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Carcinogenicity of antioxidants BHA, caffeic acid, sesamol, 4-methoxyphenol and catechol at low doses, either alone or in combination, and modulation of their effects in a rat medium-term multi-organ carcinogenesis model

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The carcinogenicity of low dietary levels of the antioxidants butylated hydroxyanisole (BHA), caffeic acid, sesamol, 4-methoxyphenol (4-MP) and catechol, known to target the forestomach or glandular stomach, were examined alone or in combination in a 2-year long-term experiment and their modifying effects assessed in a medium-term multi-organ model. In the carcinogenicity study, groups of 30–31 male F344 rats were treated with 0.4% BHA, 0.4% caffeic acid, 0.4% sesamol, 0.4% 4-MP and 0.16% catechol either alone or in combination for up to 104 weeks and then killed. In the medium-term multi-organ model, groups of 10 to 15 male F344 rats were given diethylnitrosamine (DEN), *N*-methylnitrosourea (MNU), 1,2-dimethylhydrazine (DMH), *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine (BBN) and 2,2′-dihydroxy-di-n-propylnitrosamine (DHPN) for a total multiple initiation period of 4 weeks (DMBDD treatment). BHA, caffeic acid, sesamol and 4-MP, each at doses of 0.4% or 0.08%, and catechol at doses of 0.16% or 0.032% were administered in the diet either alone or in combination after completion of the initiation regimen. All surviving animals were killed at the end of week 28, and major organs were examined histopathologically. In the carcinogenicity study, slightly increased incidences of forestomach papillomas were found in the sesamol- (15.8%), caffeic acid- (14.8%), catechol- (3%) and 4-MP- (11.5%) treated groups as compared with basal diet (0%), and a significant increase was observed with the five antioxidants in combination (42.9%, $P < 0.001$). In a medium-term multi-organ carcinogenesis model, incidences of forestomach papillomas and/or carcinomas were increased in each high dose group, but additive or synergistic effects were not found in the combination group. In the low dose case, the incidence of forestomach papillomas was significantly increased only in the combination group. With regard to other organs, the incidence of colon tumors was significantly decreased only in the high dose combination group. The results indicate that even at low dose levels phenolic compounds can exert additive/synergistic effect on carcinogenesis.

***Abbreviations:** BHA, butylated hydroxyanisole; 4-MP, 4-methoxyphenol; DEN, diethylnitrosamine; MNU, *N*-methylnitrosourea; DMH, 1,2-dimethylhydrazine; BBN, *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine; DHPN, 2,2′-dihydroxy-di-n-propylnitrosamine; DMBDD, multiple initiation regimen of DEN, MNU, BBN, DHPN and DMH; EHEN, *N*-ethyl-*N*-hydroxyethylnitrosamine; MNNG, *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine; HTHQ, 1-*O*-hexyl-2,3,5-trimethylhydroquinone; PN, papillary or nodular.

Introduction

Since the first demonstration of forestomach carcinogenicity for the synthetic phenolic antioxidant butylated hydroxyanisole (BHA*) (1), several phenolic compounds have been examined for their capacity to influence cell proliferation in the forestomach and glandular stomach of rats or hamsters (2–4). Based on these results, several compounds that induced strong cell proliferation were selected for long-term carcinogenicity studies. It was found that continuous oral treatment with 2% caffeic acid, sesamol or 4-methoxyphenol (4-MP) is carcinogenic to the forestomach, 4-methylcatechol is a carcinogen for the forestomach and glandular stomach, and that 0.8% catechol causes glandular stomach tumors in F344 rats (5–8). Although catechol was not carcinogenic to the forestomach of F344 rats, a significant increase in the incidence of papilloma was shown in a carcinogenesis study using Sprague–Dawley rats (9). In addition, 0.8% catechol was found to enhance tongue, esophagus, forestomach and glandular stomach carcinogenesis at the post-initiation stage in rats pretreated with *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG) (10) or methyl-*N*-amylnitrosamine (11). BHA has been shown to enhance urinary bladder carcinogenesis at high dose levels (1–2% in diet) (12–14), and forestomach carcinogenesis at lower doses (2–0.5% in diet) in the post-initiation stage (13–17). Of these compounds, caffeic acid, sesamol, catechol and 4-methylcatechol are present in our environment as food constituents, cigarette smoke or industrial chemicals (18–22). Thus, man is exposed to mixtures of these carcinogens on a chronic basis. There are some reports that indicate synergistic or additive effects of a mixture of environmental carcinogens (23–25), and therefore the present experiment was conducted to examine whether combined treatment with low doses of carcinogenic phenolic compounds might induce tumors.

