Table 4. PCNA labeling index, cyclin D1–positive index, and apoptotic index in the prostatic lesions

Group no.	Treatment	PCNA labeling index (%)			Cyclin D1 positive index (%)			Apoptotic index (%)		
		PIN	ADC	Nonlesional area	PIN	ADC	Nonlesional area	PIN	ADC	Nonlesional area
1	DMAB	8.8 ± 2.9* (9)	10.0 ± 2.4 (9)	4.6 ± 1.5 (5)	28.4 ± 7.2 (9)	35.7 ± 6.0 (9)	4.0 ± 1.6 (5)	1.3 ± 0.3 (9)	2.0 ± 0.5 (9)	1.2 ± 0.3 (5)
2	DMAB → 100 ppm silymarin	6.8 ± 1.7 (4)	7.4 ± 2.4 (5)	4.2 ± 2.4 (5)	25.5 ± 9.9 (4)	27.8 ± 4.4† (5)	3.6 ± 0.5 (5)	1.3 ± 0.3 (4)	2.2 ± 0.4 (5)	1.2 ± 0.3 (5)
3	DMAB → 500 ppm silymarin	6.8 ± 2.2 (11)	6.3 ± 1.5† (3)	4.0 ± 1.2 (5)	24.7 ± 6.3 (11)	23.0 ± 3.6† (3)	3.4 ± 1.1 (5)	1.6 ± 0.3† (11)	3.8 ± 0.9‡ (3)	1.1 ± 0.5 (5)
4	500 ppm silymarin	—	—	3.4 ± 1.1 (5)	—	—	0.6 ± 0.5 (5)	—	—	1.2 ± 0.3 (5)
5	None	—	—	3.8 ± 0.8 (5)	—	—	0.6 ± 0.3 (5)	—	—	1.1 ± 0.2 (5)

NOTE: Numbers in parentheses are nos. of lesions or areas examined.
Abbreviation: ADC, adenocarcinoma.
*Mean ± SD.
†Significantly different from group 1 by Student's t test, $P < 0.05$.
‡Significantly different from group 1 by Student's t test, $P < 0.02$.

relevant molecular biomarker in cancer chemoprevention (43, 44). Silymarin and silibinin were reported to decrease in protein levels of cyclin D1 in prostate cancer cells (15, 17). In the present study, silymarin also suppressed cyclin D1 overexpression in prostate adenocarcinoma.

Also, treatment with silymarin inhibits the increase in cell proliferation activity caused by a radical-generating tumor promoter (20). Silymarin is known to exert an antipromoting effect on skin tumorigenesis in mice mediated by impairment of receptor and nonreceptor tyrosine kinase signaling pathway (19). Moreover, in an *in vivo* preclinical prostate cancer model, silibinin inhibits advanced human prostate carcinoma growth (30). In this study, the incidence of adenocarcinoma was decreased by the treatment with silymarin, whereas that of PIN in group 3 was slightly higher than group 1 without statistical significance. The reason for this is unknown, but it may be possible that silymarin feeding at a dose of 500 ppm inhibits progression of PIN to invasive adenocarcinoma. Feeding with silymarin lowered the PCNA labeling indices in the preneoplasms and/or carcinomas of prostate, suggesting that silymarin in diet could suppress the high-proliferative activity of cells initiated with a carcinogen. The other significant finding of this study is the apoptotic index of PIN and adenocarcinoma, which was found to be significantly greater in silymarin-fed rats. The results are in accordance with our previous studies (23, 25) and suggest that, in addition to inhibiting proliferation, apoptosis plays a significant role in inhibition of DMAB-induced prostate carcinogenesis by silymarin. Thus, in the current study, the inhibition of carcinogen-induced prostate malignancies for rats consuming silymarin in part is explained by the alteration of cell proliferating activity and/or apoptosis.

