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STAGE AND ORGAN DEPENDENT EFFECTS OF 1-*O*-HEXYL-2,3,5-TRIMETHYLHYDROQUINONE, ASCORBIC ACID DERIVATIVES, N-HEPTADECANE-8,10-DIONE AND PHENYLETHYL ISOTHIOCYANATE IN A RAT MULTIORGAN CARCINOGENESIS MODEL

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The effects of 1-*O*-hexyl-2,3,5-trimethylhydroquinone (HTHQ), phenylethyl isothiocyanate (PEITC), 3-*O*-ethylascorbic acid, 3-*O*-dodecylcarbomethylascorbic acid and n-heptadecane-8,10-dione were analyzed in a rat multiorgan carcinogenesis model. Groups of 15 animals were given a single intraperitoneal (i.p.) injection of diethylnitrosamine and 4 i.p. injections of N-methylnitrosourea as well as N-butyl-N-(4-hydroxybutyl)nitrosamine in the drinking water during the first 2 weeks. Then 4 subcutaneous (s.c.) injections of dimethylhydrazine and 2,2′-dihydroxy-di-n-propylnitrosamine were given in the drinking water over the next 2 weeks for initiation. Test compounds were administered during the initiation or post-initiation periods. The dietary dose was 1% except for n-heptadecane-8,10-dione and PEITC (0.1%). Complete autopsy was performed at the end of experimental week 28. All 5 compounds reduced the number of lung hyperplasia, particularly PEITC when given during the initiation period. In addition, HTHQ lowered the incidence of esophageal hyperplasia in the initiation period, and of small and large intestinal adenomas in the post-initiation period. However, it also enhanced the development of hyperplasia and papilloma in the forestomach and tongue. PEITC lowered the induction of esophageal hyperplasia, kidney atypical tubules and liver glutathione S-transferase placental form (GST-P)-positive foci when given during the initiation period but enhanced the development of liver GST-P positive foci and urinary bladder tumors in the post-initiation period. Moreover, it induced hyperplasia of the urinary bladder. Our results indicate minor adverse effects for HTHQ in the forestomach and tongue, and demonstrate that PEITC, which inhibits carcinogenesis at the initiation stage in several organs, also exhibits promotion potential in liver and urinary bladder in the post-initiation stage under the present experimental conditions. ***Int. J. Cancer*** **76:851–856, 1998.**
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Many antioxidants have been investigated for their potentials as cancer chemopreventive agents. Since these act as radical scavengers, or phase I or II enzyme inducers or inhibitors, they would be expected to mainly modify carcinogenesis during the initiation phase.

1-*O*-hexyl-2,3,5-trimethylhydroquinone (HTHQ) is a novel synthetic strong antioxidant with lipophilicity, which has a strong anti-mutagenic activity against 8 carcinogenic heterocyclic amines (HCAs) contained in cooked meat, using the Ames assay in the presence of an S9 mixture (Hirose *et al.,* 1993*a,* 1995*c*). Simultaneous administration of HTHQ decreases the induction of mammary adenocarcinomas by 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), a major HCA, in female F344 rats (Hirose *et al.,* 1995*a*). Furthermore, a clear reduction of preneoplastic glutathione S-transferase placental form (GST-P)-positive foci caused by 2-amino-6-methyldipyrido[1,2-*a*:3′,2′-*d*]imidazole (Glu-P-1) or 2-amino-3,8′-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx) has been observed in rats cotreated with HTHQ. In contrast, this antioxidant does not modify the mutagenicity of N-methyl-N′-nitro-N-nitrosoguanidine, benzo[a]pyrene and 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (AF-2) (Hirose *et al.,* 1995*c*) and its administration is not effective in preventing dimethylnitrosamine enhancement of hepatocarcinogenesis after initiation with diethylnitrosamine (DEN) (Hirose *et al.,* 1995*b*). These findings suggest that the preventive effects of HTHQ might be specific for certain chemicals such as HCAs.