Recently, it has been suggested that thresholds exist with regard to dose levels for promotion or inhibition of carcinogenesis. For example, promotion of ethylnitrosourethane-initiated forestomach carcinogenesis by the antioxidant 1-*O*-hexyl-2,3,5-trimethylhydroquinone (HTHQ) was found with a dietary dose level of 0.25% but not at doses of 1and 0.5% (unpublished data). In the inhibition of methylnitrosourea or azoxymethane-induced colon carcinogenesis by green tea components, lower doses were more effective (26,27). Therefore, a second aim of the present experiment was to examine the modifying effects of carcinogenic antioxidants at dose levels lower than the carcinogenic doses. Our medium-term multi-organ carcinogenesis model, in which modifying effects of chemicals in major organs can be evaluated in a single experiment (28,29), was selected for this purpose.

Materials and methods

Animals

Male F344 rats, aged 5 weeks, were obtained from Charles River Japan Inc. (Kanagawa Japan), and housed at five to a plastic cage with wood chips for bedding in an air-conditioned room at 24 ± 2°C with a 12 h light–12 h dark

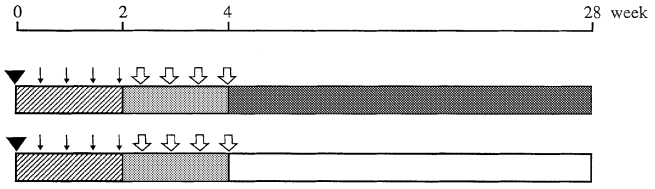

Fig. 1. Experimental protocol for the medium-term multi-organ carcinogenesis model. Animals: male F344 rats, 6 weeks old. ▼; DEN, 100 mg/kg body wt i.p., ↓ MNU, 20 mg/kg body wt i.p., ⇩ DMH, 40 mg/kg body wt s.c., ▨; BBN, 0.05% in drinking water, ▤; DHPN, 0.1% in drinking water, □; Basal diet, ■; BHA (0.4, 0.08%), caffeic acid (0.4, 0.08%), sesamol (0.4, 0.08%), 4-methoxyphenol (0.4, 0.08%), catechol (0.16, 0.032%), or low or high dose combinations of the five compounds.

cycle. They were maintained on Oriental MF powdered basal diet (Oriental Yeast Co., Tokyo, Japan) and tap water *ad libitum*. Experimentation was started after 1 week of aclimatization.

Chemicals

N-Diethylnitrosamine (DEN), *N*-methylnitrosourea (MNU), 1,2-dimethylhydrazine (DMH), *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine (BBN), 4-MP, catechol and caffeic acid were obtained from Tokyo Kasei Kogyo Co. Ltd., BHA was from Wako Pure Chemical Industries (Osaka), sesamol was from Fluka Chemie AG (Switzerland) and 2,2′-dihydroxy-di-n-propylnitrosamine (DHPN) was from Nakalai Chemical Co. (Osaka, Japan).