Chemoprevention of cancer might be defined as the deliberate introduction of these selected nontoxic substances into the diet for the purpose of reducing cancer development. Silymarin is clinically used to as antihepatotoxic agents and devoid of any toxicity and untoward effects in both animal and human studies (45). In the present study, the estimated daily silymarin intakes in rats given diet containing 100 and 500 ppm silymarin were ~5 and 25 mg/kg. In a direct extrapolation to a 60 kg person, these doses are equivalent to the estimated doses of clinical use as an antihepatotoxic agent (45). Recently, Singh et al. (30, 46) reported that dietary feeding of silibinin (up to 1%) to nude mice did not show any adverse effect. In the present study, administration of 500 ppm silymarin did not also show any adverse effect on diet consumption, body weight gain, prostate weight, and pathologic alteration for 40 weeks. On the other hand, silibinin is physiologically achievable in different organs including prostate as well as in plasma, and the achievable levels of total slibinin has been found in the range of 15 to 100 μmol/L in plasma by feeding with 0.05% to 1% silibinin/silymarin in rodents (30, 47, 48). The achievable levels (15-100 μmol/L) of silibinin showed inhibition of human prostate cancer cells growth in culture (16, 17, 30). These observations showed that the efficacy of silymarin at dietary dose levels without any adverse effects could have a direct practical and translational relevance to human prostate cancer patients. However, silymarin is a mixture of three structural isomers of flavonoids. Among the flavonoids, silibinin (also called silybin, silibin, or sibilinin) is suggested to be the most active constituent. Because the cancer chemopreventive and anticarcinogenic effects of silymarin seem to be due to the main constituent silibinin (16, 30, 37, 49, 50), further studies of the chemopreventive effects of silibinin itself are necessary.

In conclusion, dietary administration of silymarin significantly suppressed the development of DMAB-induced rat prostate carcinomas. Such cancer protective effect of silymarin might relate to the modulation of cell growth and apoptosis in the prostate neoplastic lesions.

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CHEMOPREVENTION OF COLON CARCINOGENESIS BY DIETARY NON-NUTRITIVE COMPOUNDS

Takuji Tanaka[1], Shingo Miyamoto[2], Rikako Suzuki[1], and Yumiko Yasui[1]

[1]Oncologic Pathology, Kanazawa Medical University, 1-1 Daigaku, Uchinada, Ishikawa 920-0293, Japan, and [2]Division of Food Science and Biotechnology, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan

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ABSTRACT: *Dietary habit is instrumental in about 40-60% of human colon cancer. Fruit and vegetable consumption is associated with decreased risk of several types of cancer, including colonic malignancy. Fruits and vegetables contain many non-nutritive as well as nutritive compounds, such as carotenoids, dithiolthiones, flavonoids, glucosinolates, indoles, isothiocyanates, monoterpenes, phenols, sterols, sulthydryls, and vitamins (including vitamin C, vitamin E, and folate). There may be other unknown non-nutritive constituents in foods that can reduce cancer development. Animal studies in experimental chemical carcinogenesis have indicated that several non-nutritive components, belonging to different chemical groups, in foods protect against certain types of cancer including colonic neoplasm. These chemicals are known as 'chemopreventive agents'. Many of them are anti-oxidants and might suppress carcinogenesis through (I) inhibiting phase I enzymes or blocking carcinogen formation, (ii) induction of phase II (detoxification) enzymes, (iii) scavenging DNA reactive agents, (iv) modulation in hormone homeostasis, (v) suppression of hyper-cell proliferation induced by carcinogen, (vi) induction of apoptosis, (vii) depression in tumor angiogenesis, and/or (viii) inhibition of certain phenotypic expression of neoplastic cells. With increasing the incidence of colon cancer rising certainly, there is an ever-increasing need to determine the most effective arms to prevent colon cancer and to understand the mechanism(s) underlying successful prevention. There are critical inter-relationships between diet, environment, and genetics that can affect cancer risk. Again, fruits, vegetables, teas, spices, and herbs consumed in the diet have ability of reducing cancer occurrence in pre-clinical animal carcinogenesis models. Although epidemiologic studies show similar associations, there are very few intervention studies to date. This article will introduce our recent studies in search for the effective chemopreventive effects of several naturally occurring non-nutritive products in edible plants on rat colon carcinogenesis.*

