	water	0.005	57.8 ± 28.5	8
2	beer-A solution (×1)	0.005	40.8 ± 22.0	8
3	beer-A solution (×2)	0.005	21.1 ± 9.60[c]	8

[a] Significantly different ([a]$p < 0.05$, [b]$p < 0.01$, and [c]$p < 0.005$) from group 1 (*t* test).

Table 2. Effects of Beer-A Solution in the Diet on the DNA-Adduct Formation in Mice Fed with MeIQx (Experiment 2)

group	beer solution added in the diet	MeIQx in the diet (%)	adducts/10^7 nucleotide	no. of mice
		Liver		
1	water	0.005	50.2 ± 12.2	8
2	beer-A solution (×1)	0.005	36.0 ± 08.5[a]	8
3	beer-A solution (×5)	0.005	29.6 ± 18.0[a]	8
		Lungs		
1	water	0.005	6.9 ± 3.2	8
2	beer-A solution (×1)	0.005	1.80 ± 2.40[c]	8
3	beer-A solution (×5)	0.005	1.30 ± 1.80[c]	8
		Kidneys		
1	water	0.005	109.3 ± 45.8	8
2	beer-A solution (×1)	0.005	52.5 ± 27.2[b]	8
3	beer-A solution (×5)	0.005	17.4 ± 13.2[d]	8

[a] Significantly different ([a]$p < 0.05$, [b]$p < 0.01$, [c]$p < 0.005$, and [d]$p < 0.001$) from group 1 (*t* test).

RESULTS

The in vivo effects of beer on DNA adduct formation in mice fed MeIQx were investigated. MeIQx-induced DNA adducts were formed in the liver, lungs, and kidneys of mice given MeIQx at 0.005% in the diet (experimets 1 and 2, Tables 1 and 2). The formation of DNA adducts in the liver and lungs of mice given MeIQx in the diet significantly decreased by administering drinking beer-A solution ad libitum compared with mice given MeIQx without beer solution (experiment 1, Table 1). Beer-A solution (×1) and (×5) administered to the diet also significantly decreased the amount of DNA adducts in the liver, lungs, and kidneys of mice given MeIQx in the diet (experiment 2, Table 2).The amount of adducts in the liver DNA of mice given Trp-P-2 with single administration on day 3 significantly decreased by the continuous administration of beer-A solution (×5) in the diet for 4 days compared with mice given Trp-P-2 without beer solution (Table 3, experiment 3). The amount of Trp-P-2 adducts in the liver DNA of mice continuously given Trp-P-2 for 5 days also decreased with the inclusion of beer-A solution (×1) and beer-A solution (×5) in the diet (Table 3, experiment 4). The reproducibility of the protective effect of another type of beer sample, lager type beer (beer-B), was also investigated (Table 3, experiment 5). Adduct formation in the liver DNA of mice fed Trp-P-2 for 5 days

Table 3. Effects of Beer Solution on DNA-Adduct Formation in Mice Liver Fed Trp-P-2

Experiment 3. Single Administration of Trp-P-2

group	beer solution added	Trp-P-2 (mg/kg of body wt)	adducts/10^7 nucleotides	no. of mice
1	water	30	2.95 ± 1.27	4
2	beer-A	30	1.34 ± 0.63	4
3	beer-A solution (×5)	30	$0.685 \pm 0.58^{a,b}$	4

Experiment 4. Continuous Feeding with Trp-P-2 for 5 Days

group	beer solution added	total Trp-P-2 given (mg/kg of body wt)	adducts/10^7 nucleotide	no. ofmice
1	water	30	3.5 ± 1.4	8
2	beer-A solution (×1)	30	1.0 ± 0.2^{d}	7
3	beer-A solution (×5)	30	2.0 ± 0.8^{c}	8

Experiment 5. Effects of Beer-B Solution

group	beer solution added	Trp-P-2 in the diet (%)	adducts/10^7 nucleotide	no. of mice
1	water	0.005[e]	8.0 ± 2.9	8
2	beer-B solution (×1)	0.005	3.5 ± 1.6^{f}	8
3	beer-B solution (×5)	0.005	2.4 ± 1.3^{f}	8