Treatment

Carcinogenicity study. Groups of 30–31 animals were administered Oriental MF powdered diet containing 0.4% BHA, 0.4% caffeic acid, 0.4% sesamol, 0.4% 4-MP or 0.16% catechol either alone or in combination for up to 104 weeks. Control animals were given basal diet alone throughout the experimental period. Animals were weighed once every 1–4 weeks during the experiment. All survivors were killed under ether anesthesia at the end of week 104, and subjected to complete autopsy. Animals that survived until the end of experiment were included in the effective numbers. Tissues were processed routinely for hematoxylin and eosin staining.

Medium-term multi-organ carcinogenesis study. The experimental protocol is shown in Figure 1. The animals were randomly divided into 25 groups of 10–15 animals each. Those in groups 1 to 13 received combined treatments with a single i.p. injection of 100 mg/kg body wt of DEN, four i.p. injections of 20 mg/kg body wt of MNU, four s.c. injections of 40 mg/kg body wt of DMH, together with 0.05% BBN for 2 weeks, and then 0.1% DHPN for 2 weeks (both given in the drinking water), during the initial 4-week period for multiple initiation (DMBDD treatment). Animals in groups 1–5 were administered 0.4% BHA, 0.4% caffeic acid, 0.4% sesamol, 0.4% 4-MP or 0.16% catechol in Oriental MF powdered basal diet from the completion of the DMBDD-treatment to the end of the experiment. Animals in group 6 were treated with mixtures of these five antioxidants. Animals in groups 7–12 were similarly administered 0.08% BHA, 0.08% caffeic acid, 0.08% sesamol, 0.08% 4-MP or 0.032% catechol, or mixtures of these five antioxidants. Animals in group 13 received the DMBDD-treatment alone as the carcinogen control. A further 12 groups of 10 animals were treated with high or low doses of antioxidants, either alone or in combination without the DMBDD pretreatment as antioxidant controls. Animals were weighed once every 1–4 weeks during the experiment after the carcinogen exposure. Those that died during the experiment or became moribund were autopsied and all those that survived for 20 weeks, when the first tumor appeared, were included in the effective numbers. All survivors were killed under ether anesthesia at the end of week 28, and subjected to complete autopsy. Livers and kidneys were weighed. Neutral buffered formalin solution was injected into the lung, esophagus, stomach, intestines and urinary bladder. One slice from each lung lobule, three from liver, four from esophagus, five from stomach, four from urinary bladder and one from each of the other organs were taken. Swiss-roll preparations were made from large and small intestines. Tissues were processed routinely for histopathological examination using hematoxylin and eosin staining. Student's *t*-test and Fisher's exact probability test were used for statistical analysis of the data.

Results

Carcinogenicity study

Final average body wts of rats treated with antioxidant alone were all lower than the basal diet values, and were lowest in the combination group. On the other hand, relative liver and/or kidney weights were increased in the BHA, sesamol, catechol and combination groups (Table I). Generally, incidences and multiplicities of the forestomach histopathological lesions were increased by the treatment with antioxidants except in the BHA case (Table II). The incidences and/or multiplicities of forestomach papillary or nodular (PN) hyperplasia were significantly increased in the groups treated with 4-methoxyphenol (31%, $P < 0.05$; 0.31 ± 0.46, not significant), caffeic acid (63%, $P < 0.01$; 1.11 ± 1.13, $P < 0.01$) and the antioxidants in combination (61%, $P < 0.01$; 1.11 ± 1.13, $P < 0.01$) as compared with the basal diet (4%; 0.08 ± 0.39) group. With regard to papillomas, significant differences in the incidence or multiplicity were found for the caffeic acid (0.19 ± 0.47, $P < 0.05$) and combination groups (43%, $P < 0.01$; 0.93 ± 1.31, $P < 0.01$) as compared with the basal diet group (0%). In the glandular stomach, incidences and multiplicities of submucosal hyperplasias and adenomas were significantly increased in the catechol and combination groups, without any difference between the two (Table III). No significant alteration in the incidences of neoplastic lesions was found in the esophagus, liver or kidneys.