3-*O*-ethylascorbic acid (EAsA) and 3-*O*-dodecylcarbomethylascorbic acid (DAsA), novel water soluble and lipophilic ascorbic acid derivatives, respectively, both have strong antioxidant activity with a low reducing potential (Nihro *et al.,* 1991). EAsA reduces mammary tumor induction by 7,12-dimethylbenz[a]anthracene (DMBA) (data not shown), while DAsA exerts chemopreventive effects in the liver when given together with Glu-P-1 (Hirose *et al.,* 1993*a,* 1995*b*) but not the mammary gland in PhIP-treated rats (Hirose *et al.,* 1995*a*).

n-Heptadecane-8,10-dione (HDD) is a new β-diketone type antioxidant, structurally similar to n-tritriacontane-16,18-dione (TTAD), extracted from the leaf wax of *Eucalyptus* (Osawa and Namiki, 1981). TTAD has been shown to inhibit pancreatic and hepatocarcinogenesis in a multiorgan carcinogenesis model in the post-initiation period (Hirose *et al.,* 1991), although not during the initiation period (Takaba *et al.,* 1997). However, accumulation of crystal deposits due to ingestion of TTAD occurs in the intestinal mucosa (Hirose *et al.,* 1991). In order to overcome this problem, the chemical structure of TTAD was modified to produce the HDD, which can be easily absorbed in the intestine. Its chemopreventive potential has hitherto remained unclear.

Phenylethyl isothiocyanate (PEITC), a primary product of thioglucosidase-catalyzed hydrolysis of gluconasturtiin, is a naturally occurring compound found in certain cruciferous vegetables (VanEtten *et al.,* 1976). It is reported to decrease DMBA induction of mammary tumors in female Sprague-Dawley rats (Wattenberg, 1977) and forestomach and pulmonary adenomas in female ICR/Ha mice (Wattenberg, 1977), N-nitrosobenzylmethylamine (NMBA)-induced esophageal carcinogenesis in male F344 rats (Siglin *et al.,* 1995; Stoner *et al.,* 1991; Wilkinson *et al.,* 1995) and N-nitrosobis(2-oxopropyl)amine-induced lung and pancreatic tumors in hamsters (Nishikawa *et al.,* 1996). Inhibiting effects of PEITC on lung tumorigenicity and DNA adduct formation in the 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-treated F344 rat and A/J mouse have also been demonstrated (Morse *et al.,* 1989*a,b*). An earlier experiment in our laboratory has shown that simultaneous treat-

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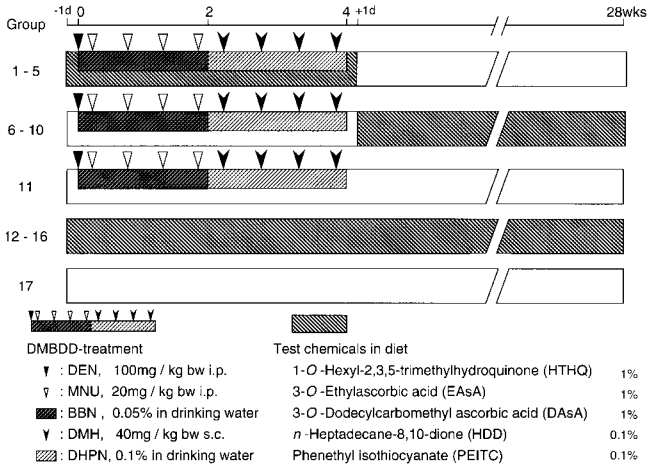

FIGURE 1 – Experimental protocol of the multiorgan carcinogenesis bioassay. The initiation treatments were performed within the first 4 weeks. Test chemicals were fed during the initiation period (groups 1–5) or post-initiation (groups 6–10). Group 11 was the initiation control and groups 12–16 were the non-initiation test chemical control.

ment with PEITC inhibited the development of GST-P-positive foci in Glu-P-1-treated rats but that it enhanced foci in the post-initiation stage in a medium-term liver bioassay system after DEN initiation (Hirose *et al.*, 1995*b*). PEITC increases the multiplicity of DMBA-induced mammary tumors (Lubet *et al.*, 1997).

