JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Chemopreventive Effects of 4-Methylthio-3-butenyl Isothiocyanate (Raphasatin) but Not Curcumin against Pancreatic Carcinogenesis in Hamsters

Toshiya Okamura,[†] Takashi Umemura,[†] Tomoki Inoue,[†] Masako Tasaki,[†] Yuji Ishii,[†] Yasushi Nakamura,[§] Eun Young Park,[§] Kenji Sato,[§] Tomoaki Matsuo,[#] Shigehisa Okamoto,[⊥] Akiyoshi Nishikawa,*[,‡] and Kumiko Ogawa[†]

[†]Division of Pathology and [‡]Biological Safety Research Center, National Institute of Health Sciences, Tokyo 158-8501, Japan

[§]Department of Food Sciences and Nutritional Health, Kyoto Prefectural University, Kyoto 606-8522, Japan

[#]Department of Biochemical Science and Technology and [⊥]Department of Agricultural Science, Kagoshima University, Kagoshima 890-0065, Japan

ABSTRACT: The modifying effects of 4-methylthio-3-butenyl isothiocyanate (MTBITC) and curcumin were investigated in *N*-nitrosobis(2-oxopropyl)amine (BOP)-initiated hamsters. Male 6-week-old Syrian hamsters were subcutaneously injected with 10 mg/kg body weight (b.w.) of BOP four times a week, and fed a diet supplemented with 80 mg/kg diet of MTBITC, equivalent to 4.6 mg/kg b.w./day for the initiation stage or 3.8 mg/kg b.w./day for the postinitiation stage administration, respectively, or 2000 mg/kg diet of curcumin, equivalent to 118.8 mg/kg b.w./day for the initiation stage or 100.8 mg/kg b.w./day for the postinitiation stage administration, respectively. The incidence of combined pancreatic lesions, including atypical hyperplasias and adenocarcinomas, was significantly decreased to 55% ($P < 0.05$) by the 80 mg/kg diet MTBITC given during the initiation stage as compared to the BOP alone group (85%) but not by the curcumin administration at 16 weeks after the BOP-treatment. In the second study, the multiplicity of combined pancreatic lesions was also significantly decreased to 0.50 ± 0.51 ($P < 0.05$) by 700 mg/kg diet MTBITC given in the initiation stage (equivalent to 59.0 mg/kg b.w./day) as compared to the BOP alone group (1.10 ± 1.02). Our results indicate that the naturally occurring isothiocyanate MTBITC may exert preventive effects against BOP-initiation of hamster pancreatic carcinogenesis.

KEYWORDS: *daikon, isothiocyanate, chemoprevention, pancreatic cancer*

INTRODUCTION

In many countries of the world, pancreatic cancer rates are steadily increasing with 5-year survival values ranging from only 5% to 36%.[1−3] Although a number of approaches have been used to treat pancreatic cancers, median survival after diagnosis is roughly ranging between 12 and 40 months.[2,4] Unfortunately, development of pancreatic cancers is clinically silent in general so that at the time of diagnosis, the vast majority of cases are incurable with a very poor prognosis. Therefore, effective new preventive approaches against this aggressive disease are urgently required.

It has been reported that some food constituents such as phenethyl isothiocyanate (PEITC) included in cruciferous vegetables efficiently exert chemopreventive effects against hamster pancreatic carcinogenesis induced by *N*-nitrosobis(2-oxopropyl)amine (BOP).[5] In addition, we have found that 4-methylthio-3-butenyl isothiocyanate (MTBITC; raphasatin), a constituent extracted from daikon (Japanese white radish), extremely familiar as an heirloom vegetable in Japan, exerts antimutagenic activity.[6,7] Previously, we also reported non-steroidal anti-inflammatory drugs including a cyclooxygenase (COX)-2 inhibitor, nimesulide, suppress BOP-induced pancreatic carcinogenesis in hamsters.[5] Curcumin is also known to be a COX-2 inhibitor and therefore might be expected to have both cancer preventive and therapeutic potential.[8,9] It remains unknown as to whether MTBIC inhibits COX-2, although some other isothiocyanates have been shown to be COX-2 inhibitors.[10−13]

BOP is known to induce pancreatic, lung, liver and kidney tumors in hamsters.[5,10] It is well documented that the hamster model has particular advantages for assessing modification effects of chemicals on pancreatic carcinogenicity because of the histological and biological similarities of the induced lesions to those observed in man.[5,10] The present experiment was performed to elucidate the effects of MTBITC and curcumin during the initiation and postinitiation stages in the BOP-induced carcinogenesis model in hamsters.