[a] Significantly different ($p < 0.05$) from group 1 (*t*-test). [b] Detection limit: 0.01 adducts/10^7 nucleotide. [c] Significantly different ([c]$p < 0.05$ and [d]$p < 0.005$) from group 1 (*t*-test). [e] 0.005%of Trp-P-2 in the diet for 3 days was equivalent of 25 mg (total)/kg body wt. [f] Significantly different ($p < 0.005$) from group 1 (*t*-test).

significantly decreased with the inclusion of beer-B solution (×1) and beer-B solution (×5) in the diet compared with mice fed with Trp-P-2 only.

The amount of Trp-P-2(NHOH) produced by the in vitro metabolic activation of Trp-P-2 (20 nmol) in the presence of beer solution following incubation for 20 min at 37 °C was measured by HPLC (Figure 1). Trp-P-2 and Trp-P-2(NHOH) eluted at 14.2 and 11.7 min, respectively. Inhibition of metabolic conversion by the beer solution was observed in a dose-dependent manner. In the absence of beer solution, 11−17% of Trp-P-2 was converted, and 1.6−1.7 nmol/tube of Trp-P-2(NHOH), representing 8% of original Trp-P-2, was detected. In the presence of beer-A (Figure 1A) and beer-C (Figure 1B) solution, the amount of Trp-P-2(NHOH) formed decreased and the amount of Trp-P-2 remaining correspondingly increased. In the presence of 100 mL of beer-A solution, 0.42 nmol of Trp-P-2(NHOH) was detected, which represents 24% of that amount detected in the absence of beer solution (Figure 1A).

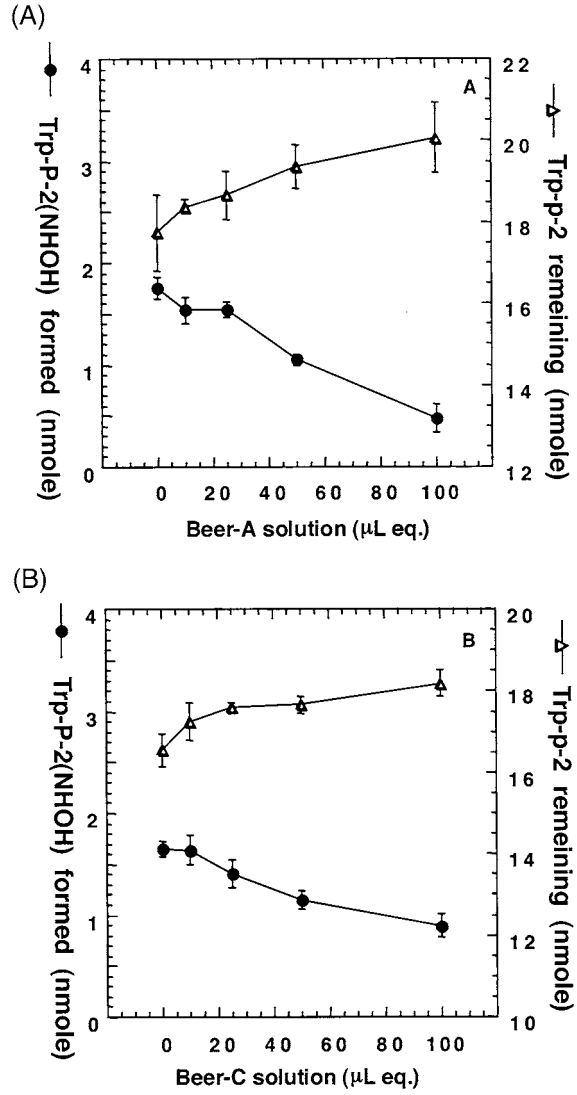

Figure 1. Effects of beer-A (A) and Beer-C (B) on the metabolic conversion of Trp-P-2 to Trp-P-2(NHOH). The amounts of Trp-P-2(NHOH) formed (●) and Trp-P-2 remaining (▽) were determined by HPLC.

