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INTAKE OF BEER INHIBITS AZOXYMETHANE-INDUCED COLONIC CARCINOGENESIS IN MALE FISCHER 344 RATS

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Modulatory effects of beer consumption on azoxymethane (AOM)-induced rat colonic carcinogenesis in male Fischer 344 rats were investigated. Single cell gel electrophoresis assay indicated that DNA damage of colonocytes, induced by a single AOM injection (15 mg/kg body weight), was significantly reduced in rats fed beer or malt extract for 2 weeks. Examination of aberrant crypt foci (ACF) formation in colonic mucosa, induced by AOM (15 mg/kg body weight; twice weekly), revealed that feeding of beer during the whole experimental period of 5 weeks significantly reduced the number of ACF by 35%. In the post-initiation protocol, a reduction in ACF formation by 26% was not significant. The efficacy in inhibition of ACF formation varied with the brand of beer. ACF formation was significantly reduced in rats treated with freeze-dried beer (FD Beer), but not with ethanol, suggesting that nonvolatile components of beer are responsible for the reduction. Significant suppression of ACF formation was observed in groups treated with hot water extract of malt, especially with extracts of colored malts, although no reduction was observed by feeding with hops extract. A long-term experiment of 42 weeks indicated that intake of beer decreased tumor incidence by 22% and decreased the number of neoplastic lesions, including adenocarcinomas and adenomas, by 44%. These results suggest that components of beer have chemopreventive effects on colonic carcinogenesis induced by AOM and that intake of beer may contribute to a reduction in the risk of cancer susceptibility.

Key words: *beer; malt; hops; chemoprevention; comet assay; aberrant crypt foci; colonic carcinogenesis; rat; azoxymethane*

Epidemiological and experimental studies have indicated that environmental factors and diet are important determinants of human cancer. Although the genetic background of individuals may affect the probability of suffering neoplastic disease, this is not of overriding importance in the majority of cases and attention must be given to daily foods with chemopreventive activity to reduce cancer risk. Epidemiological studies have shown an association between alcohol consumption and mortality from coronary heart disease and cancer as J-shaped: the relative risk of mortality decreases for moderate drinkers compared to nondrinkers and then increases with higher alcohol intake.[1,2] Several epidemiological studies showing an association between beer consumption and cancer risk have been published. Some suggest a positive relationship between beer intake and colorectal cancer[3,4] or prostate cancer.[5] However, some reports indicate that moderate intake of beer is not associated with an elevated risk of colon or prostate cancer.[6–8]

Colon cancer is one of the most prevalent neoplastic diseases and may be related to intake of a high fat/low fiber diet and dietary carcinogens. Dietary consumption of vegetables and fruits is considered to lower the risk of colon cancer[9] and natural components from plant sources have been shown to have inhibitory effects on the initiation, promotion and progression stages in experimental colon carcinogenesis models.[10–14] Some plant constituents, including antioxidative vitamins and phenolics, possess many biological functions, such as anti-inflammatory,[15] anti-oxidative,[16] anti-mutagenic,[17,18] and anti-angiogenetic[19] actions, which may be involved in cancer chemopreventive activity. It is important to examine the efficacy of dietary factors in the multistages of carcinogenesis, especially in short-term and long-term *in vivo* experiments, in order to evaluate chemopreventive potential.

The single cell gel electrophoresis assay (comet assay) can be used to monitor DNA damage in individual organs of rodents exposed to mutagens.[20,21] This assay is based on the ability of denatured DNA fragments to migrate out of the nucleus to form comet tails during electrophoresis under alkaline conditions (pH>12.6) and has been used to examine the efficacy of chemopreventive agents in the initiation stage of carcinogenesis.[22–24] Aberrant crypt foci (ACF) are early morphological changes or hyperproliferative lesions found in the colon of humans and carcinogen-treated rodents; they are considered to be putative preneoplastic lesions for colon cancer.[25–27] Azoxymethane (AOM)-induced colonic ACF in rats have been used to identify chemical agents that prevent colon cancer, including many dietary factors, especially those derived from plant sources.[28,29]

Two previous reports investigate the cancer preventive efficacy of beer in experimental carcinogenesis: one report indicated that chronic intake of beer can decrease gastrointestinal tumors induced by dimethylhydrazine in a rodent model.[30] The second report documented evidence of a decrease in incidence of AOM-induced tumors with beer intake.[31] The authors in the latter report suggest that the effect was due to alcohol rather than other beverage constituents.[31] Beer is a low-alcohol beverage brewed from natural ingredients rich in vitamins, amino acids, minerals and micronutrients, such as ferulic acid and polyphenols, which may be involved in the prevention of carcinogenesis.[32–35] Beer has antioxidative effect *in vivo*[36] and anti-mutagenic effects against several mutagens in *Salmonella* mutation assays.[18] The intake of beer also reduced the number of DNA adducts in the liver of mice administered Trp-P-2.[18] Prenylflavonoids, from hops, inhibited metabolic activation of heterocyclic amines by a P450 enzyme, CYP1A2, in the Ames *Salmonella* assay, and showed cytotoxic effects in human cancer cell lines.[37–39] Humulone, also from hops, was shown to have anti-tumor promotion activity and inhibit angiogenesis.[40,41] It also suppressed expression of cyclooxygenase-2 (COX-2),[42] one of the key enzymes involved in carcinogenesis. It was demonstrated that xanthohumol, several other chal-

Abbreviations: ACF, aberrant crypt foci; AD, adenomas; ADC, adenocarcinomas; AIN, AIN-76A; AOM, azoxymethane; COX-2, cyclooxygenase-2; FD Beer, freeze-dried Beer; HBSS, Hanks' balanced salt solution Ca-Mg-free; (I), initiation; (I/PI), initiation and post-initiation; (PI), post-initiation.

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cones and lupulones from hops inhibit LPS/IFN-γ-induced NO production in murine macrophage RAW 264.7 cells.[43] Therefore, it is important to clarify if beer has cancer preventive activities in experimental carcinogenesis.

In our study, we investigated the modifying effects of beer on AOM-induced rat colonic carcinogenesis using *in vivo* single cell gel electrophoresis assays, ACF analysis and pathological analysis of colon tumors in a long-term experiment. We demonstrate that intake of beer reduced the formation of colorectal tumors and preneoplastic lesions in AOM-induced experimental carcinogenesis in male Fischer rats.