MATERIALS AND METHODS

Chemicals. BOP and curcumin (purity was indicated as 70%) were obtained from Nacalai Tesque (Kyoto, Japan) and Sigma-Aldrich (St. Louis, MO, USA), respectively. MTBITC was extracted and purified from the root of the heirloom varieties (Momoyama and Karami) of daikon (*Raphanus sativus*) according to the method described previously.[6] MTBITC purified from daikon was analyzed with gas chromatography-electron ionization-mass spectrometry (GC-EIMS). GC-EIMS showed two peaks on total ion chromatograph (94:6 in

Received: April 19, 2012
Revised: January 15, 2013
Accepted: February 7, 2013
Published: February 7, 2013

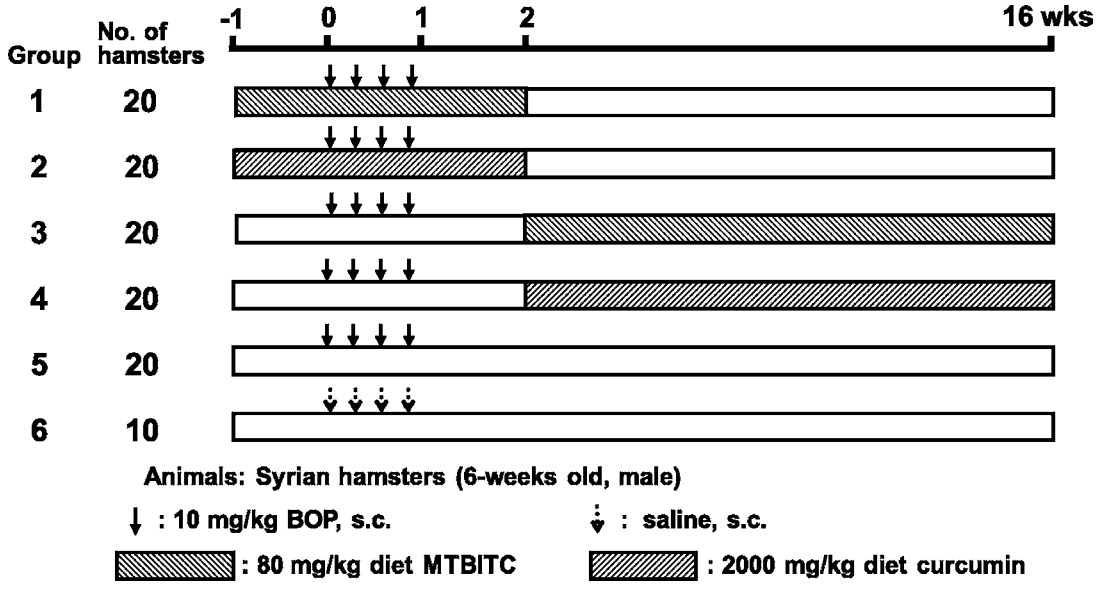

Figure 1. Experimental design for experiment 1. Male Syrian hamsters were given BOP (groups 1−5) or saline (group 6) subcutaneously 4 times within one week. The animals at the groups 1 and 2 were given 80 mg/kg diet MTBITC or 2000 mg/kg diet curcumin for 3 weeks, from 1 week before the BOP-treatment. Groups 3 and 4 were given 80 mg/kg diet MTBITC or 2000 mg/kg diet curcumin for 14 weeks, from 1 week after the end of BOP-treatment. Groups 5 and 6 were given basal diet throughout the experimental period as positive or negative control, respectively. All surviving animals were sacrificed 16 weeks after the start of BOP treatment.

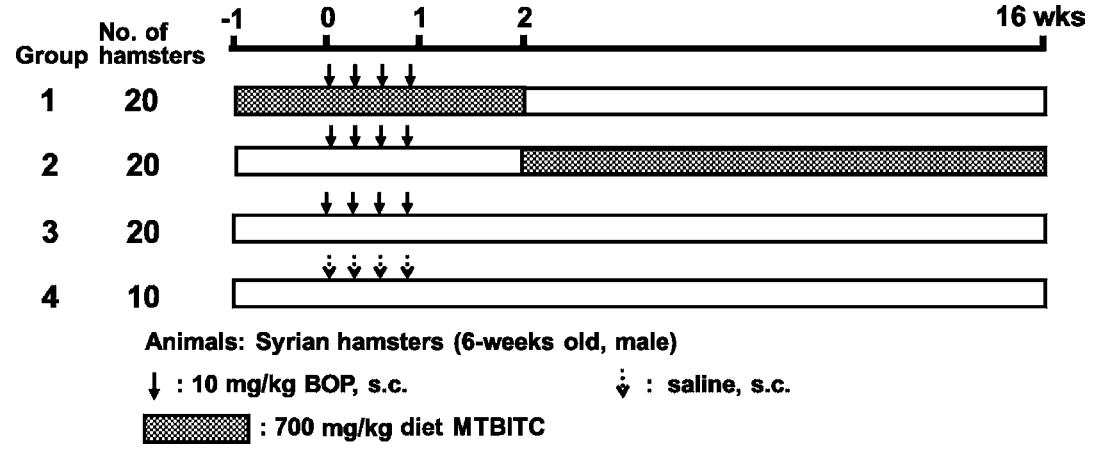

Figure 2. Experimental design for sxperiment 2. Male Syrian hamsters were given BOP (groups 1−3) or saline (group 4) subcutaneously 4 times within one week. The animals in group 1 were given 700 mg/kg diet MTBITC for 3 weeks, from 1 week before the BOP-treatment. Group 2 was given 700 mg/kg diet MTBITC for 14 weeks, from 1 week after the end of BOP-treatment. Groups 3 and 4 were given basal diet throughout the experimental period as positive or negative control, respectively. All surviving animals were sacrificed 16 weeks after the start of BOP treatment.