DISCUSSION

Considering the relevance to human dietary habits, the effects of beer solution given as drinking beverages were investigated. Beer-A solution in drinking water decreased DNA adduct formation in the liver and lungs of mice fed with MeIQx in the diet (Table 1). The addition of beer-A solution in the diet also decreased the amount of DNA adducts formed in the liver, lungs, and kidneys of mice given MeIQx in the diet (Table 2). Ohgaki et al. (*13*) showed that mice fed with MeIQx at 0.06% in the diet developed liver tumor, lymphoma, and leukemia in males, while liver and lung tumors developed in females. The results suggested that beer components both in the drinking water and in the diet of mice could suppress the formation of MeIQx DNA adducts in target organs associated with tumorigenesis (liver and lungs) and nontarget organs (kidneys).

Trp-P-2 at 0.02% in the diet induced a high incidence of hepatocellular tumours in female CDF1 mice (*13*). The formation of DNA adducts in the liver both with single administration (po) and continuous feeding of Trp-P-2 was significantly inhibited by administration of beer-A solution in the diet (Table 3, experiments 1 and 2). DNA-adduct formation in the liver DNA of C57BL mice following administration of Trp-P-2 at 0.005% in the diet was observed with smaller amounts of Trp-P-2 than that observed in relation to carcinogenicity in CDF1 mice. It will be interesting to determine if beer could inhibit DNA adduct formation in the target organ of Trp-P-2 tumorigenesis, i.e., the liver. This observation suggests that protective effects can also be expected in humans when beer components are daily consumed with occasional or periodic intake of fried meats. In addition to the stout-type beer (beer-A), the lager-type beer (beer-B) solution also inhibited Trp-P-2-induced DNA adduct formation in vivo (Table 3, experiment 3). The effects of beer-A and beer-B solutions were similar to the preventive effects on Trp-P-2-induced DNA adduct formation. These results suggest that antigenotoxic components might be commonly present in beer.

MeIQx and Trp-P-2 are metabolically activated through a process involving N-hydroxylation by CYP1A1, CYP1A2, and CYP1B1 (*14*, *15*). The genotoxicity of Trp-P-2 in mice is also dependent on the induction of CYP enzymes and phase II enzymes (*4*, *16*−*18*). Although the activity of CYP1A is

frequently assayed with *O*-demethylase and *O*-deethylase activity, this would provide a better estimation of the effect of beer on CYP enzymes by measuring the activity associated with the *N*-hydroxylation of Trp-P-2. We analyzed the production of Trp-P-2(NHOH) from Trp-P-2 in vitro in the presence of beer solution. The suppression of the metabolic conversion of Trp-P-2 to Trp-P-2(NHOH) indicated that the antimutagenic effects of beer toward Trp-P-2 was linked with the inhibition of metabolic activation.

We previously found that a green tea component, epigallocatechin gallate, prevented MeIQx-induced DNA damage and adduct formation in insect DNA, and was associated with accelerated degradation of Glu-P-1(NHOH) in vitro (*19*). Miranda et al. (*20*) reported that 8-prenylnaringenin and xanthohumol from hops could inhibit the mutagenic activation of IQ mediated by CYP1A2. These components from hops could be candidates responsible for the inhibition of CYP enzyme by beer. However, xanthohumol was only mildly effective in inhibiting the mutagenicity of Trp-P-2(NHOH) (data not shown). Although the antimutagenic compounds toward heterocyclic amines have not yet been identified, it suggests that beer components have at least one target associated with the observed antimutagenicity, that is inhibition of the activity of CYP enzymes for Trp-P-2 and MeIQx.

In the process involving carcinogenesis by genotoxic agents, DNA adduct formation and the resulting genetic changes play critical roles (*21*). Our results provide possible candidates as modulators of heterocyclic amine-induced carcinogenesis.

ABBREVIATIONS USED

MeIQx, 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline; Trp-P-2, 3-amino-1-methyl-5*H*-pyrido[4.3-*b*]indole; Trp-P-2(NHOH), (3-hydroxyamino-1-methyl-5*H*-pyrido[4.3-*b*]indole; Glu-P-1(NHOH), 2-hydroxyamino-6-methyldipyrido[1,2-*a*:3′,2′-*d*]imidazole; MNNG, *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine.

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