MATERIAL AND METHODS

Materials

AOM was purchased from Sigma Chemical Co. (St. Louis, MO), 10% neutral-buffered formalin, ethanol and methylene blue was from Wako Pure Chemical Co. (Osaka, Japan) and piroxicam was from ICN Biomedicals, Inc. (Costa Mesa, CA). Low melting point agarose (Agarose-LGT) and normal melting agarose (Agarose GP-42), for use in the comet assays, were obtained from Nacalai Tesque Co. (Kyoto, Japan). Frosted glass slides were purchased from Matsunami Glass Co. (Tokyo, Japan). Four types of malts, Pilsner, Munich, Chocolate and Caramel malts, and Aroma hops (Saaz) were obtained from a brewery. Pilsner malt, popularly used for brewing of pilsner-type beer, is produced by kilning (drying) germinated barley for shorter periods of time and at lower temperatures than darker malts. Munich, Chocolate and Caramel malts are dark roasted, kilned for a longer period and at a higher temperature than pilsner malt, in order to produce a dark brown color and a special flavor to the malt. Four types of commercial beers (beer A, B, C and D), purchased in a liquor shop, were used in the present study. Beer A was brewed from Munich malt, Pilsner malt and hops. Beers B and C were brewed from Pilsner malt , hops, rice and cornstarch. Beer D was brewed from Pilsner malt, Caramel malt and hops.

Experimental samples

Beers were degassed by stirring at room temperature before aliquoting into feeding bottles. Five percent (v/v) ethanol, which was the same concentration as the alcohol content in the beers used, was also aliquoted into feeding bottles. Ground hops and malt were mixed with hot water (hops 1%, malt 5%, w/v) and extracted for 30 minutes at boiling point. Filtered solutions were aliquoted into feeding bottles. Hot water extracts of hops at higher concentrations could not be used since rats did not drink them and reductions in body weights were observed. FD Beer was mixed with the AIN-76A (AIN) basal diet (Dyets, Inc. Bethlehem, PA) at various concentrations. For the experiments using FD Beer, cornstarch was added to the control and experimental diets to mimic isocaloric conditions since most of the calories in the solid component of beer are derived from carbohydrates. Piroxicam was used as a control to compare the efficacy in the inhibitory effect and was added to the basal diet at a concentration of 200 ppm, as used in a previous report.[29]

Treatment of animals

Male Fischer 344 rats at 4 weeks old were purchased from Charles River Japan and maintained in wire cages in an air-conditioned room under constant conditions of temperature (22±2 OC) and humidity (55±10%). The animals were allowed free access to a basal diet [CE-2; CLEA Japan (Tokyo, Japan) or AIN] and were randomized into experimental groups after 4 days of acclimatization. The animals were treated in accordance with Kirin Pharmaceutical's ethical guidelines for animal care, handling and termination.

Single cell gel electrophoresis assay (Comet assay)

Rats were fed a basal diet (CE2) and experimental drinks (beer, 1% hop extract solution and 5% malt extract solution) or a control drink (water) *ad libitum* for 2 weeks (Fig. 1*a*). Each treatment group consisted of 4 animals. They received subcutaneous injections of AOM at a dose of 15 mg/kg body weight at 16 hr before sacrifice. Three animals used as the vehicle controls were administered an equal volume of saline. Immediately after the sacrifice, colons were excised and washed with Hanks' balanced salt solution Ca-Mg-free (HBSS). They were then filled with HBSS containing proteinase K (17 units/ml) and incubated at 37°C for 30 min. The resultant cell suspensions were passed through a 70 μm mesh and used for slide preparation.[44] Comet assays were conducted according to an established procedure.[20,45] Comet lengths of 50 nuclei per slide and 2 slides per rat, for a total of 100 nuclei per rat, were measured using an image analysis program (Keio Co., Japan).

Experimental procedures for ACF assays

ACF formation was investigated by 3 protocols over 5 weeks, as indicated in Figure 1. Test samples were given to the animals during the whole experimental period of 5 weeks initiation and post-initiation (I/PI), during initiation phase (I) and during post-initiation (PI) phase. For the initiation (I) protocol, experimental diets were given for 17 days from the beginning of the experiment. For the post-initiation protocol, experimental diets were given 3 days after the second AOM injection until the end of the experiment (Fig. 1*b*). In all protocols for 1 week after the start of experiments, rats were treated with subcutaneous injections of AOM (15 mg/kg body weight) once a week for 2 weeks. Experimental drinks, beer, 5% (v/v) ethanol and water were fed *ad libitum* with the basal diet. FD Beer was mixed in the basal diet, AIN, at various concentrations. Ingested amounts of the experimental diets and drinks were recorded to estimate the consumption of the test materials. All rats were weighed weekly until the end of the experimental period, at which point the animals were sacrificed and colons were excised for subsequent analysis of ACF. After fixation in 10% buffered formalin, colons were stained with 0.2% methylene blue and ACF were scored under a light microscope at 40× magnification. ACF were distinguished from normal crypts by their increased size, more prominent epithelial cells and their

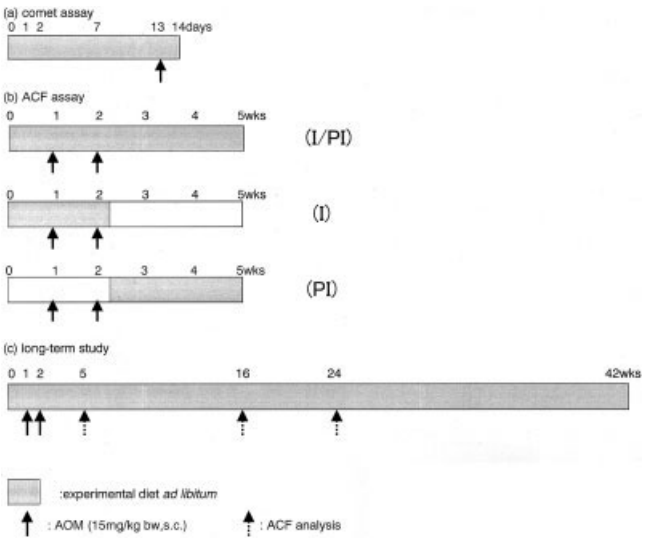

FIGURE 1 – Experimental protocols for the treatment of animals used. Five-week-old male F344 rats were used and sacrificed at the end of the experimental periods. Animals received subcutaneous injections of AOM at a dose of 15 mg/kg body weight at the indicated time points, shown by arrows in each experiment. In the ACF experiments, rats received experimental diets for the whole 5 week period (I/PI protocol), 17 days from the start of the experiment (I protocol) or for 18 days from 3 days after the second AOM injection until the end of the experiment (PI protocol).

increased pericryptal area.[25] The number of ACF and the number of aberrant crypts (ACs) per rat were quantified.

Long-term experiment

Ninety-three rats were randomly divided into 3 groups, 40 rats for the AOM+water group, 40 rats for the AOM+beer group and 13 rats for the AOM untreated group (Saline+water group). Rats received the basal diet (CE2) with water or beer *ad libitum* during the experimental period (Fig. 1*c*). Rats in the AOM+water and AOM+beer groups received 2 subcutaneous injections of AOM (15 mg/kg body weight per week), rats in the Saline+water group were injected with saline. Six rats each were taken from the AOM+beer and AOM+water groups for the analysis of ACF at 5, 16 and 24 weeks. At 42 weeks, all rats were sacrificed and colons were excised and examined for the presence of neoplastic lesions after fixation in 10% neutral-buffered formalin. Each colon was divided into 3 parts: rectum colon (3 cm from anus), middle colon (3 cm to 15cm part) and proximal colon (rest of the colon including cecum). The locations of all lesions were scored and these lesions were embedded in paraffin blocks, sectioned and stained with hematoxylin and eosin (H&E) for histopathological analysis. Tumors were classified into carcinomas and adenomas according to published criteria.[46]

Statistical analysis

Data are expressed as the means±SD. The data in the comet assay and in the ACF experiment were analyzed by 1-way ANOVA followed by the Dunnett's test or Kruskal-Wallis test followed by the Bonferroni Correction. In the long-term experiment, ACF and tumor multiplicity were compared by the Mann-Whitney-U-test. Tumor incidence was compared using χ^2 test and Fischer's exact probability test. Significant differences were concluded when the p value was less than 0.05.