Table I. Final body and relative organ wts (carcinogenicity study)

Treatment	No. of rats	Body wt (g)	Relative organ wts (g/100 g body wt)	
			Liver	Kidney
Combination	25	375 ± 24**	2.87 ± 0.25**	0.32 ± 0.04
BHA	26	405 ± 33**	2.95 ± 0.29**	0.30 ± 0.02**
4-Methoxyphenol	26	396 ± 25**	3.07 ± 0.59	0.34 ± 0.04
Caffeic acid	27	384 ± 34**	3.16 ± 0.32	0.35 ± 0.04
Sesamol	19	398 ± 34**	2.84 ± 0.54**	0.30 ± 0.03**
Catechol	29	385 ± 40**	3.05 ± 0.40	0.31 ± 0.03*
Basal diet	28	444 ± 38	3.24 ± 0.40	0.33 ± 0.04

Significantly different at *$P < 0.05$, **$P < 0.01$ vs basal diet group values.

Medium-term multi-organ carcinogenesis study

A significant decrease in the final body wts was evident in the combination high-dose and sesamol low-dose groups. The relative liver weights were increased in the high- and low-dose combination and BHA groups (Table IV). Histopathological findings are summarized in Tables V and VI. In the high-dose case, forestomach PN hyperplasias and papillomas were significantly increased by caffeic acid (100 and 73%, $P < 0.01$) and 4-methoxyphenol (87 and 60%, $P < 0.01$), and papillomas by catechol (30%, $P < 0.05$). Tendencies for increase were also observed for the BHA and sesamol groups. Significant increase was also noted in the combination group (93 and 67%, $P < 0.01$), but no additive or synergistic enhancement was apparent since data for the lesions, including carcinomas, were not different from those of the caffeic acid group. In the large intestine, each antioxidant alone tended to decrease the incidence of tumors, and reduction was demonstrated in the combination group (7%, $P < 0.05$) as compared with the basal diet case (53%). In the low-dose groups, forestomach hyperplasia and papilloma tended to increase in each antioxidant group, and were significantly increased in the combination group (60 and 30% respectively, $P < 0.05$). In the urinary bladder, a slight but significant increase in the incidence of PN hyperplasia was demonstrated by the BHA (33%, $P < 0.05$) and sesamol (33%, $P < 0.05$) groups. In the

Table II. Histopathological findings in the forestomach (carcinogenicity study)

Treatment	No. of rats	PN hyperplasia incidence (%)	Multiplicity (no./slide)	Papilloma incidence (%)	Multiplicity (no./slide)	Carcinoma incidence (%)
Combination	28	17 (61)**	1.04 ± 1.05**	12 (43)**	0.93 ± 1.31**	1 (4)
BHA	26	0	–	0	–	0
4-Methoxyphenol	26	8 (31)*	0.31 ± 0.46	3 (12)	0.19 ± 0.62	0
Caffeic acid	27	17 (63)**	1.11 ± 1.13**	4 (15)	0.19 ± 0.47*	0
Sesamol	19	5 (26)	0.37 ± 0.67	3 (16)	0.21 ± 0.52	0
Catechol	29	6 (21)	0.69 ± 2.73	1 (3)	0.03 ± 0.18	0
Basal diet	25	1 (4)	0.08 ± 0.39	0	–	0

Significantly different at $*P < 0.05$, $**P < 0.01$ vs basal diet group values.

Table III. Histopathological findings in the glandular stomach (carcinogenicity study)

Treatment	No. of rats	Submucosal hyperplasia incidence (%)	Multiplicity (no./slide)	Adenoma incidence (%)	Multiplicity (no./slide)
Combination	28	6 (21)*	0.21 ± 0.49*	16 (57)**	0.57 ± 0.50**
BHA	26	0	–	0	–
4-Methoxyphenol	26	0	–	0	–
Caffeic acid	27	0	–	0	–
Sesamol	19	0	–	1 (5)	0.05 ± 0.22
Catechol	29	8 (28)**	0.32 ± 0.67*	13 (45)**	0.48 ± 0.57**
Basal diet	25	0	–	0	–

Significantly different at $*P < 0.05$, $**P < 0.01$ vs basal diet group values.