It is well known that modifying effects of chemicals may differ with the organ and the timing of administration (Ito and Hirose, 1989), so that systematic analysis is required before chemopreventive agents can be considered for application in humans. Effects of these antioxidants on carcinogenesis in the post-initiation period have not been studied systemically. In the present study, the effects of HTHQ, EAsA, DAsA and HDD in the initiation as well as post-initiation stages have been investigated using PEITC as a positive control for chemoprevention by a medium-term multiorgan carcinogenesis bioassay in rats, utilizing a wide spectrum initiation protocol (Ito *et al.*, 1991).

MATERIALS AND METHODS

Animals

Male F344 rats, aged 5 weeks, were purchased from Charles River (Kanagawa, Japan). They were housed 5 to a plastic cage with wood chips and maintained in an air-conditioned room at 22 ± 2°C with a 12 hr light/12 hr dark cycle. Powdered semisynthetic basal diet (Oriental MF, Oriental Yeast, Tokyo, Japan) and tap water were made available *ad libitum.*

Chemicals

DEN (purity > 99%), N,N′-dimethylhydrazine (DMH; purity > 98%), N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN; purity > 97%) and PEITC (purity > 98%) were purchased from Tokyo Kasei Kogyo (Japan), N-methylnitrosourea (MNU; purity about 50% in water) from Sigma-Aldrich (Tokyo, Japan) and 2,2′-dihydroxy-di-n-propylnitrosamine (DHPN; purity 85–90%) from Nakarai (Osaka, Japan). HTHQ (purity > 99.9%), EAsA (purity > 97%) and DAsA (purity > 97%) were synthesized at Dainippon Ink (Tokyo, Japan). HDD (purity > 98%) was a generous gift from Eisai (Tsukuba, Japan).

Treatment

As shown in Figure 1, at the age of 6 weeks, 15 animals each in groups 1–11 received the DMBDD initiation treatment (Hasegawa *et al.*, 1992), composed of a single intraperitoneal (i.p.) injection of 100 mg/kg body weight (bw) DEN, 4 i.p. injections of 20 mg/kg bw MNU and 4 subcutaneous (s.c.) injections of 40 mg/kg bw DMH within 4 weeks, together with 0.05% BBN in the drinking water during the first 2 weeks and 0.1% DHPN in the water over the next 2 weeks. HTHQ, EAsA, DAsA and HDD, as well as PEITC, were given in the diet at concentrations of 1%, 1%, 1%, 0.1% and 0.1%, respectively. Diets were prepared every 2 weeks using a mixer. HTHQ, EAsA and DAsA are stable at room temperature, and PEITC is reported to be stable for at least 10 days in a refrigerator at 4°C (Morse *et al.*, 1989*b*). PEITC and HDD were kept at 4°C and other diets were stored at room temperature. All diets were changed twice a week throughout the experiment. For the simultaneous administration groups (1–5), the compounds were fed from 1 day before the start of the 4-week initiation schedule until 1 day after the completion of initiation. For the post-initiation treatment groups (6–10), exposure to test compounds started 1 day after completion of the initiation schedule and continued until the sacrifice. Animals in group 11 were treated with DMBDD alone as