RESULTS

General observations

The body weights of rats in the control and experimental groups in the short-term experiments were not significantly different. In the long-term experiment, rats in the AOM-injected groups had significantly reduced body weights compared to the saline-injected controls at the time of sacrifice. There were no differences in body weights between the AOM+beer and AOM+water groups (see Table VII). Chronic administration of beer was not associated with any gross changes or signs of toxicity.

Prevention of DNA damage in colon cells

Figure 2 shows the result of a single cell gel electrophoresis assay with colon cells. Comet lengths of the colonocytes from untreated control animals were, on average, 40 μm. AOM treatment induced significant DNA damage in colon cells: comet lengths in the group receiving water were 2-fold longer than those in the saline-treated control. Comet lengths in the groups fed either beer or malt extract were significantly shorter than those in the AOM-treated rats (37% and 36%, respectively), suggesting beer or malt extract inhibits AOM-induced DNA damage. A reduction in comet length of only 15% was observed in the hops extract group: this was not significant.

Inhibitory effects of beer on AOM-induced ACF formation

Daily ingestion of beer (Beer A) during the whole experimental period of 5 weeks (I/PI) significantly reduced the number of ACF/colon by 35%, the total number of ACs/colon by 40% and the number of ACF with 4 or more crypts/focus by 67% (Table I). The inhibitory effect was less pronounced when beer was introduced after the second AOM injection (PI): 26% reduction in ACF/colon, 33% reduction in ACs/colon and 62% reduction in ACF with 4 or more crypts/focus. In the post-initiation protocol, a reduction in the total number of ACF was not significant. Intake of 5% ethanol in both protocols slightly reduced the number of ACF/colon, ACs/colon and ACF with 4 or more crypts, but these effects were not significant. Inhibitory effects of several pilsner-type beers on ACF formation were examined (Table II). The results indicated that the extent of inhibition of beer on AOM-induced ACF formation varied among the brands of beer. Although feeding of Beer A significantly inhibited ACF formation (54% reduction), almost no reduction in the number of ACF was observed with Beer C. The reduction in the number of ACF with Beer B (29% reduction) and D (21% reduction) were not significant.

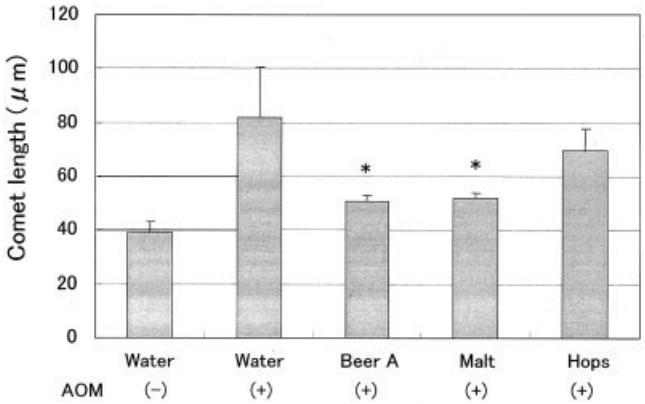

FIGURE 2 – Effects of beer (Beer A), malt extract and hops extract on DNA damage in the rat colonocyte induced by AOM. Experimental drinks were fed to the animals (n=4 for AOM treated and n=3 for saline treated) for 2 weeks and colons were excised 16 hr after AOM injection. One hundred nuclei were analyzed for each animal in the Comet assay. Bars indicate means±SD values. Significantly different from the AOM control at *p<0.01.

Inhibitory effects of solid constituents of beer on AOM-induced ACF formation

Since ethanol had only a small effect on AOM-induced ACF formation, the inhibitory effects of beer were thought to be attributable to the solid components. We evaluated the effects of various concentrations of FD Beer on AOM-induced ACF formation (Table III). Animals were fed the AIN basal diet containing 0.1, 0.25, 0.5, 1, 2 or 4% FD Beer for 5 weeks (I/PI). There were no significant differences in the daily consumptions of diets and final body weights among the experimental and control groups. A significant decrease in the number of ACF was observed by feeding FD Beer at 1, 2 and 4% in the basal diet (28, 31 and 20% reduction, respectively). Diets containing 0.1, 0.25 and 0.5% FD Beer were less effective with the same protocol (Table III), suggesting the optimal effective dose to be between 1% and 2%. We then compared the inhibitory effect of FD Beer on AOM-induced ACF formation with that of piroxicam, a nonsteroidal anti-inflammatory drug, in the 3 different protocols (I/PI, I, and PI in Table IV). Results indicated that feeding of 1% FD Beer in the basal diet during the whole experimental period (I/PI) and initiation phase (I) significantly suppressed ACF formation; the total number of ACF was decreased by 28% and 30%, respectively. A weak suppressive effect was observed when the experimental diet was fed in the post-initiation phase (PI) (14%). Piroxicam exhibited strong inhibition of ACF formation in the (I/PI) and (PI) protocols, the total number of ACF was decreased by 46 and 53%, respectively: no significant inhibition was observed in the (I) protocol.

Inhibitory effects of raw materials on AOM-induced ACF formation

To investigate the nature of the inhibitory components in beer, AOM-injected animals were given malt or hops extracts during the experimental period of 5 weeks (I/PI). Treatment of animals with 5% malt extract significantly reduced the number of ACF, no decrease was observed in the group treated with 1% hops extract (Table V). In addition, 4 different types of malt, Pilsner, Munich,

TABLE I – EFFECTS OF BEER A ON AOM-INDUCED ACF FORMATION IN THE (I/PI) AND (PI) PROTOCOLS

Group	Incidence	Body weight (g)	Number of ACF/ colon	Number of ACs/colon	Number of ACs/focus	Number of ACF > 3AC/colon
AOM + Water	8/8	221.4 ± 6.6[1]	97.9 ± 25.7	213.3 ± 54.0	2.2 ± 0.1	8.8 ± 3.5
AOM + 5%Ethanol (I/PI)	8/8	217.5 ± 8.2	92.6 ± 25.4	186.0 ± 46.4	2.0 ± 0.2	5.6 ± 2.4
AOM + Beer A (I/PI)	8/8	212.5 ± 14.6	63.3 ± 12.7[3]**	126.6 ± 28.6[4]***	2.0 ± 0.2	2.9 ± 1.7[3]**
AOM + 5%Ethanol (PI)	8/8	219.4 ± 7.4	95.3 ± 23.0	193.8 ± 47.2	2.0 ± 0.1	5.5 ± 3.3
AOM + Beer A (PI)	8/8	217.6 ± 9.8	72.4 ± 18.7	142.8 ± 39.6[2]*	2.0 ± 0.2	3.3 ± 2.6[3]**

[1]Values are given as mean ± SD.–[2–4]Significantly different from the AOM + Water group.–*$p < 0.05$.–**$p < 0.005$.–***$p < 0.001$.