peak area) with same proportion of fragment ions: 159 $[M]^+$ (22), 87 (95), 72 (25), 59 (18), 53 (23), 47 (21), 45 (100). Those major and minor peaks were geometrical isomers *trans*- and *cis*-MTBITC, respectively. The purity of the MTBITC was 98.0% as estimated by HPLC (Shimadzu, Kyoto, Japan) model LC-20AT with a YMC ODS-H80 (⌀ 4.6 × 250 mm) column using 55% acetonitrile in 0.1% trifluoroacetate mobile phase, SPD-20A UV detector, and a C-R8A integrator. The UV absorbance at 254 nm was used to detect total (trans- and cis-) MTBITC at 5.3 min.

Animals. A total of 180 male Syrian hamsters (Japan SLC, Inc., Shizuoka, Japan), 5 weeks old and weighing about 70 g at the commencement, were used in the present experiment. The animals were housed, five per polycarbonate cage, in an air-conditioned room at 23 ± 2 °C and 60 ± 5% humidity under a daily cycle of alternating 12-h periods of light and darkness. A standard basal diet Oriental MF (Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water were available ad libitum.

Experimental Protocol. The protocol for this study was approved by the Animal Care and Utilization Committee of the National Institute of Health Sciences (Tokyo).

In experiment 1, as shown in Figure 1, groups 1−5 each consisting of 20 hamsters (6 weeks old) were given BOP subcutaneously 4 times within one week at a dose of 10 mg/kg body wt dissolved in saline. For the initiation stage, from 1 week before the BOP treatment, the animals were simultaneously given 80 mg/kg diet MTBITC or 2000 mg/kg diet curcumin for 3 weeks, and thereafter fed a basal diet (groups 1 and 2). During the postinitiation treatment, starting 1 week after the end of BOP-treatment, the animals were continuously fed diet supplemented with 80 mg/kg diet MTBITC or 2000 mg/kg diet curcumin for 14 weeks (groups 3 and 4). Groups 5 and 6 each consisting of 20 and 10 animals were fed a basal diet throughout the experiment with BOP initiation or vehicle, respectively. The doses of MTBITC used in the present study were determined with reference to practical daily consumption.[6] The concentration of MTBITC in the various kinds of daikons was reported as a range from 36.7 ± 3.5 to

dx.doi.org/10.1021/jf3003174 | J. Agric. Food Chem. 2013, 61, 2103−2108

Table 1. Final Body Weight and Relative Organ Weights (Experiment 1)

group	treatment: initiation	treatment: postinitiation	no. of animals	body weight (g)	lung (g%)	liver (g%)	kidney (g%)
1	BOP+MTBITC	basal diet	20	156.3 ± 14.7[a]	0.59 ± 0.13	4.75 ± 0.28	0.61 ± 0.04[b]
2	BOP+curcumin	basal diet	20	160.7 ± 16.6	0.60 ± 0.11	4.89 ± 0.42[b]	0.62 ± 0.04[b]
3	BOP	MTBITC	20	164.4 ± 14.9[b]	0.58 ± 0.10	4.77 ± 0.31	0.60 ± 0.04[c]
4	BOP	curcumin	19	166.4 ± 25.0[b]	0.59 ± 0.13	4.81 ± 0.41	0.63 ± 0.07
5	BOP	basal diet	20	150.5 ± 23.5	0.57 ± 0.11	4.62 ± 0.40	0.66 ± 0.06
6	saline	basal diet	10	176.4 ± 25.6[b]	0.54 ± 0.08	4.88 ± 0.43	0.64 ± 0.04

[a]Mean ± SD. [b]$p < 0.05$ vs group 5. [c]$p < 0.01$ vs group 5.

Table 2. Incidence and Multiplicity Data for Pancreatic Atypical Hyperplasias and Adenocarcinomas (Experiment 1)

group	treatment: initiation	treatment: postinitiation	no. of animals	no. of animals with (%): AH[a]	ADC	total	no. of tumors/animal (mean ± SD): AH	ADC	total
1	BOP+MTBITC	basal diet	20	11 (55)	2 (10)	11 (55)[b]	0.95 ± 1.05	0.10 ± 0.31	1.05 ± 1.15
2	BOP+curcumin	basal diet	20	12 (60)	3 (15)	13 (65)	1.55 ± 1.88	0.15 ± 0.37	1.70 ± 1.92
3	BOP	MTBITC	20	16 (80)	2 (10)	16 (80)	1.50 ± 1.10	0.10 ± 0.31	1.60 ± 1.27
4	BOP	curcumin	19	12 (63)	3 (16)	13 (68)	0.95 ± 0.91	0.16 ± 0.37	1.11 ± 0.99
5	BOP	basal diet	20	16 (80)	1 (5)	17 (85)	1.55 ± 1.39	0.05 ± 0.22	1.60 ± 1.35
6	saline	basal diet	10	0	0	0			

[a]AH; atypical hyperplasia, ADC; adenocarcinoma. [b]$p < 0.05$ vs group 5.