TABLE I – BODY AND RELATIVE ORGAN WEIGHTS AT THE END OF EXPERIMENT

Group[1]	Treatment	Number of rats	Body weight (g)	Liver (% of bw)	Kidney (% of bw)
	DMBDD plus				
1	HTHQ	14	341.3 ± 16.2	2.6 ± 0.1	0.30 ± 0.02
2	EAsA	15	332.6 ± 23.8	2.7 ± 0.1	0.32 ± 0.03
3	DAsA	14	325.1 ± 37.0	2.7 ± 0.3	0.35 ± 0.17
4	HDD	14	341.0 ± 14.4	2.8 ± 0.2**	0.31 ± 0.02
5	PEITC	13	368.7 ± 21.4***	2.8 ± 0.1*	0.29 ± 0.01
	DMBDD →				
6	HTHQ	14	307.1 ± 19.4**	3.0 ± 0.2***	0.34 ± 0.01**
7	EAsA	15	320.6 ± 16.9	2.6 ± 0.1	0.32 ± 0.04
8	DAsA	15	318.2 ± 15.0	2.9 ± 0.1**	0.31 ± 0.02
9	HDD	14	333.8 ± 27.6	2.8 ± 0.2**	0.32 ± 0.04
10	PEITC	14	317.0 ± 33.1	3.2 ± 0.5***	0.35 ± 0.07
11	DMBDD alone	15	329.6 ± 17.3	2.6 ± 0.3	0.31 ± 0.05
	Without DMBDD				
12	HTHQ	10	353.0 ± 11.1***	2.9 ± 0.1***	0.31 ± 0.01***
13	EAsA	8	376.9 ± 23.4	2.9 ± 0.2**	0.29 ± 0.02
14	DAsA	10	374.7 ± 15.2	2.6 ± 0.1	0.28 ± 0.01
15	HDD	10	397.2 ± 21.0	2.7 ± 0.1*	0.28 ± 0.01
16	PEITC	10	375.7 ± 12.4	3.0 ± 0.1***	0.29 ± 0.01*
17	Non-treatment	10	387.1 ± 21.6	2.6 ± 0.1	0.28 ± 0.01

[1]In groups 1–5 and 6–10, test chemicals were given during the initiation period and after the initiation period, respectively. Significant differences: groups 1–10 *vs.* 11 or 12–16 *vs.* 17: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

TABLE II – INCIDENCE (%) OF THE HISTOPATHOLOGICAL FINDINGS FOR THE DIGESTIVE ORGANS, URINARY BLADDER AND THYROID[1]

Organs and lesions	Group 1 HTHQ (n = 14)	2 EAsA (n = 15)	3 DAsA (n = 14)	4 HDD (n = 15)	5 PEITC (n = 13)	6 HTHQ (n = 14)	7 EAsA (n = 15)	8 DAsA (n = 15)	9 HDD (n = 15)	10 PEITC (n = 13)	11 DMBDD alone (n = 15)
	DMBDD plus					DMBDD →					
Tongue											
Hyperplasia	0	0	0	7	14	29*	0	0	0	0	0
Papilloma	0	0	0	0	0	29*	0	0	0	8	0
SCC	0	0	0	0	0	0	0	0	0	8	0
Esophagus											
Hyperplasia	14*	47	36	67	15*	50	60	67	50	54	67
Papilloma	0	7	0	0	0	0	7	0	0	0	0
Forestomach											
Simple hyperplasia	35	13	57	33	8	93**	33	13	21	54	33
PN-hyperplasia	0	0	29	7	0	50*	0	7	0	0	7
Papilloma	0	0	7	0	0	36*	0	7	0	0	0
SCC	0	0	0	0	0	14	0	0	0	0	0
Small intestine											
Adenoma	14	7	21	29	31	0*	13	20	14	50	33
Carcinoma	14	7	14	21	23	0	0	20	14	14	7
Large intestine											
Adenoma	43	33	29	29	54	0*	47	13	14	7	27
Carcinoma	0	40	7	43*	0	7	13	0	7	0	7
Urinary bladder											
Simple hyperplasia	57	53	47	50	21	29	73	13	36	100**	40
PN-hyperplasia	14	7	20	29	7	36	47	7	7	100***	13
Papilloma	21	7	0	7	0	0	7	0	0	33	7
Carcinoma	14	7	0	0	0	0	0	0	0	33	7
Thyroid											
Hyperplasia	23	7	14	43**	15	15	7	21	14	23	0
Adenoma	0	0	7	7	8	0	0	0	7	15	0
Carcinoma	0	0	0	21	8	8	0	0	21	8	7