TABLE II – EFFECTS OF 4 TYPES OF BEERS ON AOM-INDUCED ACF FORMATION IN THE (I/PI) PROTOCOLS

Group	Incidence	Body weight (g)	Number of ACF/ colon	Number of ACs/colon	Number of ACs/focus	Number of ACF > 3AC/colon
AOM + Water	6/6	222.1 ± 6.0[1]	145.5 ± 35.5	339.2 ± 90.0	2.3 ± 0.1	18.0 ± 8.1
AOM + Beer A (I/PI)	6/6	221.3 ± 6.1	67.2 ± 17.3[2]*	139.3 ± 44.9[3]**	2.1 ± 0.3	4.8 ± 5.0[2]*
AOM + Beer B (I/PI)	6/6	217.6 ± 15.9	103.3 ± 36.3	230.0 ± 95.0	2.2 ± 0.3	10.8 ± 5.4
AOM + Beer C (I/PI)	6/6	220.3 ± 4.6	142.5 ± 16.9	323.2 ± 39.0	2.3 ± 0.1	11.8 ± 3.3
AOM + Beer D (I/PI)	6/6	212.2 ± 8.9	114.3 ± 30.4	249.2 ± 73.3	2.2 ± 0.2	9.0 ± 3.8

[1]Values are given as mean ± SD.–[2,3]Significantly different from the AOM + Water group.–*$p < 0.05$.–**$p < 0.01$.

TABLE III – EFFECTS OF FREEZE-DRIED BEER A ON AOM-INDUCED ACF FORMATION IN THE (I/PI) PROTOCOL

Group	Incidence	Body weight (g)	Daily diet intake (g/rat)	Number of ACF/ colon	Number of ACs/colon	Number of ACs/focus	Number of ACF > 3AC/colon
AOM + AIN	8/8	217.4 ± 14.5[1]	12.2	97.5 ± 16.1	222.1 ± 40.7	2.3 ± 0.2	10.3 ± 4.2
AOM + 0.1% FD Beer A (I/PI)	8/8	210.1 ± 12.7	11.8	100.9 ± 17.6	208.9 ± 31.9	2.1 ± 0.2	5.9 ± 4.3
AOM + 0.25%FD Beer A (I/PI)	8/8	216.7 ± 14.3	12.1	86.3 ± 16.3	187.4 ± 34.9	2.2 ± 0.2	7.0 ± 3.7
AOM + 0.5%FD Beer A (I/PI)	8/8	218.9 ± 13.5	11.7	83.9 ± 17.3	173.9 ± 42.0[2]*	2.1 ± 0.1	5.4 ± 3.2[2]*
AOM + 1%FD Beer A (I/PI)	8/8	214.3 ± 12.7	12.1	69.8 ± 10.9[3]**	150.1 ± 23.5[3]**	2.2 ± 0.1	3.8 ± 2.4[3]**
AOM + 2%FD Beer A (I/PI)	8/8	213.1 ± 11.4	11.3	67.0 ± 19.3[2]*	139.0 ± 39.1[3]**	2.1 ± 0.2	3.1 ± 1.1[3]**
AOM + 4%FD Beer A (I/PI)	8/8	212.2 ± 6.0	11.5	77.8 ± 10.0[2]*	159.6 ± 18.2[3]**	2.1 ± 0.2	4.6 ± 2.5[3]**

[1]Values are given as mean ± SD.–[2,3]Significantly different from the AOM + AIN group.–*$p < 0.05$.–**$p < 0.005$.

TABLE IV – EFFECTS OF FREEZE-DRIED BEER A AND PIROXICAM ON AOM-INDUCED ACF FORMATION IN THE (I), (PI) AND (I/PI) PROTOCOLS

Group	Incidence	Body weight (g)	Number of ACF/colon	Number of ACs/colon	Number of ACs/focus	Number of ACF > 3AC/colon
AOM + AIN	8/8	217.4 ± 14.5[1]	97.5 ± 16.1	222.1 ± 40.7	2.3 ± 0.2	10.3 ± 4.2
AOM + 1%FD Beer A (I/PI)	8/8	214.3 ± 12.7	69.8 ± 10.9[3]**	150.1 ± 23.5[3]**	2.2 ± 0.1	3.8 ± 2.4[3]**
AOM + 200ppm Piroxicam(I/PI)	8/8	205.2 ± 6.3	44.8 ± 16.4[4]***	94.4 ± 38.7[4]***	2.1 ± 0.2	3.3 ± 2.4[3]**
AOM + 1%FD Beer A (I)	8/8	217.3 ± 13.8	68.3 ± 17.4[3]**	136.1 ± 32.8[3]**	2.0 ± 0.3	4.8 ± 4.1[2]*
AOM + 200 ppm Piroxicam(I)	8/8	209.2 ± 8.3	89.9 ± 17.1	189.1 ± 35.7	2.1 ± 0.1	6.6 ± 3.3
AOM + 1%FD Beer A (PI)	8/8	214.7 ± 9.0	83.9 ± 16.9	172.1 ± 34.2[2]*	2.1 ± 0.1	3.9 ± 1.6[3]**
AOM + 200 ppm Piroxicam(PI)	8/8	209.7 ± 6.5	46.9 ± 14.2[4]***	96.6 ± 31.3[4]***	2.1 ± 0.2	1.6 ± 2.6[4]***

[1]Values are given as mean ± SD.–[2–4]Significantly different from the AOM + AIN group.–*$p < 0.05$.–**$p < 0.005$.–***$p < 0.001$.

TABLE V – EFFECTS OF HOT WATER EXTRACTS OF HOPS AND PILSNER MALT ON AOM-INDUCED ACF FORMATION IN THE (I/PI) PROTOCOL

Group	Incidence	Body weight (g)	Number of ACF/ colon	Number of ACs/ colon	Number of ACs/focus	Number of ACF > 3AC/colon
AOM + Water	6/6	236.9 ± 5.6[1]	153.5 ± 19.5	350.5 ± 63.0	2.3 ± 0.2	15.7 ± 8.0
AOM+Hops (I/PI)	6/6	229.0 ± 10.6	174.3 ± 60.8	380.2 ± 142.9	2.2 ± 0.1	12.7 ± 7.2
AOM + Pilsner Malt (I/PI)	6/6	240.6 ± 10.9	91.7 ± 23.5[2]*	221.7 ± 65.4	2.4 ± 0.2	11.8 ± 5.0

[1]Values are given as mean ± SD.–[2]Significantly different from the AOM + Water group.–*$p < 0.05$.