KEY WORDS: Chemoprevention, Colon carcinogenesis, Diet, Non-nutritive compounds, Rats

Corresponding Author: Dr. Takuji Tanaka, Department of Oncologic Pathology, Kanazawa Medical University, 1-1 Daigaku, Uchinada, Ishikawa 920-0293, Japan E-mail: takutt@kanazawa-med.ac.jp

INTRODUCTION

Prevention of disease is an old and important concept. An important consideration in cancer research today is that exposure to pharmacologically active chemicals may play an important role in reducing the relative risks resulting from exposure to carcinogenic chemicals. Chemoprevention of cancer might be defined as the deliberate introduction of these selected non-toxic substances into the diet for the purpose of reducing cancer development. Numerous epidemiological studies on the relationship between diet and carcinogenesis have demonstrated a protective effect of the consumption of fruits and vegetables against various forms of cancers (Block et al., 1992; Hebert et al., 1993; Steinmetz and Potter, 1996). A number of compounds (Table 1) in diet are known to modulate the development of tumors in experimental animal models (Slaga, 1980). Epidemiological studies also suggest that specific, pharmacologically active agents present in diet might reduce or increase the relative risk of cancer development. As to colon cancer, marked variations in dietary habits among populations of different cultures and life-styles have been associated with a risk of this malignancy (Reddy, 1986; Weisburger, 1991). Also, there is an inverse correlation between the intake of vegetables/fruits and human colon cancer (Block et al., 1992; Hirayama, 1979; Steinmetz and Potter, 1991a; Steinmetz and Potter, 1991b). Thus, a relationship between the risk of the development of colon cancer and dietary habits is important (Reddy, 1993; Tanaka, 1997b), although etiology of colon cancer is multi-factorial and complex. Among the dietary components, fiber is found to reduce the risk of colorectal cancer development (Bingham et al., 2003; Fuchs et al., 1999; Giovannucci et al., 1994; Peters et al., 2003). Also, green tea could inhibit colorectal tumorigenesis (Dashwood et al., 1999; Weisburger, 1999; Weisburger, 2000; Williams et al., 1999). However, several epidemiological data have suggested no effects of dietary fiber on the recurrence of colorectal adenomas (Alberts et al., 2000;

Schatzkin et al., 2000) and no influence of green tea on the risk of stomach cancer (Tsubono et al., 2001).

Table 1. Potential non-nutritive compounds that inhibit chemical carcinogenesis, in fruits, vegetables, and spices

	Class	Compounds	Major food sources
Flabonoids	Flavones	Tangeretin, Nobiletin, Apigenin, Chrysin, Diosmetin, Luteolin	Celery, parsley, sweet red pepper, thyme
	Flavonols	Quercetin, Rutin, Myricetin, Kaempferol	Apples, berries, broccoli cherries, fennel, kale, red wine, sorrel, grains, onions, tea
	Catechins	Epigallocatechin-3-gallate (EGCG) Epigallocatechin Epicatechin-3-gallate	Tea, apples, cocoa, red wine
	Flavanones	Naringenin, Fisetin, Hesperidin, Hesperitin, Taxifolin	Citrus fruit, prunes, citrus peel
	Isoflavones	Genistein, Daidzein	Soybeans, legumes
	Anthocyanidins	Cyanidin, Malvidin, Pelargonidin	Grapes, cherries, raspberries
Indoles		Indole-3-carbinol (I3C) 3,3'-diindoylmethane (DIM)	Cruciferous vegetables
Isothiocyanates			Cruciferous vegetables
Lignans		Matairesinol, Secoisolariciresinol, Enterodiol, Enterolactone	Grains, flaxseed, berries, chives, beverages
Organozulfur		Diallyl disulfide (DADS)	Allium vegetables: garlic and onions
Terpenes		D-Limonene	Citrus, spices