[1]n = number of rats. SCC = squamous cell carcinoma. Significantly different from group 11: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

a carcinogen control. Six further groups of 10 rats each were fed HTHQ, EAsA, DAsA, HDD, PEITC or basal diet at the same concentrations as for groups 1–10 without DMBDD treatment. Animals which died during the experiment or were sacrificed upon becoming moribund were autopsied. All surviving rats were killed under ether anesthesia at the end of week 28 and complete autopsies were performed. All those which were alive at the 19-week time point were included in the effective numbers. Formalin-fixed and paraffin-embedded sections of tongue, stomach, small intestine, large intestine, liver, lungs, kidneys, urinary bladder and thyroid glands were prepared routinely and stained with hematoxylin-eosin (H&E) for histological analysis. In addition, 3 slices of each liver were fixed in ice-cold acetone and immunohistochemically stained for evaluation of GST-P-positive foci using an image analyzer (Olympus VIP-21). The Student's *t*-test and Fisher's exact probability test were used for statistical analysis of the data.

TABLE III – QUANTITATIVE DATA FOR GST-P-POSITIVE FOCI IN THE LIVER

Group	Treatment	Number of rats	GST-P-positive foci Number (number/cm²)	GST-P-positive foci Area (mm²/cm²)
	DMBDD plus			
1	HTHQ	14	16.8 ± 6.6*	1.6 ± 1.1
2	EAsA	15	13.3 ± 3.8	1.2 ± 0.5
3	DAsA	14	13.3 ± 4.2	1.0 ± 0.4
4	HDD	14	15.8 ± 7.1	1.4 ± 0.6*
5	PEITC	13	12.1 ± 2.0	0.7 ± 0.2***
	DMBDD →			
6	HTHQ	14	13.5 ± 4.0	1.2 ± 0.4
7	EAsA	15	15.2 ± 3.4**	1.3 ± 0.3**
8	DAsA	15	12.5 ± 3.4	1.2 ± 0.4
9	HDD	14	12.2 ± 3.3	1.0 ± 0.3
10	PEITC	14	16.9 ± 4.2***	1.5 ± 0.4***
11	DMBDD alone	15	11.8 ± 2.5	1.0 ± 0.2

Significantly different from group 11: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

RESULTS

Final average body and relative liver and kidney weights are shown in Table I. Increases in body weight of rats given PEITC during the initiation period and decreases in those given HTHQ in the post-initiation period and without initiation were observed. The relative liver weights were increased in some groups treated with these chemicals during initiation, in the post-initiation period or without initiation. The kidney weights were increased in rats given HTHQ in the post-initiation period or without initiation, and in the PEITC-treated rats without initiation.

Quantitative data for tumors and preneoplastic lesions in the DMBDD-treated groups are summarized in Tables II–V. In the esophagus (Table II), the incidence of hyperplasia was reduced in groups 1 and 5. Significant increase in the incidences of hyperplasia and papilloma in the tongue and hyperplasias and papillomas in the forestomach were observed in group 6 (Table II). In the small and large intestines (Table II), significant reduction in the tumor incidence was observed in group 6. In group 4, the incidence of large intestinal adenocarcinomas was increased. In the liver (Table III), numbers of GST-P-positive foci were increased in groups 1, 7 and 10, and areas of foci were increased in groups 4, 7 and 10, but decreased in group 5. Liver tumors were not found in any of the groups. The numbers of lung hyperplasias were reduced in groups 2, 3, 5, 6, 7, 8, 9 and 10, but the incidences of adenomas and carcinomas did not significantly differ (Table IV). In the kidney (Table V), the numbers and/or incidence of nephroblastomas were reduced in groups 1, 4, 5 and 7, and the number of atypical tubules was decreased in group 5. Adenomas were not found in this group. In the urinary bladder (Table II), the incidences of papillary or nodular (PN)-hyperplasias and tumors were also increased in group 10. The incidence of hyperplasias in thyroid glands was slightly