Chocolate and Caramel, were examined for their ability to inhibit ACF formation. While all types of malt extract significantly reduced the number of ACF, dark roasted malts were more effective than pilsner malt in reducing the number of ACF: Munich, Chocolate, and Caramel malts exhibited 28%, 28% and 29% reduction, respectively (Table VI).

Effects of beer on colonic carcinogenesis

The results of colon tumor development examined at week 42 are summarized in Tables VII and VIII. Tumors in the colon were classified as adenomas or adenocarcinomas. The incidence of adenomas and adenocarcinomas, and total tumor incidence in the entire colon were lower in the beer-fed rats than in the control group (41%, 18% and 22% reduction, respectively) (Table VII). A decrease in the incidence of adenomas was detected in the proxi-

TABLE VI – EFFECTS OF HOT WATER EXTRACTS OF 4 TYPES OF MALTS ON AOM-INDUCED ACF FORMATION IN THE (I/PI) PROTOCOL

Group	Incidence	Body weight (g)	Number of ACF/colon	Number of ACs/colon	Number of ACs/focus	Number of ACF > 3AC/colon
AOM + Water	8/8	218.3 ± 4.9[1]	171.3 ± 27.0	390.1 ± 70.2	2.3 ± 0.2	20.1 ± 8.9
AOM + Pilsner Malt (I/PI)	8/8	218.9 ± 14.9	139.3 ± 15.3[2]*	309.8 ± 29.5[2]*	2.2 ± 0.1	11.5 ± 4.4
AOM + Munich Malt (I/PI)	8/8	213.6 ± 12.9	123.3 ± 30.3[3]**	267.0 ± 70.4[3]**	2.2 ± 0.1	11.9 ± 4.9
AOM + Chocolate Malt (I/PI)	8/8	212.2 ± 8.6	124.0 ± 20.7[3]**	287.9 ± 62.2[3]**	2.3 ± 0.3	16.0 ± 7.2
AOM + Caramel Malt (I/PI)	8/8	212.1 ± 9.6	121.5 ± 21.4[3]**	256.3 ± 43.5[4]***	2.1 ± 0.1	9.1 ± 5.5

[1]Values are given as mean ± SD.–[2–4]Significantly different from the AOM + Water group.–*$p < 0.05$.–**$p < 0.01$.–***$p < 0.005$.

TABLE VII – EFFECTS OF BEER A ON AOM-INDUCED TUMOR INCIDENCE IN THE COLON OF F344 RATS

Group	Animals (*n*)	Body weight (g)	Tumor incidence(%)								
			Rectum		Middle colon		Proximal colon		Entire colon		
			AD	ADC	AD	ADC	AD	ADC	AD	ADC	total
Saline + Water	13	416.2 ± 21.5[1]	0	0	0	0	0	0	0	0	0
AOM + Water	22	393.7 ± 24.7[2]*	14	9	32	64	9	32	46	82	86
AOM + Beer A	22	385.2 ± 22.8[2]*	0	9	5[3]**	59	0	18	5[4]***	64	64

[1]Values are given as mean ± SD.–[2]Significantly different from the Saline + Water group.–[3,4]Signficantly different from the AOM + Water group.–*$p < 0.05$.–**$p < 0.05$.–***$p < 0.01$.

mal, middle and rectum colons. The incidence of adenocarcinomas was not reduced in the middle and rectum colons, while there was a slight reduction in the proximal colon. Tumor multiplicity in the entire colon was significantly suppressed, by 44%, in the group given beer (Table VIII). The tumor multiplicity of adenocarcinomas in the proximal colon was significantly reduced by beer intake. A decrease in the number of ACF was observed at weeks 5, 16 and 24 in the beer-fed group (by 31, 26 and 20%, respectively), and the decrease was significant at week 16 (Fig. 3).

DISCUSSION

In our study, 3 experimental protocols, *in vivo* single cell gel electrophoresis assay, 5 week ACF assays and a 42 week colon carcinogenesis experiment, were employed to investigate the effect of beer on AOM-induced rat colonic carcinogenesis. The results indicated that beer components, possibly derived from malt, have chemopreventive effects, particularly at the early stage of carcinogenesis induced by AOM injection.

The single cell gel electrophoresis assay (Comet assay) was used to monitor DNA damage in the colon of rat induced by AOM treatment in order to evaluate the anti-mutagenic activities of beer and raw materials. The comet assay indicated that treatment of rats with a single AOM injection significantly induced elongation of the comet tail, which corresponds with its strong mutagenicity in colonic mucosa, detected by DNA adduct analysis.[11,47] The results shown in Figure 2 suggested that intake of beer and malt extract protected against DNA damage induced by AOM in the colonic mucosa. These results are consistent with the results of the ACF experiment, *i.e.*, the inhibitory effects of beer or FD Beer on ACF formation were greater in the initiation phase (I) than in the post-initiation phase (PI) (Tables I and IV).

Daily ingestion of beer (beer A) significantly reduced the number of ACF and the ACF with 4 or more crypts/focus (Table I). However, the result using several brands of beers as experimental samples indicated that there are considerable variations in inhibitory actions on ACF formation among brands of beer (Table II). This variation may be due to the contents of components responsible for the preventive functions on ACF formation. The result that malt extract reduced ACF formation in the 5 week ACF assay (Table V) with the above observation suggests that malt extract may be one source of the protective components in beer. Malt, which is germinated barley, contains several phytochemicals, which may have cancer preventive activity, such as ferulic acid, catechins, proanthocyanidines, folate, GABA and soluble or insoluble dietary fiber.[32–35] Several reports demonstrated that ferulic acid prevents colorectal and oral cancer in rodent carcinogenesis models.[29,48–50] Although ferulic acid may partly contribute to the inhibitory action of beer, it is likely that other components contribute to the inhibitory effects of beer on AOM-induced rat colonic carcinogenesis since the concentration of ferulic acid in beer (0.5–2.5 ppm) was not sufficient to account for the inhibition. In our study, hot water extracts of dark-colored malts, including Munich, Chocolate and Caramel, exhibited stronger effects in the inhibition of ACF formation than that of Pilsner malt, suggesting that components produced during the kilning process at higher temperatures may be important for inhibition (Table VI). It was reported that the antioxidant activities of malts, which affect the efficacy of cancer chemoprevention, were produced at least partly by the Maillard reaction and were altered by kilning conditions.[51] It should be noted that inhibition of ACF formation by dark-colored beer (Beer D), in which dark-colored malt (Caramel malt) was used, was weaker than that by pilsner-type beer (using Pilsner and Munich malts). This suggests that components in Beer D generated from brewing process, other than those rich in dark-colored malts, are responsible for the adverse effect on ACF formation.