Potential chemopreventive agents are to be found both among nutrients and non-nutrients in diet. Epidemiological and experimental studies have revealed that a number of micronutrients may have cancer preventive properties in several organs including large bowel (Micozzi, 1989). Examples are vitamins A, C, and b-carotene, selenium, and calcium. We have demonstrated cancer chemopreventive ability of two xanthophylls without pro-vitamin A activity in the rat colon and oral cavity (Tanaka et al., 1995a; Tanaka et al., 1995b). Most of these compounds are anti-oxidants that could serve as an explanation for their mode of action. The well-known non-nutritive chemopreventives in colon tumorigenesis is dietary fiber, a variety of ingestible carbohydrates (Weisburger et al., 1993). Since the modifying effects of the major dietary factors on rodents colon carcinogenesis resulted in heterogeneous (Angres and Beth, 1991), we focus on other non-nutritive inhibitors derived from vegetables and fruits in experimental colon carcinogenesis. Wattenberg also suggested that some minor non-nutrients in the diet have protective effects on colon tumorigenesis (Wattenberg, 1983). In 1985, he roughly classified chemopreventive agents into blocking and suppressing agents based on the time period that agents appear to have activity in animal models of carcinogenesis (Wattenberg, 1985). Since then, several naturally occurring compounds and synthetic chemicals have been intensively investigated for their chemopreventive ability on chemically induced malignant epithelial neoplasms including colon carcinoma. These include the inorganic and organic selenium salts, phenolic anti-oxidants, non-steroidal anti-inflammatory drugs (NSAIDs), ornithine decarboxylase (ODC) inhibitors, *etc.* Our group also found several natural chemopreventive agents against colon carcinogenesis (Table 2-4). Indeed food chemists and natural product scientists have identified hundreds of 'phytochemicals' that are being evaluated for the prevention of cancer (American, 1996; Huang et al., 1994). Among the non-nutrients dietary components believed to exert a chemopreventive effect are flavonoids, polyphenolic derivatives of benzo(*a*)pyrene that are widely distributed in edible plants (Formica and Regelson, 1995). There are several major classes of flavonoids, which may occur as glycosides or aglycones. Total dietary intake of flavonoids has been estimated as high as 1 g/day, equivalent to 50,000 ppm in diet (Pierpoint, 1986), although more recent studies have indicated that intake varies widely (Hertog et al., 1995).

Table 2. Non-nutritive compounds that inhibit aberrant crypt foci (ACF) formation in an ACF bioassay in our laboratory

Compounds	Dose	Carcinogens	Animals	% inhibition of ACF	Reference
Rebaudioside A Liquiritin Phyllodulcin Hydrangenol Oleanoic acid Costunolide Soyasaponin A_2	200 ppm	Azoxymethane (AOM)	Rat	19 6 8 24 36 22 16	Kawamori et al. 1995
Safflower oil Perilla oil Perilla + Olive oil	12% 6% + 6% 3% + 9%	AOM	Rat	47 74 49 41	Onogi et al. 1996
Olive oil + β-carotene* Perilla + Olive oil +β-carotene* Perilla oil + β-carotene*	12% + 50 or 200 mg/kg/day* 3% + 9% +50 mg/kg/day* 12% + 50 mg/kg/day*	AOM	Rat	27 or 38 87 91	Komaki et al. 1996
d-Limonen	5000 ppm	AOM	Rat	32	Kawamori et al. 1996
β-cryptoxanthin and hesperidin rich powder	500 ppm	AOM	Rat	20	Kohno et al. 1999
Caffeine Quercetin	500 ppm	AOM	Rat	30 48	Tanaka et al. 1999
Garcinol	100 ppm 500 ppm	AOM	Rat	26 40	Tanaka et al. 2000
Zerumbone	100 ppm 500 ppm	AOM	Rat	14 46	Tanaka et al. 2001
Chalcone 2-Hydroxychalcone	500 ppm	AOM	Rat	51 56	Kohno et al. 2002
Extract of leaves of ginkgo (*Ginkgo biloba*) Bilobalide	50 ppm 500 ppm 15 ppm 150 ppm	AOM	Rat	31 47 26 33	Suzuki et al. 2004
Powderd broccoli sprout	20 ppm 100 ppm	AOM	Rat	47 40	Suzuki et al. 2004