TABLE IV – HISTOPATHOLOGICAL FINDINGS FOR THE LUNG

Group	Treatment	Number of rats	Hyperplasia (%)		Tumor incidence (%)	
			Incidence	Number/rat	Adenoma	Carcinoma
	DMBDD plus					
1	HTHQ	13	13 (100)	23.9 ± 4.8	2 (15)	0
2	EAsA	15	15 (100)	16.2 ± 3.8***	0	0
3	DAsA	15	15 (100)	19.1 ± 5.5***	3 (20)	0
4	HDD	14	14 (100)	27.1 ± 5.9	1 (7)	0
5	PEITC	13	13 (100)	8.9 ± 3.0***	0	0
	DMBDD →					
6	HTHQ	13	13 (100)	19.9 ± 6.1**	1 (8)	0
7	EAsA	14	14 (100)	22.0 ± 6.0*	1 (7)	0
8	DAsA	14	14 (100)	20.5 ± 6.0**	2 (14)	0
9	HDD	14	14 (100)	18.6 ± 6.1***	1 (7)	0
10	PEITC	13	13 (100)	22.7 ± 5.2*	3 (23)	1 (8)
11	DMBDD alone	15	15 (100)	26.9 ± 4.4	0	1 (7)

Significantly different from group 11: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

TABLE V – HISTOPATHOLOGICAL FINDINGS FOR THE KIDNEYS

Group	Treatment	Number of rats	Atypical tubules		Adenoma		Nephroblastoma	
			Incidence	Number/rat	Incidence	Number/rat	Incidence	Number/rat
	DMBDD plus							
1	HTHQ	15	14 (93)	5.1 ± 3.5	3 (20)	0.2 ± 0.4	6 (40)*	0.6 ± 0.7*
2	EAsA	15	15 (100)	4.4 ± 2.3	3 (20)	0.2 ± 0.4	9 (60)	0.7 ± 0.7
3	DAsA	14	14 (100)	5.3 ± 2.9	3 (21)	0.2 ± 0.4	11 (79)	0.9 ± 0.5
4	HDD	14	14 (100)	4.9 ± 2.5	4 (29)	0.4 ± 0.6	6 (43)*	0.6 ± 0.8
5	PEITC	13	12 (92)	1.5 ± 1.0***	0	0	4 (31)**	0.5 ± 0.8*
	DMBDD →							
6	HTHQ	15	15 (100)	5.7 ± 2.0	1 (7)	0.1 ± 0.2	12 (80)	1.1 ± 0.7
7	EAsA	15	15 (100)	4.3 ± 2.3	3 (20)	0.3 ± 0.7	6 (40)*	0.5 ± 0.6*
8	DAsA	15	15 (100)	4.7 ± 2.1	1 (7)	0.1 ± 0.5	12 (80)	0.9 ± 0.6
9	HDD	14	14 (100)	5.3 ± 2.3	3 (21)	0.3 ± 0.6	9 (64)	0.7 ± 0.6
10	PEITC	13	13 (100)	4.9 ± 2.5	3 (23)	0.4 ± 0.7	9 (69)	0.8 ± 0.6
11	DMBDD alone	15	15 (100)	4.7 ± 2.3	2 (13)	0.1 ± 0.3	13 (87)	1.2 ± 0.8

Significantly different from group 11: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

TABLE VI – SUMMARY OF THE EFFECTS OF THE POTENTIAL CHEMOPREVENTIVE AGENTS ON VARIOUS ORGANS[1]