Table IV. Final body and relative organ wts (medium-term multi-organ carcinogenesis study)

			Relative organ wt (%)	
Chemical	No. of rats	Body wt (g)	Liver	Kidney
High-dose groups (0.4%)				
Combination	14	299 ± 26**	2.9**	1.0
BHA	12	321 ± 19	2.7**	0.7
Caffeic acid	14	319 ± 20	2.4	0.8
Sesamol	14	328 ± 17	2.4	0.6
4-Methoxyphenol	14	316 ± 45	2.4	0.7
Catechol	14	319 ± 20	2.5	1.0
Low-dose groups (0.08%)				
Combination	15	318 ± 29	2.6*	0.6
BHA	13	326 ± 19	2.6**	0.7
Caffeic acid	15	318 ± 19*	2.5	0.6
Sesamol	12	332 ± 21	2.3	0.6
4-Methoxyphenol	13	338 ± 20	2.4	0.6
Catechol	13	325 ± 21	2.4	0.7
Basel diet	15	332 ± 17	2.4	0.7

Significantly different at $*P < 0.05$, $**P < 0.01$ vs basal diet group values.

non-initiation groups, hyperplasias in the lung and forestomach were found sporadically.

Discussion

In the present long-term carcinogenicity study, all compounds, except BHA, tended to increase the development of PN hyperplasias or papillomas, and a significant increase was found with 4-MP and caffeic acid, indicating weak tumorigenic activities of these compounds at a low dose. The lack of tumorigenicity of BHA at the dose of 0.4% is consistent with the previous finding that continuous oral treatment with 0.5% for 2 years did not induce forestomach tumors (30). Although additive or synergistic effects were not apparent with regard to forestomach PN hyperplasia, a clear additive effect was demonstrated for the incidence of papillomas, and synergism was noted for the multiplicity of papillomas in the combination group. In addition, carcinoma development was only found in this combination case. Therefore, clear additive/synergistic enhancement was demonstrated for forestomach carcinogenesis. The lack of any such effect in the glandular stomach indicates that only catechol is a glandular stomach carcinogen.

In the medium-term multi-organ carcinogenesis model, all compounds at high doses enhanced forestomach carcinogenesis, although the effect was weak with BHA and sesamol. This corresponds with the previous report that threshold concentrations exist for promotion of forestomach carcinogenesis with compounds like BHA after initiation with MNNG (15,17,31). The higher incidences of hyperplasias and papillomas in the caffeic acid and 4-MP groups also correlates well with the higher values for incidence or multiplicity of forestomach lesions in the long-term carcinogenicity study. Additive or synergistic enhancement of carcinogenesis was not shown in the present experiment, indicating that the synergism found earlier (17) might be influenced by duration of experimental period or the carcinogen used for initiation. The observed inhibition of colon tumor development in the combination group may partly be due to caloric restriction, since a decrease in body-weight gain was observed during the experiment.

A weak promotion effect of all antioxidants, except BHA, was shown with the low-dose groups in terms of incidences of forestomach PN hyperplasia and papillomas, and a significant increase in these lesions was apparent in the combination group. The incidence of urinary bladder PN hyperplasia was significantly increased in the BHA and sesamol groups, but no clear increase in the incidence of tumors was found. Promotion of urinary bladder carcinogenesis by BHA has only

Table V. Histopathological findings in major organs (medium-term multi-organ carcinogenesis study, high dose groups)