While treatment with hops extract did not exhibit any significant inhibition of AOM-induced ACF formation, there have been several reports that components from hops may have chemopreventive potential. For example, prenylflavonoids exhibit cytotoxic effects in human cancer cell lines,[39] humulone suppresses the expression of cyclooxygenase-2 (COX-2)[42] and inhibits angiogenesis,[41] xanthohumols inhibit the LPS/IFN-γ-induced production of NO in murine macrophage RAW 264.7 cells.[43] It has also been reported that many nonsteroidal anti-inflammatory drugs (NSAIDs) reduce the number of AOM-induced ACF,[29,52–55] and these agents exhibit their chemopreventive activities mainly in the post-initiation phase, consistent with our results for piroxicam (Table IV). Treatment with beer in the postinitiation phase also reduced the number of ACF, suggesting that components with anti-promotion activity exist in beer. It remains unclear whether the concentration of hops extract at 1% was suitable for treatment and it is possible that hops extract at lower doses might inhibit AOM-induced ACF formation in the animal experiments, since suppression of COX-2 and angiogenesis were observed at low concentrations.[41,42]

In the long-term experiments, the number of colon tumors, including adenocarcinomas and adenomas, was significantly reduced by feeding with beer. Beer also decreased the number of ACF at weeks 5, 16 and 24 (by 31, 26 and 20%, respectively). These results suggest that the cancer chemopreventive effects of

TABLE VIII – EFFECTS OF BEER A ON AOM-INDUCED TUMOR MULTIPLICITY IN THE COLON OF F344 RATS

Group	Animals (n)	Tumor multipulicity(tumors/animals)									
		Rectum		Middle colon		Proximal colon		Entire colon			
		AD	ADC	AD	ADC	AD	ADC	AD	ADC	total
Saline + Water	13	0	0	0	0	0	0	0	0	0
AOM + Water	22	0.14 ± 0.35[1]	0.14 ± 0.47	0.32 ± 0.48	0.86 ± 0.83	0.09 ± 0.29	0.41 ± 0.73	0.55 ± 0.67	1.41 ± 1.10	1.95 ± 1.50
AOM + Beer A	22	0	0.13 ± 0.47	0.09 ± 0.43[2]*	0.73 ± 0.70	0	0.14 ± 0.35[2]*	0.09 ± 0.43[3]**	1.00 ± 0.98[2]*	1.09 ± 1.15[3]**

[1]Values are given as mean ± SD.–[2,3]Significantly different from the AOM + Water group.–*$p < 0.05$.–**$p < 0.005$.

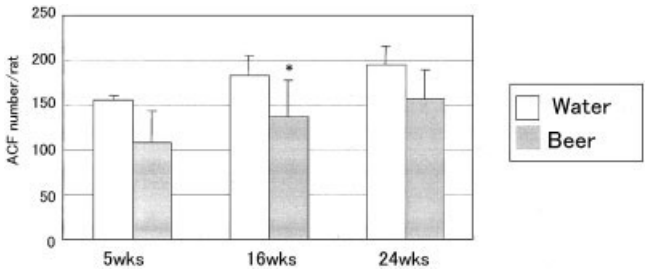

FIGURE 3 – Effects of beer on AOM-induced ACF formation in the long-term experiment. Six rats were taken from the water- and beer-fed groups at 5, 16 and 24 weeks after the start of the experiment. Bars indicate means±SD values. Significantly different from the water-control at *$p<0.05$.

beer in the initiation phase are related to a reduced number of ACF in the later phases (weeks 16 and 24) and a reduction in colon tumors. The present results indicate that tumor incidence and multiplicity in the proximal colon (right colon) was reduced by intake of beer, similar to a previous observation by Hamilton *et al.*[31] They reported that tumor incidence in the left colon increased in groups given diets containing low alcohol (LO-BEER or LO-EtOH). In our study, the incidence and multiplicity of adenomas in the middle and rectum colon (left colon) was reduced and no adverse effect was observed by beer intake.

AOM is metabolized to methylazoxymethanol (MAM) by CYP2E1 in the liver;[56,57] ethanol is also partly metabolized by CYP2E1.[58] Therefore, the slight reduction in the number of ACF with ethanol feeding found in our experiments may be attributable to inhibitory effects on AOM metabolism. There are conflicting reports on the influence of dietary ethanol on colon carcinogenesis in experimental animal models, with tumor promotion found in one study[59] and an inverse relationship in another.[31] It is worth noting that ethanol was given as 36% of the total calories in the former experiment and as 18% (Hi-EtOH group) in the latter experiment. Ethanol intake differed between the 2 experiments and it has been suggested that the influence of dietary ethanol consumption on the incidence of tumors varies with the concentration of ethanol in the liquid diet.[31]

A significant decrease in the total number of ACF and ACs/colon in animals fed diets containing FD Beer indicated that nonvolatile components are responsible for the inhibition of colonic carcinogenesis (Tables III and IV). It was estimated that the solid content of the beer used in this experiment was approximately 3% (w/w) and the average amount of daily diet intake by rats was about 12 g (Table III). Therefore, the intake of beer by animals fed diets containing 1 and 2% FD Beer, the most effective concentrations, could be calculated to be approximately 4 and 8 ml/day/rat. Although it is difficult to estimate the effective dose of beer for humans from the animal experiments, one can estimate the daily dose for humans on the basis of body surface area. Supposing the effective dose is proportional to the size of body surface [approximately calculated as (body weight value)$^{0.7}$ and the body weights of rat and human are 0.15 kg and 60 kg, respectively, the daily intake of 4 and 8 ml/rat could be estimated as 265 and 530 ml for humans.[60] The intake of beer in the experiments in which beer was fed *ad libitum* by feeding bottles was approximately 10 ml/day, indicating that the amount of beer constituents taken by the rats with reduced number of ACF were not so different between the solid and liquid forms. It should be noted that the number of ACF in the group with 4% FD Beer in the diet was higher than those with 1 and 2% diets, suggesting adverse effects on ACF inhibition may be due to high amounts of beer constituents. These complicated effects on ACF formation may be attributable to the complexity of beer ingredients. Beer may contain some components that enhance ACF formation and the fluctuations of those contents as well as the contents of components with chemopre-

vention activity may cause the variation in the ACF inhibitory activities among beers. It should also be emphasized that excess intake of beer may increase the cancer risk due to the disadvantageous effects of alcohol.

In conclusion, the cancer preventive effects of beer were observed using an AOM-induced colonic carcinogenesis model. A significant decrease in the number of adenomas in animals treated with beer, together with the reduction in the number of developed ACF with more than 4 aberrant crypts in the beer-fed group in the post-initiation phase, may suggest that the components of beer inhibit the growth of initiated cells. Further studies are needed to clarify the components responsible and the underlying mechanisms. The results suggest that daily moderate consumption of beer may reduce the risk of cancer susceptibility in colon.