Group	Treatment	Tongue	Esophagus	Forestomach	Small intestine	Large intestine	Lung	Kidney		Urinary bladder	Thyroid	Liver
								Epithelial	Nephroblastoma			
	DMBDD plus											
1	HTHQ	—	▼	—	—	—	—	—	▼	—	—	△
2	EAsA	—	—	—	—	—	▼	—	—	—	—	—
3	DAsA	—	—	—	—	—	▼	—	—	—	—	—
4	HDD	—	—	—	—	△	—	—	▼	—	△	△
5	PEITC	—	▼	—	—	—	—	▼	▼	—	—	▼
	DMBDD →											
6	HTHQ	△	—	△	▼	▼	▼	—	—	—	—	—
7	EAsA	—	—	—	—	—	▼	—	▼	—	—	△
8	DAsA	—	—	—	—	—	▼	—	—	—	—	—
9	HDD	—	—	—	—	—	▼	—	—	—	—	—
10	PEITC	—	—	—	—	—	▼	—	—	△	—	△

[1]▼ = inhibitory effect; △ = enhancing effect; — = no effect.

increased in group 4 (Table II). The results for groups 1–10 are summarized in Table VI. In the groups fed each test chemical alone without DMBDD treatment, there was no significant induction of lesions, except forestomach simple hyperplasia (9/9; $p < 0.001$, compared with non-treated control group) in the HTHQ-treated group and urinary bladder simple hyperplasia (10/10; $p < 0.001$) and PN-hyperplasia (6/10; $p < 0.05$) in the PEITC-treated group.

There were no pathological findings which could account for decrease in body weight or increase in liver and kidney weight in the non-initiation groups.

DISCUSSION

The identification of chemopreventive compounds is presently given a high priority. Since many antioxidants act as radical scavengers, phase I or II enzyme inducers or inhibitors, cell cycle mediators or protein kinase inhibitors, we have concentrated our attention on this group of compounds as possible chemopreventive agents and found great efficacy, especially for HTHQ (Hirose *et al.*, 1993*a*, 1995*a–c*). In the present study, positive effects of 4 antioxidants and PEITC were observed when administered during the initiation or post-initiation periods in a medium-term multiorgan carcinogenesis model, but as in earlier studies, adverse influence was also noted for most of the compounds.

In our previous study, HTHQ showed a very strong chemopreventive action on Glu-P-1 or MeIQx-induced rat hepatocarcinogenesis (Hirose *et al.*, 1995*b*) and PhIP-induced rat mammary gland carcinogenesis when given simultaneously (Hirose *et al.*, 1995*a*). It is considered a primary candidate for chemoprevention of HCA-induced carcinogenesis. At a dietary dose of 1%, it decreases the

size of DMBA-initiated rat mammary carcinomas when given in the post-initiation stage (data not shown), and in the present experiments, it inhibited the development of small and large intestinal tumors after initiation. It also slightly depressed initiation of esophageal carcinogenesis. On the other hand, HTHQ itself induced simple hyperplasia in the forestomach with a high incidence, and slightly enhanced forestomach and tongue carcinogenesis with administration during the post-initiation period. The enhancing effect of HTHQ on forestomach carcinogenesis appears to be much weaker than that of the phenolic antioxidant butylated hydroxyanisole (BHA) and comparable to that of *tert*-butylhydroquinone (TBHQ) (Hirose *et al.,* 1993*b*). BHA was found to enhance urinary bladder carcinogenesis (Imaida *et al.,* 1983) and butylated hydroxytoluene (BHT) to promote urinary bladder (Imaida *et al.,* 1983), esophageal (Fukushima *et al.,* 1987) and thyroid carcinogenesis (Hirose *et al.,* 1993*b*). TBHQ also enhances forestomach and esophageal carcinogenesis (Hirose *et al.,* 1993*b*) when high doses are given. Anti-mutagenic activity of HTHQ against Glu-P-1-induced mutagenesis using the Ames assay in the presence of an S9 mixture and antioxidative effects of HTHQ in a liver microsome Fe^{3+}/ADP system were both found to be highest among the phenolic antioxidants BHA, BHT, TBHQ and propyl gallate (Hirose *et al.,* 1997). Considering that humans do not possess a forestomach, HTHQ may still have the greatest potential of the phenolic antioxidants for chemopreventive application, particularly against HCAs. A dose-response study (0.125–1%) of HTHQ in male rats initiated with ethylnitrosourethane showed enhancing effects on forestomach carcinogenesis at the 0.25% dose, but findings for the tongue were equivocal (data not shown).