Table 3. Non-nutritive compounds that inhibit colonic adenocarcinoma (ADC) in a long-term bioassay in our laboratory

Compounds	Dose	Carcinogens	Animals	% inhibition of ADC		Reference
				Initiation	Post-initiation	
Chlorogenic acid	250 ppm	Methylazoxymethanol (MAM) acetate	Hamster	ND*	100	Mori et al. 1986
$Mg(OH)_2$	500 ppm 1000 ppm	MAM acetate	Rat	ND	77 47	Tanaka et al. 1989
Astaxanthin Canathaxanthin	100 ppm 500 ppm 100 ppm 500 ppm	Azoxymethane (AOM)	Rat	ND	39 54 31 69	Tanaka et al. 1995
Juglone Plumbagin Hydrangenol	200 ppm	AOM	Rat	7 26 17	ND	Sugie et al. 1998
Satuma mandarin juice	MJ** MJ2*** MJ5****	AOM	Rat	ND	49 64 78	Tanaka et al. 2000
Columbin	4 ppm 20 ppm 100 ppm	AOM	Rat	36 55 82	ND	Kohno et al. 2002
Seed oil from bitter melon (*Momordica charantia*)	100 ppm 1000 ppm 1%	AOM	Rat	47 40 17		Kohno et al. 2004
Pomegranate (*Punica granatum L.*) seed oil	100 ppm 1000 ppm 1%	AOM	Rat	46 53 31		Kohno et al. 2004

Not determined; ** MJ contains 0.8 mg β-cryptoxantin and 79 mg hesperidin in 100 g juice; *** MJ2 contains 1.7 mg β-cryptoxantin and 84 mg hesperidin in 100 g juice, and **** MJ5 contains 3.9 mg β-cryptoxantin and 100 mg hesperidin in 100 g juice.

Table 4. Non-nutritive compounds that suppress aberrant crypt foci (ACF) formation in an ACF bioassay and colonic adenocarcinoma (ADC) development in a long-term bioassay in our laboratory

Compounds	Dose	Carcinogens	Animals	% inhibition of ACF Initiation	% inhibition of ADC		Reference
					Initiation	Post initiation	
1'-Acetoxychavicol acetate	100 ppm	Azoxymethane (AOM)	Rat	41	54	45	Tanaka et al. 1997;
	200 ppm			37	ND*		
500 ppm			ND	77	93	Tanaka et al	1997
Diosmin	1000 ppm	AOM	Rat	56	70	93	Tanaka et al. 1997
Hesperidin	1000 ppm			63	93	79	
Diosmin + Hesperidin	900 + 100 ppm			61	73	93	
Auraptene	100 ppm	AOM	Rat	41	49	58	Tanaka et al. 1997; Tanaka et al 1998
	500 ppm			56	65	65	
Morin	500 ppm	AOM	Rat	63	43	61	Tanaka et al. 1999
Defatted rice-germ	2.5%	AOM	Rat	20	ND		Kawabata et al. 1999
γ-Aminobutyric (GABA) - enriched defatted rice-germ				32	43	73	
Rice-germ				57	61	64	
Ferulic acid	250 ppm	AOM	Rat	26	61	46	Kawabata et al. 2000
	500 ppm			31	54	39	
Capsaicon	500 ppm	AOM	Rat	40	60	28	Yoshitani et al. 2001
Rotenone				60	47	68	
Obacunone	200 ppm	AOM	Rat	65	ND		Tanaka et al. 2001
	500 ppm			65	78	89	
Limonin	200 ppm			55	ND		
	500 ppm			56	94	89	
Nobiletin	100 ppm	AOM	Rat	50	ND	18	Kohno et al. 2004; Suzuki et al. 2004
	500 ppm			55		48	
Silymarin	100 ppm	AOM	Rat	47	24	29	Kohno et al. 2002
	500 ppm			54	80	73	
	1000 ppm			64	ND		
Conjugated linoleic acid	100 ppm	AOM	Rat	19			Kohno et al. 2002; 2004
	1000 ppm			36	ND		
	1%			63	38		
Ethyl acetate extract of 'Kurosu'	500 ppm	AOM	Rat	21	ND	38	Shimoji et al. 2003; 2004
	1000 ppm			37		56	
	2000 ppm			67	ND		