421 ± 27.1 micro mol/100 g.[6] According to a calculation from the food consumption data in this experiment, 80 mg/kg diet of MTBITC diet in hamster was equivalent to 300 g of daikon per day in human. Food was prepared once a week and kept in 4 °C refrigerator and the contents of feeding jar were exchanged every 3−4 days. In experiment 2, as shown in Figure 2, groups 1−3, each consisting of 20 hamsters, were given BOP subcutaneously 4 times within one week at a dose of 10 mg/kg body wt. For the initiation stage, the animals were simultaneously given 700 mg/kg diet MTBITC for 3 weeks overlapping with the 1-week BOP treatment, and thereafter fed a basal diet (group 1). During the postinitiation treatment, starting 1 week after the end of BOP-treatment, the animals were continuously fed diet supplemented with 700 mg/kg diet MTBITC for 14 weeks (group 2). The doses of MTBITC used in the present study were selected on the basis of previous experimental results where PEITC, another ITC compound, showed preventive effects on pancreatic carcinogenesis in hamsters[11,12] and lung carcinogenesis in rats.[13] The dose 700 mg/kg diet was applied to confirm the chemopreventive effect shown at 80 mg/kg diet, the rationale being based on a little less than 10 times. Groups 3 and 4 consisting of 20 and 10 animals, respectively, were fed a basal diet alone with and without prior BOP initiation.

The hamsters were observed daily and weighed once every week. Food consumption was measured weekly. At the end of week 16, all surviving animals were sacrificed and examined histopathologically.

Histological Examination. At autopsy, the pancreas, lung, liver and kidney were carefully examined macroscopically, removed, and fixed in 10% phosphate buffered formalin. Before fixation, the latter three organs were weighed. After routine processing, paraffin embedded tissue sections 3 μm thick were stained with hematoxylin and eosin. For the purpose of thorough detection of pancreatic proliferative lesions, four parts of the pancreas (splenic, duodenal and gastric lobes, and the head portion) were serially sectioned according to our routine method.[5] Neoplastic and preneoplastic lesions were histopathologically diagnosed as adenocarcinomas and atypical hyperplasias and counted in representative sections as standardized in our laboratory.[5]

Statistical Evaluation. Quantitative results were statistically evaluated by analysis of variance (ANOVA) and the Fisher's exact probability test for comparisons between BOP-initiated control and treated groups. Significance was inferred at the less than 5% level.

RESULTS

In experiment 1, one animal in group 4 was found dead without significant lesion and the final body weights were significantly increased in groups 3 (MTBITC postinitiation) and 4 (curcumin postinitiation) as compared to group 5 (BOP alone). In the BOP-treated groups in general, the final body weights tended to be smaller than in the nontreatment (saline) controls (Table 1). Among them, the final body weight in group 5 (BOP alone) was significantly lower ($p < 0.05$) than that in group 6 (saline) and those in groups 3 and 4 (additional MTBITC- or curcumin-treatment in the postinitiation phase) were significantly increased ($p < 0.05$) than those in group 5 (BOP alone). Significant increase in liver weights relative to body weights in group 2 (curcumin in initiation phase) and significant decrease in relative kidney weight in groups 1−3 (MTBITC initiation, curcumin initiation and MTBITC post-initiation, respectively) were observed as compared to group 5 (BOP alone, Table 1). From the data on food consumption, the doses of MTBITC in the initiation stage, MTBITC in the postinitiation stage, curcumin in the initiation stage and Curcumin in the postinitiation stage were estimated as 4.6, 3.8, 118.8, and 100.8 mg/kg b.w./day, respectively. Preneoplastic pancreatic lesions occurred in the splenic and gastric lobes and head portion without any clear skewing in lobe distribution (data not shown), and were histopathologically diagnosed as atypical hyperplasias, consisting of proliferation of atypical ductules accompanied by inflammatory cell infiltration, predominantly lymphocyte in character (Figure 3a). Pancreatic adenocarcinomas were well to moderately differentiated, showing distinct glandular patterns with severely atypical columnar or cuboidal epithelia (Figure 3b). Incidence and multiplicity data for histopathologically diagnosed pancreatic lesions observed in each group of hamsters are summarized in Table 2. The incidence of combined adenocarcinomas/atypical hyperplasias was significantly ($p < 0.05$) reduced in group 1 (MTBITC in initiation phase) as compared to group 5 (BOP alone). The curcumin treatment showed a tendency to reduce the incidence of pancreatic lesions but without statistical