In the present study, EAsA was only effective at inhibiting induction of nephroblastomas, an uncommon tumor mostly found in childhood in humans. EAsA increased the number and area of preneoplastic GST-P-positive foci, but not tumors, indicating that it may weakly enhance hepatocarcinogenesis in the post-initiation stage. DAsA was without effect in the present experiment. Therefore, of the 2 ascorbic acid derivatives tested, only EAsA might have any chemopreventive effect.

In many of the groups treated with antioxidants or PEITC either during or after initiation, reduction in the number of lung hyperplasias was apparent but the incidence of lung tumors was not reduced except with EAsA or PEITC treatment during the initiation period.

Weak enhancement of colon, thyroid and liver carcinogenesis was found in rats treated with HDD during the initiation period. A similar β-diketone derivative, TTAD, and curcumin did not show any modifying effects when given during multiple initiation with 3,2′-dimethyl-4-aminobiphenyl, N-ethyl-N-hydroxyethylnitrosamine and DHPN (Takaba *et al.,* 1997); in addition, TTAD forms crystal deposits in the intestinal mucosa (Hirose *et al.,* 1991). Although curcumin was found to reduce the incidence of benzo(a)pyrene-induced forestomach carcinomas and azoxymethane-induced colon adenocarcinomas in initiation and post-initiation stages (Huang *et al.,* 1994), it might not be practical to use TTAD or HDD as chemopreventive agents.

PEITC inhibited carcinogenesis in the esophagus, lung, kidney, liver and pancreas during the initiation period, in line with previous results using nitrosamines such as 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (Morse *et al.,* 1989*b*), N-nitrosomethylbenzylamine (Siglin *et al.,* 1995; Stoner *et al.,* 1991) and *N*-nitrosobis(2-oxopropyl)amine (Nishikawa *et al.,* 1996).

The possible mechanisms of inhibition by PEITC include interference with metabolic activation (Staretz and Hecht, 1995), induction of phase II enzymes (Zhang and Talalay, 1994), activation of JNK1 which can phosphorylate various transcription factors including phase II detoxifying enzyme genes (Yu *et al.,* 1996) or influence DNA alkylation (Morse *et al.,* 1989*a*,*b;* Wilkinson *et al.,* 1995). However, administration of PEITC in the post-initiation period enhances urinary bladder and hepatocarcinogenesis and PEITC causes high incidences of simple and PN-hyperplasia in the urinary bladder, even without initiation, as shown in the present study and as reported previously by Lubet *et al.* (1997). In a long-term carcinogenicity study, allyl isothiocyanate was also shown to induce hyperplasia and papillomas in the urinary bladder (Dunnick *et al.,* 1982). PEITC (Hirose *et al.,* 1995*b*) and benzyl isothiocyanate (data not shown) at a dose of 0.1% were also found to enhance hepatocarcinogenesis in a medium-term liver bioassay. Another isothiocyanate, 6-phenylhexyl isothiocyanate, enhances N-methylbenzyl nitrosamine-induced esophageal (Stoner *et al.,* 1995) and azoxymethane-induced colon (Rao *et al.,* 1995) carcinogenesis. Further studies are required for evaluation of the human hazard of isothiocyanates.

In conclusion, 5 chemicals which have been reported to be chemopreventors demonstrated different spectra in terms of modifying potential and/or organ specificity as summarized in Table VI. Our data further confirmed that for the overall evaluation of chemopreventive compounds, consideration of all organs and treatment timing are critical.

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