Organs and lesions	Combination $n = 15$	BHA $n = 15$	Caffeic acid $n = 15$	Sesamol $n = 15$	4-Methoxyphenol $n = 15$	Catechol $n = 15$	Basal diet $n = 15$
Thyroid							
Adenoma	1 (7)	1 (7)	3 (20)	3 (20)	0*	3 (20)	5 (33)
Carcinoma	0	0	1 (7)	0	1 (7)	2 (13)	1 (7)
Lung							
Adenoma	4 (27)	4 (27)	4 (27)	7 (47)	4 (27)	8 (53)	10 (67)
Carcinoma	0	3 (20)	2 (13)	1 (7)	2 (13)	3 (20)	1 (7)
Tongue							
Papilloma	0	0	1 (7)	0	0	0	0
Carcinoma	0	0	0	0	0	1 (7)	0
Forestomach							
PN hyperplasia	14 (93)**	7 (47)	15 (100)**	0	13 (87)**	8 (53)	2 (13)
Papilloma	10 (67)**	4 (27)	11 (73)**	4 (27)	9 (60)**	5 (30)*	0
Carcinoma	4 (27)	1 (7)	3 (20)	0	0	2 (13)	0
Small intestine							
Adenoma	0	0	1 (7)	0	1 (7)	1 (7)	0
Carcinoma	3 (20)	1 (7)	4 (27)	0	1 (7)	3 (20)	3 (20)
Large intestine							
Adenoma	0	0 1 (7)	2 (13)	0	1 (7)	1 (7)	
Carcinoma	1 (7)*	2 (13)	6 (40)	5 (33)	3 (20)	5 (33)	7 (47)
Adenoma/carcinoma	1 (7)*	2 (13)	7 (47)	6 (40)	3 (20)	6 (40)	8 (53)
Liver							
Hepatocellular adenoma	0	0	0	0	1 (7)	0	1 (7)
Hepatocellular carcinoma	0	0	0	0	0	0	1 (7)
Kidney							
Atypical tubules	13 (87)	7 (50)[a]	9 (64)[a]	10 (67)	13 (87)	10 (67)	13 (87)
Renal cell tumors	0	1 (7)	1 (7)	2 (13)	1 (7)	2 (13)	0
Nephroblastoma	3 (20)	5 (36)	6 (43)	6 (40)	9 (60)	9 (60)	8 (53)
Urinary bladder							
PN hyperplasia	3 (20)	3 (20)	3 (20)	2 (13)	1 (7)	2 (13)	0
Papilloma	0	0	0	0	1 (7)	1 (7)	1 (7)
Carcinoma	1 (7)	0	0	0	0	0	0
All sites							
Malignant lymphoma/ leukemia	2 (13)	0	0	0	1 (7)	1 (7)	0

Significantly different at $*P < 0.05$, $**P < 0.01$ vs basal diet group values.
[a]No. of rats is 14.

been reported with high doses of 1% or more (12–14), and in another experiment, BHA at a dose of 0.1% did not enhance bladder carcinogenesis in the same medium-term multi-organ model (unpublished data). Therefore, it is difficult to conclude that these antioxidants enhance urinary bladder carcinogenesis at low-dose levels. In usual carcinogenicity studies, doses of chemicals are very high compared with possible human intake if the toxicity level of the compound is low, because they are determined on the basis of toxicity data. Therefore, effects of chemicals at low doses close to human intake levels have not been well investigated. Assessment of such low-dose effects of chemicals using initiation–promotion models appears to be important for the determination of chemical risk as well as to establish optical doses of chemopreventive agents.

Previously, we have shown that although 4-MP at a dose of 2% in the diet was carcinogenic for forestomach epithelium, it did not enhance forestomach carcinogenesis at doses between 2–0.25% in rats pretreated with MNNG (32). No clear enhancing effect of 0.5% caffeic acid was found in the MNNG model (17). In the present experiment, 4-MP at a dose of 0.4%, and caffeic acid at a dose of 0.4%, clearly enhanced forestomach carcinogenesis after initiation with DEN, MNU, BBN, DHPN and DMH. Of these five carcinogens, MNU is the only one that targets forestomach. Therefore, it is suggested that 4-MP selectively enhanced MNU-initiated forestomach carcinogenesis and caffeic acid responded more sensitively in MNU initiation. Generally, promotion activity of chemicals is not influenced by the initiator, although some exceptions have been reported: one example is that antioxidant BHT did not enhance hepatocarcinogenesis in rats pretreated with dibutylnitrosamine (33), aflatoxin B1 (34) or DEN (35), while it was inhibited in a multi-organ model after initiation with BBN, DHPN and *N*-ethyl-*N*-hydroxyethylnitrosamine (EHEN) (36). However, it enhanced hepatocarcinogenesis at a dietary dose of 0.6% after initiation with 2-acetylaminofluorene (37). Treatment with 4-MP after MNNG initiation induces more severe forestomach damage than does 4-MP alone. Such complexity in the evoked reaction may be responsible for the lack of promotion in a case of 4-MP after MNNG initiation (32).