dx.doi.org/10.1021/jf3003174 | J. Agric. Food Chem. 2013, 61, 2103−2108

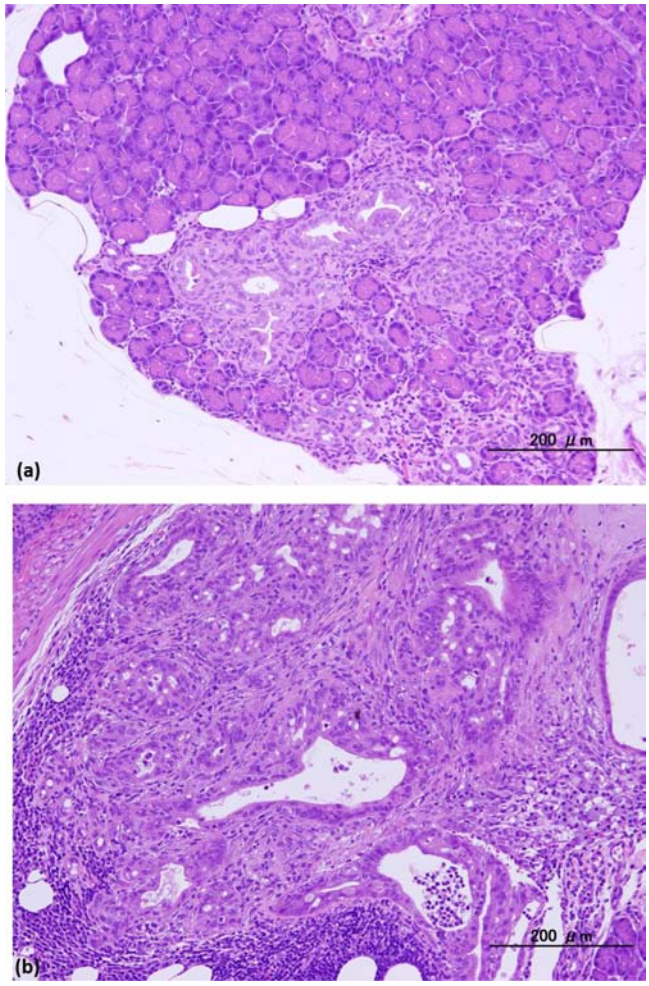

Figure 3. Representative appearance of the pancreatic atypical hyperplasia (a) and adenocarcinoma (b) in the hamster given BOP subcutaneously 4 times within one week at a dose of 10 mg/kg body wt (experiment 2).

significance. BrdU-labeling indices were comparable between BOP-treated groups (data not shown). In the incidence and multiplicity data for proliferative lesions in liver, lung and kidneys, effects of test chemicals were not significant (Table 3).

In experiment 2, the final body weights were significantly reduced in the group 2 (MTBITC in postinitiation phase) as compared to the group 3 (BOP alone, data not shown). Relative lung or kidney weights were significantly decreased in group 1 (MTBITC initiation) as compared to those in group 3 (BOP alone, data not shown). From the data of food consumption, the doses of MTBITC in the initiation stage and MTBITC in the postinitiation stage were estimated as 59.0 and 42.2 mg/kg b.w./day, respectively. Incidence and multiplicity data for histopathologically diagnosed pancreatic lesions observed in each group of hamsters are summarized in Table 4. The incidence of pancreatic atypical hyperplasias was increased in the BOP-treated groups as compared to the nontreatment control group, but there were no significant differences among groups 1−3. However, the multiplicity of atypical hyperplasias or combined adenocarcinomas/atypical hyperplasias was significantly ($p < 0.05$) lower in group 1 (MTBITC initiation) than in group 3 (BOP alone). The incidence and multiplicity of pancreatic atypical hyperplasias or adenocarcinomas also showed a tendency for decrease in group 2 (MTBITC

Table 3. Incidences of Proliferative Lesions in the Liver, Lung, and Kidney (Experiment 1)

group	treatment: initiation	treatment: post-initiation	no. of animals		liver: cholangiocellular AH[a]	liver: cholangiocellular adenoma	liver: cholangiocellular carcinoma	lung: hyperplasia	lung: adenoma	lung: adenocarcinoma	kidney: renal tubule hyperplasia
1	BOP+MTBITC	basal diet	20	incidence (%)	1 (5)	1 (5)	0	3 (15)	2 (10)	0	1 (5)
				no. lesions/animal	0.05 ± 0.22[b]	0.05 ± 0.22		0.15 ± 0.37	0.10 ± 0.31		0.05 ± 0.22
2	BOP+curcumin	basal diet	20	incidence (%)	2 (10)	1 (5)	0	3 (15)	1 (5)	2 (10)	0
				no. lesions/animal	0.10 ± 0.31	0.05 ± 0.22		0.15 ± 0.37	0.05 ± 0.22	0.10 ± 0.31	
3	BOP	MTBITC	20	incidence (%)	2 (10)	1 (5)	0	4 (20)	4 (20)	1 (5)	0
				no. lesions/animal	0.10 ± 0.31	0.05 ± 0.22		0.20 ± 0.41	0.20 ± 0.41	0.05 ± 0.22	
4	BOP	curcumin	19	incidence (%)	0	1 (5)	2 (11)	5 (26)	2 (11)	2 (11)	1 (5)
				no. lesions/animal		0.05 ± 0.23	0.11 ± 0.32	0.26 ± 0.45	0.11 ± 0.32	0.11 ± 0.32	0.05 ± 0.23
5	BOP	basal diet	20	incidence (%)	2 (10)	0	0	5 (25)	2 (10)	0	0
				no. lesions/animal	0.10 ± 0.31			0.25 ± 0.44	0.10 ± 0.31		
6	saline	basal diet	10	incidence (%)	0	0	0	0	0	0	0
				no. lesions/animal							