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REFERENCES

1. Rimm EB, Giovannucci EL, Willett WC, Colditz GA, Ascherio A, Rosner B, Stampfer MJ. Prospective study of alcohol consumption and risk of coronary disease in men. Lancet 1991;338:464–8.
2. Tsugane S, Fahey MT, Sasaki S, Baba S. Alcohol consumption and all-cause and cancer mortality among middle-aged Japanese men: seven-year follow-up of the JPHC study Cohort I. Japan Public Health Center. Am J Epidemiol 1999;150:1201–7.
3. Riboli E, Cornee J, Macquart-Moulin G, Kaaks R, Casagrande C, Guyader M. Cancer and polyps of the colorectum and lifetime consumption of beer and other alcoholic beverages. Am J Epidemiol 1991;134:157–66.
4. Sharpe CR, Siemiatycki J, Rachet B. Effects of alcohol consumption on the risk of colorectal cancer among men by anatomical subsite (Canada). Cancer Causes Control 2002;13:483–91.
5. Sharpe CR, Siemiatycki J. Case-control study of alcohol consumption and prostate cancer risk in Montreal, Canada. Cancer Causes Control 2001;12:589–98.
6. La Vecchia C, Negri E, Franceschi S, D'Avanzo B. Moderate beer consumption and the risk of colorectal cancer. Nutr Cancer 1993;19: 303–6.
7. Jain MG, Hislop GT, Howe GR, Burch JD, Ghadirian P. Alcohol and other beverage use and prostate cancer risk among Canadian men. Int J Cancer 1998;78:707–11.
8. Sesso HD, Paffenbarger RS, Jr., Lee IM. Alcohol consumption and risk of prostate cancer: The Harvard Alumni Health Study. Int J Epidemiol 2001;30:749–55.
9. Block G, Patterson B, Subar A. Fruit, vegetables, and cancer prevention: a review of the epidemiological evidence. Nutr Cancer 1992;18:1–29.
10. Chen W, Sohn OS, Fiala ES, Weisburger JH. Effect of tea on the formation of DNA adducts by azoxymethane. Xenobiotica 1998;28: 213–7.
11. Suaeyun R, Kinouchi T, Arimochi H, Vinitketkumnuen U, Ohnishi Y. Inhibitory effects of lemon grass (Cymbopogon citratus Stapf) on formation of azoxymethane-induced DNA adducts and aberrant crypt foci in the rat colon. Carcinogenesis 1997;18:949–55.
12. Weisburger JH, Rivenson A, Reinhardt J, Aliaga C, Braley J, Pittman B, Zang E. Effect of black tea on azoxymethane-induced colon cancer. Carcinogenesis 1998;19:229–32.
13. Tanaka T, Kohno H, Tsukio Y, Honjo S, Tanino M, Miyake M, Wada K. Citrus limonoids obacunone and limonin inhibit azoxymethane-induced colon carcinogenesis in rats. Biofactors 2000;13:213–8.
14. Kohno H, Tanaka T, Kawabata K, Hirose Y, Sugie S, Tsuda H, Mori H. Silymarin, a naturally occurring polyphenolic antioxidant flavonoid, inhibits azoxymethane-induced colon carcinogenesis in male F344 rats. Int J Cancer 2002;101:461–8.
15. Middleton E, Jr., Kandaswami C. Effects of flavonoids on immune and inflammatory cell functions. Biochem Pharmacol 1992;43:1167–79.
16. Ho CT, Chen Q, Shi H, Zhang KQ, Rosen RT. Antioxidative effect of polyphenol extract prepared from various Chinese teas. Prev Med 1992;21:520–5.
17. Shiraki M, Hara Y, Osawa T, Kumon H, Nakayama T, Kawakishi S. Antioxidative and antimutagenic effects of theaflavins from black tea. Mutat Res 1994;323:29–34.
18. Arimoto-Kobayashi S, Sugiyama C, Harada N, Takeuchi M, Takemura M, Hayatsu H. Inhibitory effects of beer and other alcoholic beverages on mutagenesis and DNA adduct formation induced by several carcinogens. J Agric Food Chem 1999;47:221–30.
19. Paper DH. Natural products as angiogenesis inhibitors. Planta Med 1998;64(8):686–95.
20. Singh NP, McCoy MT, Tice RR, Schneider EL. A simple technique for quantitation of low levels of DNA damage in individual cells. Exp Cell Res 1988;175:184–91.
21. Sasaki YF, Tsuda S, Izumiyama F, Nishidate E. Detection of chemically induced DNA lesions in multiple mouse organs (liver, lung, spleen, kidney, and bone marrow) using the alkaline single cell gel electrophoresis (Comet) assay. Mutat Res 1997;388:33–44.
22. Kassie F, Rabot S, Uhl M, Huber W, Qin HM, Helma C, Schulte-Hermann R, Knasmuller S. Chemoprotective effects of garden cress (Lepidium sativum) and its constituents towards 2-amino-3-methylimidazo[4,5-f]quinoline (IQ)-induced genotoxic effects and colonic preneoplastic lesions. Carcinogenesis 2002;23:1155–61.
23. Guglielmi F, Luceri C, Giovannelli L, Dolara P, Lodovici M. Effect of 4-coumaric and 3,4-dihydroxybenzoic acid on oxidative DNA damage in rat colonic mucosa. Br J Nutr 2003;89:581–7.
24. Lazze MC, Pizzala R, Savio M, Stivala LA, Prosperi E, Bianchi L. Anthocyanins protect against DNA damage induced by tert-butylhydroperoxide in rat smooth muscle and hepatoma cells. Mutat Res 2003;535:103–15.
25. Bird RP. Observation and quantification of aberrant crypts in the murine colon treated with a colon carcinogen: preliminary findings. Cancer Lett 1987;37:147–51.
26. Pretlow TP, Cheyer C, OTapos;Riordan MA. Aberrant crypt foci and colon tumors in F344 rats have similar increases in proliferative activity. Int J Cancer 1994;56:599–602.
27. Roncucci L, Stamp D, Medline A, Cullen JB, Bruce WR. Identification and quantification of aberrant crypt foci and microadenomas in the human colon. Hum Pathol 1991;22:287–94.
28. Olivo S, Wargovich MJ. Inhibition of aberrant crypt foci by chemopreventive agents. In Vivo 1998;12:159–66.
29. Wargovich MJ, Jimenez A, McKee K, Steele VE, Velasco M, Woods J, Price R, Gray K, Kelloff GJ. Efficacy of potential chemopreventive agents on rat colon aberrant crypt formation and progression. Carcinogenesis 2000;21:1149–55.
30. Nelson RL, Samelson SL. Neither dietary ethanol nor beer augments experimental colon carcinogenesis in rats. Dis Colon Rectum 1985; 28:460–2.
31. Hamilton SR, Hyland J, McAvinchey D, Chaudhry Y, Hartka L, Kim HT, Cichon P, Floyd J, Turjman N, Kessie G, Nair PP, Dick J. Effects of chronic dietary beer and ethanol consumption on experimental colonic carcinogenesis by azoxymethane in rats. Cancer Res 1987;47: 1551–9.
32. Madigan D MI, Smyth MR. Rapid determination of 4-vinyl guaiacol and ferulic acid in beers and worts by high-performance liquid chromatography. J Am Soc Brew Chem 1994;52:152–5.
33. Lapcik O HM, Hampl R, Wahala K, Adlercreutz H. Identification of isoflavonoids in beer. Steroids 1998;63:14–20.
34. Madigan D MI, Smyth MR. Determination of proanthocyanidins and catechins in beer and barley by high-performance liquid chromatography with dual-electrode electrochemical detection. Analyst 1994;119:863–8.
35. Whittle N EH, Bartley J. Identification of the polyphenols in barley and beer by HPLC/MS and HPLC/Electrochemical Detection. J. Inst. Brew. 1999;105:89–99.
36. Ghiselli A, Natella F, Guidi A, Montanari L, Fantozzi P, Scaccini C. Beer increases plasma antioxidant capacity in humans 2000;11:76–80.
37. Miranda CL, Yang YH, Henderson MC, Stevens JF, Santana-Rios G, Deinzer ML, Buhler DR. Prenylflavonoids from hops inhibit the metabolic activation of the carcinogenic heterocyclic amine 2-amino-3-methylimidazo[4, 5-f]quinoline, mediated by cDNA-expressed human CYP1A2. Drug Metab Dispos 2000;28:1297–302.
38. Henderson MC, Miranda CL, Stevens JF, Deinzer ML, Buhler DR. In vitro inhibition of human P450 enzymes by prenylated flavonoids from hops, Humulus lupulus. Xenobiotica 2000;30:235–51.
39. Miranda CL, Stevens JF, Helmrich A, Henderson MC, Rodriguez RJ, Yang YH, Deinzer ML, Barnes DW, Buhler DR. Antiproliferative and cytotoxic effects of prenylated flavonoids from hops (Humulus lupulus) in human cancer cell lines. Food Chem Toxicol 1999;37:271–85.
40. Yasukawa K, Takeuchi M, Takido M. Humulon, a bitter in the hop, inhibits tumor promotion by 12-O-tetradecanoylphorbol-13-acetate in two-stage carcinogenesis in mouse skin. Oncology 1995;52:156–8.
41. Shimamura M, Hazato T, Ashino H, Yamamoto Y, Iwasaki E, Tobe H, Yamamoto K, Yamamoto S. Inhibition of angiogenesis by humulone, a bitter acid from beer hop. Biochem Biophys Res Commun 2001;289:220–4.
42. Yamamoto K, Wang J, Yamamoto S, Tobe H. Suppression of cyclo-