In conclusion, these synergistic/additive effects are exerted on the forestomach with low doses of carcinogenic antioxidants in combination. In organs other than forestomach, although no clear low dose-enhancement or inhibition of carcinogenesis was demonstrated by individual carcinogens, modification was apparent when they were given in combination in the post-initiation stage.

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Table VI. Histopathological findings in major organs (medium-term multi-organ carcinogenesis study, low-dose groups)

Organs and lesions	Combination $n = 15$	BHA $n = 15$	Caffeic acid $n = 15$	Sesamol $n = 15$	4-Methoxyphenol $n = 15$	Catechol $n = 15$	Basal diet $n = 15$
Thyroid							
Adenoma	2 (13)	2 (13)	2 (13)	0*	2 (13)	5 (33)	5 (33)
Carcinoma	3 (20)	3 (20)	1 (7)	1 (7)	1 (7)	1 (7)	1 (7)
Lung							
Adenoma	9 (60)	7 (47)	7 (47)	8 (53)	8 (53)	6 (40)	10 (67)
Carcinoma	3 (20)	0	0	0	0	0	1 (7)
Tongue							
Papilloma	1 (7)	0	0	0	0	0	0
Forestomach							
PN hyperplasia	9 (60)	2 (13)	4 (27)	4 (27)	4 (27)	7 (47)	2 (13)
Papilloma	5 (30)*	0	1 (7)	2 (13)	3 (13)	3 (20)	0
Carcinoma	1 (7)	0	1 (7)	0	0	0	0
Small intestine							
Adenoma	3 (20)	1 (7)	0	0	0	1 (7)	0
Carcinoma	1 (7)	4 (27)	5 (33)	4 (27)	3 (20)	5 (33)	3 (20)
Large intestine							
Adenoma	0	0	0	0	0	0	1 (7)
Carcinoma	2 (13)	2 (13)	4 (27)	4 (27)	2 (13)	3 (30)	7 (47)
Adenoma/carcinoma	2 (13)	2 (13)	4 (27)	4 (27)	2 (13)	3 (20)	8 (53)
Liver							
Hepatocellular adenoma	0	1 (7)	0	1 (7)	0	0	1 (7)
Hepatocellular carcinoma	0	0	1 (7)	0	1 (7)	0	1 (7)
Kidney							
Atypical tubules	13 (87)	11 (79)[a]	12 (80)	11 (85)[b]	11 (79)[a]	13 (87)	13 (87)
Renal cell tumors	1 (7)	1 (7)	1 (7)	1 (7)	2 (14)	0	0
Nephroblastoma	4 (27)	5 (36)	6 (40)	8 (62)	3 (21)	5 (33)	8 (53)
Urinary bladder							
PN hyperplasia	2 (13)	5 (33)*	0	5 (33)*	1 (7)	0	0
Papilloma	1 (7)	3 (20)	1 (7)	0	0	2 (13)	1 (7)
Carcinoma	0	0	0	1 (7)	0	0	0
All sites							
Malignant lymphoma/ leukemia	0	0	1 (7)	0	1 (7)	1 (7)	0

Significantly different at $*P < 0.05$, $**P < 0.01$ vs basal diet group values.
[a]No. of rats is 14
[b]No of rats is 13.

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