[a]Atypical hyperplasia. [b]Mean ± SD.

dx.doi.org/10.1021/jf3003174 | J. Agric. Food Chem. 2013, 61, 2103−2108

Table 4. Incidence and Multiplicity Data for Atypical Hyperplasias and Pancreatic Adenocarcinomas (Experiment 2)

	treatment			no. of animals with (%)			no. of tumors/animal (Mean ± SD)		
group	initiation	postinitiation	no. of animals	AH[a]	ADC	total	AH	ADC	total
1	BOP+MTBITC	basal diet	20	9 (45)	1 (5)	10 (50)	0.45 ± 0.51[b]	0.05 ± 0.22	0.50 ± 0.51[b]
2	BOP	MTBITC	20	11 (55)	1 (5)	12 (60)	0.55 ± 0.51	0.05 ± 0.22	0.60 ± 0.50
3	BOP	basal diet	20	13 (65)	2 (10)	14 (70)	1.00 ± 1.03	0.10 ± 0.31	1.10 ± 1.02
4	saline	basal diet	10	0	0	0			

[a]AH, atypical hyperplasia; ADC, adenocarcinoma. [b]$p<0.05$ vs group 3.

Table 5. Incidences of Proliferative Lesions in the Liver, Lung, and Kidneys (Experiment 2)

	treatment				liver		lung		kidney
group	initiation	post-initiation	no. of animals		cholangiocellular atypical hyperplasia	cholangiocellular adenocarcinoma	hyperplasia	adenoma	renal tubule hyperplasia
1	BOP+MTBITC	basal diet	20	incidence (%)	1 (5)	0	6 (30)	0	5 (25)
				no. lesions/animal	0.05 ± 0.22[a]		0.40 ± 0.68		0.25 ± 0.44
2	BOP	MTBITC	20	incidence (%)	3 (15)	0	1 (5)[b]	0	3 (15)
				no. lesions/animal	0.20 ± 0.52		0.10 ± 0.45[b]		0.20 ± 0.52
3	BOP	basal diet	20	incidence (%)	0	1 (5)	8 (40)	1 (5)	2 (10)
				no. lesions/animal		0.05 ± 0.22	0.60 ± 0.99	0.05 ± 0.22	0.10 ± 0.31
4	saline	basal diet	10	incidence (%)	0	0	0	0	0
				no. lesions/animal					

[a]Mean ± SD. [b]$p<0.05$ vs group 3.

postinitiation) compared to the group 3 (BOP alone) value, although this was not statistically significant. As summarized in Table 5, neoplastic and preneoplastic lesions were also observed in the lungs, liver, and kidneys of hamsters receiving BOP. The incidence and multiplicity of lung bronchioloalveolar hyperplasias were significantly ($p < 0.05$) reduced in group 2 (MTBITC postinitiation) as compared to those in group 3 (BOP alone), but there are no significant changes in data for other lesions.

DISCUSSION

In the present study, treatment with MTBITC suppressed the development of pancreatic cancers and/or precancerous lesions especially when applied in the initiation phase. This effect is in good agreement with findings for other isothiocyanates such as PEITC and PBITC.[5,11,12] It is likely that the mechanism underlying anti-initiation effect of MTBITC might be related to its antimutagenic actions.[7,14] In addition, recent reports suggest that some other mechanisms such as inhibition of cell proliferation,[15−17] induction of apoptosis,[18,19] antioxidant activity,[17,18] and induction of detoxification enzymes[20,21] might be involved in the chemoprevention effects of MTBITC.

MTBITC can be extracted with *n*-hexane from many varieties of *Raphanus sativus*, including the roots and sprouts of Japanese white radish, Tunisian radish, and Spanish black radish.[6,7,14,16,17,19,20] Previously, it has been reported that MTBITC has antimicrobial,[22] antimutagenic,[7,14] and anticarcinogenic[7] properties in in vitro studies. Recently, it was also found that MTBITC inhibits cell proliferation[15−17] and induces apoptosis in human cancer cells by modulating genes related to apoptotic pathways.[18,19] In addition to antiproliferative effects on mouse leukemia cells and human colon cancer cells and reduction of micotoxic damage on mouse keratinocyte cells, MTBITC exerts free radical scavenging effects.[17,18] Inhibition of genotoxicity has been reported in vivo as well as in vitro assay systems.[7,14] Similarly to other isothiocyanates such as PEITC, MTBITC might induce xenobiotic metabolizing enzymes (e.g., CYP1A1, CYP1A2, quinone reductase, microsomal epoxide hydrolase, glutathione S-transferase α2, and UGT1A6).[20,21,23] Among them, induction of detoxification enzymes such as glutathione S-transferase and UGT might play significant roles in the suppression of BOP-induced pancreatic carcinogenesis in hamsters.