oxygenase-2 gene transcription by humulon of beer hop extract studied with reference to glucocorticoid. FEBS Lett 2000;465:103–6.
43. Zhao F, Nozawa H, Daikonnya A, Kondo K, Kitanaka S. Inhibitors of Nitric Oxide Production from Hops (Humulus lupulus L.). Biol Pharm Bull 2003;26:61–5.
44. Pool-Zobel BL, Bertram B, Knoll M, Lambertz R, Neudecker C, Schillinger U, Schmezer P, Holzapfel WH. Antigenotoxic properties of lactic acid bacteria in vivo in the gastrointestinal tract of rats. Nutr Cancer 1993;20:271–81.
45. Tice RR, Agurell E, Anderson D, Burlinson B, Hartmann A, Kobayashi H, Miyamae Y, Rojas E, Ryu JC, Sasaki YF. Single cell gel/comet assay: guidelines for in vitro and in vivo genetic toxicology testing. Environ Mol Mutagen 2000;35:206–21.
46. Ward JM. Morphogenesis of chemically induced neoplasms of the colon and small intestine in rats. Lab Invest 1974;30:505–13.
47. Hong MY, Lupton JR, Morris JS, Wang N, Carroll RJ, Davidson LA, Elder RH, Chapkin RS. Dietary fish oil reduces O6-methylguanine DNA adduct levels in rat colon in part by increasing apoptosis during tumor initiation. Cancer Epidemiol Biomarkers Prev 2000;9:819–26.
48. Mori H, Kawabata K, Yoshimi N, Tanaka T, Murakami T, Okada T, Murai H. Chemopreventive effects of ferulic acid on oral and rice germ on large bowel carcinogenesis. Anticancer Res 1999;19:3775–8.
49. Kawabata K, Yamamoto T, Hara A, Shimizu M, Yamada Y, Matsunaga K, Tanaka T, Mori H. Modifying effects of ferulic acid on azoxymethane-induced colon carcinogenesis in F344 rats. Cancer Lett 2000;157:15–21.
50. Han BS, Park CB, Takasuka N, Naito A, Sekine K, Nomura E, Taniguchi H, Tsuno T, Tsuda H. A ferulic acid derivative, ethyl 3-(4′-geranyloxy-3-methoxyphenyl)-2-propenoate, as a new candidate chemopreventive agent for colon carcinogenesis in the rat. Jpn J Cancer Res 2001;92:404–9.
51. Woffenden HM, Ames JM, Chandra S, Anese M, Nicoli MC. Effect of kilning on the antioxidant and pro-oxidant activities of pale malts. J Agric Food Chem 2002;50:4925–33.
52. Pereira MA. Prevention of colon cancer and modulation of aberrant crypt foci, cell proliferation, and apoptosis by retinoids and NSAIDs. Adv Exp Med Biol 1999;470:55–63.
53. Li H, Kramer PM, Lubet RA, Steele VE, Kelloff GJ, Pereira MA. Termination of piroxicam treatment and the occurrence of azoxymethane-induced colon cancer in rats. Cancer Lett 1999;147:187–93.
54. Yoshimi N, Shimizu M, Matsunaga K, Yamada Y, Fujii K, Hara A, Mori H. Chemopreventive effect of N-(2-cyclohexyloxy-4-nitrophenyl)methane sulfonamide (NS-398), a selective cyclooxygenase-2 inhibitor, in rat colon carcinogenesis induced by azoxymethane. Jpn J Cancer Res 1999;90:406–12.
55. Shpitz B, Bomstein Y, Kariv N, Shalev M, Buklan G, Bernheim J. Chemopreventive effect of aspirin on growth of aberrant crypt foci in rats. Int J Colorectal Dis 1998;13:169–72.
56. Sohn OS, Ishizaki H, Yang CS, Fiala ES. Metabolism of azoxymethane, methylazoxymethanol and N-nitrosodimethylamine by cytochrome P450IIE1. Carcinogenesis 1991;12:127–31.
57. Sohn OS, Fiala ES, Requeijo SP, Weisburger JH, Gonzalez FJ. Differential effects of CYP2E1 status on the metabolic activation of the colon carcinogens azoxymethane and methylazoxymethanol. Cancer Res 2001;61:8435–40.
58. Lieber CS. Biochemical and molecular basis of alcohol-induced injury to liver and other tissues. N Engl J Med 1988;319:1639–50.
59. Seitz HK, Czygan P, Waldherr R, Veith S, Raedsch R, Kassmodel H, Kommerell B. Enhancement of 1,2-dimethylhydrazine-induced rectal carcinogenesis following chronic ethanol consumption in the rat. Gastroenterology 1984;86:886–91.
60. Adolph E. Quantitative relations in the physiological constitutions of mammals. Science 1949;109:579–85.