In contrast, curcumin failed to modulate pancreatic carcinogenesis in hamsters in the present study not only when applied in the initiation stage but also postinitiation. It is well documented that curcumin exerts significant suppressive effects against carcinogenesis induced in several organs, with pleiotropic effects including COX-2 inhibition as suggested mechanisms.[8] In fact, a COX-2 inhibitor nimesulide has been shown to be effective in suppression of hamster pancreatic carcinogenesis when given in the postinitiation stage.[24] It is likely that curcumin is not as active as nimesulide in terms of COX inhibition and thus failed to modulate pancreatic carcinogenesis.

In the present study, lung hyperplasias were also reduced by the MTBITC treatment in the postinitiation stage. Taken together with the tendency for decrease in lung adenomas, the data thus suggested that MTBITC may also possess antitumorigenic effects against lung tumorigenesis. Because MTBITC is a naturally occurring constituent extracted from Japanese white radish, it may be an excellent candidate for a cancer chemopreventive agent. It should also be stressed that MTBITC was effective at both 700 mg/kg diet and 80 mg/kg diet, which means that inhibitory effects may be exerted at low doses within the range of daily consumption levels.

AUTHOR INFORMATION

Corresponding Author

*Tel: +81-3-3700-1564. Fax: +81-3-3700-1622. E-mail: nishikaw@nihs.go.jp.

Funding

This work was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Health, Labour and Welfare and the Ministry of Education, Culture, Sports, Science & Technology in Japan.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Aki Kijima, Ayako Saikawa, and Yoshimi Komatsu for expert technical assistance.

■ ABBREVIATIONS

MTBITC, 4-methylthio-3-butenyl isothiocyanate; BOP, *N*-nitrosobis(2-oxopropyl)amine; PEITC, phenethyl isothiocyanate; COX, cyclooxygenase

■ REFERENCES

(1) Saif, M. W. Pancreatic neoplasm in 2011: an update. *J. Pancreas* **2011**, *12*, 316−321.

(2) Chua, T. C.; Saxena, A. Preoperative chemoradiation followed by surgical resection for resectable pancreatic cancer: A review of current results. *Surg. Oncol.* **2011**, *20*, e161−e168.

(3) Yamamoto, M.; Ohashi, O.; Saitoh, Y. Japan pancreatic cancer registry: current status. *Pancreas* **1998**, *16*, 238−242.

(4) Broese, M.; Hoy, L.; Jaehne, J. Surgical therapy of exocrine pancreatic carcinoma −results at an academic teaching hospital and evaluation of prognostic factors. *Hepatogastroenterology* **2011**, *58*, 593−598.

(5) Nishikawa, A.; Furukawa, F.; Lee, I. S.; Tanaka, T.; Hirose, M. Potent chemopreventive agents against pancreatic cancer. *Curr. Cancer Drug Targets* **2004**, *4*, 373−384.

(6) Nakamura, Y.; Nakamura, K.; Asai, Y.; Wada, T.; Tanaka, K.; Matsuo, T.; Okamoto, S.; Meijer, J.; Kitamura, Y.; Nishikawa, A.; Park, E. Y.; Sato, K.; Ohtsuki, K. Comparison of the glucosinolate-myrosinase systems among daikon (*Raphanus sativus*, Japanese white radish) varieties. *J. Agric. Food Chem.* **2008**, *56*, 2702−2707.

(7) Nakamura, Y.; Iwahashi, T.; Tanaka, A.; Koutani, J.; Matsuo, T.; Okamoto, S.; Sato, K.; Ohtsuki, K. 4-(Methylthio)-3-butenyl isothiocyanate, a principal antimutagen in daikon (*Raphanus sativus*; Japanese white radish). *J. Agric. Food Chem.* **2001**, *49*, 5755−5760.

(8) Shehzad, A.; Wahid, F.; Lee, Y. S. Curcumin in cancer chemoprevention: molecular targets, pharmacokinetics, bioavailability, and clinical trials. *Arch. Pharm. (Weinheim)* **2010**, *343*, 489−499.

(9) Aggarwal, B. B.; Kumar, A.; Bharti, A. C. Anticancer potential of curcumin: preclinical and clinical studies. *Anticancer Res.* **2003**, *23*, 363−398.

(10) Pour, P.; Althoff, J.; Krüger, F. W.; Mohr, U. A potent pancreatic carcinogen in Syrian hamsters: *N*-nitrosobis(2-oxopropyl)amine. *J. Natl. Cancer Inst.* **1977**, *58*, 1449−1453.

(11) Nishikawa, A.; Furukawa, F.; Uneyama, C.; Ikezaki, S.; Tanakamaru, Z.; Chung, F. L.; Takahashi, M.; Hayashi, Y. Chemopreventive effects of phenethyl isothiocyanate on lung and pancreatic tumorigenesis in *N*-nitrosobis(2-oxopropyl)amine-treated hamsters. *Carcinogenesis* **1996**, *17*, 1381−1384.

(12) Son, H. Y.; Nishikawa, A.; Furukawa, F.; Lee, I. S.; Ikeda, T.; Miyauchi, M.; Nakamura, H.; Hirose, M. Modifying effects of 4-phenylbutyl isothiocyanate on *N*-nitrosobis(2-oxopropyl)amine-induced tumorigenesis in hamsters. *Cancer Lett.* **2000**, *160*, 141−147.

(13) Hecht, S. S.; Trushin, N.; Rigotty, J.; Carmella, S. G.; Borukhova, A.; Akerkar, S.; Rivenson, A. Complete inhibition of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced rat lung tumorigenesis and favorable modification of biomarkers by phenethyl isothiocyanate. *Cancer Epidemiol. Biomarkers Prev.* **1996**, *5*, 645−652.

(14) Ben Salah-Abbès, J.; Abbès, S.; Ouanes, Z.; Abdel-Wahhab, M. A.; Bacha, H.; Oueslati, R. Isothiocyanate from the Tunisian radish (*Raphanus sativus*) prevents genotoxicity of zearalenone *in vivo* and *in vitro*. *Mutat. Res.* **2009**, *677*, 59−65.

(15) Tokiwa, H.; Hasegawa, T.; Yamada, K.; Shigemori, H.; Hasegawa, K. A major factor in gravitropism in radish hypocotyls is the suppression of growth on the upper side of hypocotyls. *J. Plant Physiol.* **2006**, *163*, 1267−1272.

(16) Yamasaki, M.; Omi, Y.; Fujii, N.; Ozaki, A.; Nakama, A.; Sakakibara, Y.; Suiko, M.; Nishiyama, K. Mustard oil in "Shibori Daikon" a variety of Japanese radish, selectively inhibits the proliferation of H-ras-transformed 3Y1 cells. *Biosci., Biotechnol., Biochem.* **2009**, *73*, 2217−2221.

(17) Salah-Abbès, J. B.; Abbès, S.; Abdel-Wahhab, M. A.; Oueslati, R. *In-vitro* free radical scavenging, antiproliferative and anti-zearalenone cytotoxic effects of 4-(methylthio)-3-butenyl isothiocyanate from Tunisian *Raphanus sativus*. *J. Pharm. Pharmacol.* **2010**, *62*, 231−239.

(18) Papi, A.; Orlandi, M.; Bartolini, G.; Barillari, J.; Iori, R.; Paolini, M.; Ferroni, F.; Grazia, F. M.; Pedulli, G. F.; Valgimigli, L. Cytotoxic and antioxidant activity of 4-methylthio-3-butenyl isothiocyanate from *Raphanus sativus* L. (Kaiware Daikon) sprouts. *J. Agric. Food Chem.* **2008**, *56*, 875−883.

(19) Barillari, J.; Iori, R.; Papi, A.; Orlandi, M.; Bartolini, G.; Gabbanini, S.; Pedulli, G. F.; Valgimigli, L. Kaiware Daikon (*Raphanus sativus* L.) extract: a naturally multipotent chemopreventive agent. *J. Agric. Food Chem.* **2008**, *56*, 7823−7830.

(20) Scholl, C.; Eshelman, B. D.; Barnes, D. M.; Hanlon, P. R. Raphasatin is a more potent inducer of the detoxification enzymes than its degradation products. *J. Food Sci.* **2011**, *76*, C504−511.

(21) Nakamura, Y.; Matsuo, T.; Okamoto, S.; Nishikawa, A.; Imai, T.; Park, E. Y.; Sato, K. Antimutagenic and anticarcinogenic properties of Kyo-yasai, heirloom vegetables in Kyoto. *Genes Environ.* **2008**, *30*, 41−47.

(22) Beevi, S. S.; Mangamoori, L. N.; Dhand, V.; Ramakrishna, D. S. Isothiocyanate profile and selective antibacterial activity of root, stem, and leaf extracts derived from *Raphanus sativus* L. *Foodborne Pathog. Dis.* **2009**, *6*, 129−136.

(23) Abdull Razis, A. F.; De Nicola, G. R.; Pagnotta, E.; Iori, R.; Ioannides, C. 4-Methylsulfanyl-3-butenyl isothiocyanate derived from glucoraphasatin is a potent inducer of rat hepatic phase II enzymes and a potential chemopreventive agent. *Arch. Toxicol.* **2012**, *86*, 183−194.

(24) Furukawa, F.; Nishikawa, A.; Lee, I. S.; Kanki, K.; Umemura, T.; Okazaki, K.; Kawamori, T.; Wakabayashi, K.; Hirose, M. A cyclooxygenase-2 inhibitor, nimesulide, inhibits postinitiation phase of *N*-nitrosobis(2-oxopropyl)amine-induced pancreatic carcinogenesis in hamsters. *Int. J. Cancer* **2003**, *104*, 269−273.