* Not determined

This review is limited to a few non-essential dietary components broadly or for those for which substantial documentation exists about an effect on the carcinogenesis process and for those in which a plausible mechanism of action can be postulated. We therefore list several non-nutritive chemopreventive agents against colon carcinogenesis in Tables 2-4 (Kawabata et al., 1999; Kawabata et al., 2000; Kawamori et al., 1995; Kawamori et al., 1996; Kawamori et al., 1994; Kohno et al., 1999; Kohno et al., 2002a; Kohno et al., 2002b; Kohno et al., 2004a; Kohno et al., 2002c; Kohno et al., 2002d; Kohno et al., 2004b; Kohno et al., 2001c; Komaki et al., 1996; Mori et al., 1986; Morishita et al., 1997; Onogi et al., 1996; Shimoji et al., 2004; Shimoji et al., 2003; Sugie et al., 1998; Suzuki et al., 2004a; Suzuki et al., 2004b; Suzuki et al., 2004c; Tanaka et al., 1999a; Tanaka et al., 1998; Tanaka et al., 1997a; Tanaka et al., 1997b; Tanaka et al., 1999b; Tanaka et al., 1995a; Tanaka et al., 2000a; Tanaka et al., 2000b; Tanaka et al., 2001c; Tanaka et al., 1997c; Tanaka et al., 1997d; Tanaka et al., 2001d; Tanaka et al., 1989; Yoshitani et al., 2001) that we found from edible plants in our laboratory. Also, The present report will introduce our recent data demonstrating chemopreventive ability of capsaicin (Yoshitani et al., 2001), rotenone (Yoshitani et al., 2001), obacunone (Tanaka et al., 2001c), limonin (Tanaka et al., 2001c), nobiletin (Kohno et al., 2001c; Suzuki et al., 2004a), and silymarin (Kohno et al., 2002c), that are present in certain edible plants using animal colon carcinogenesis models. It must be noted that the response to individual components is assumed to be consistent with that occurring in a complex food matrix. Whether this is true or not remains to be adequately verified.

GENE-ENVIRONMENT INTERACTION AND CANCER CHEMOPREVENTION

Malignant epithelial neoplasm (cancer) is now considered to be primarily determined by the interaction of environmental factors (including dietary habit) with genetic, epigenetic, and posttranslational events involved in the cancer process (Greenwald et al., 2002; Knudson, 1997; Loktionov, 2003; Muller and Kersten, 2003; Raunio et al., 1995; Wynder and Gori, 1977). Because dominantly inherited or familial cancers probably contribute only a small percent of total cases, it is quite important to identify those environmental modulators that influence non-familial risks (Knudson, 1997; Muller and Kersten, 2003; Raunio et al., 1995). Dietary habits are possibly a variable that markedly influences non-familial cancer risk. Some have estimated that dietary habits are instrumental in about 60% of cancers in women and about 40% of cancers in men (Wynder and Gori, 1977). Although these are significant contributions, the true effect depends on the individual's genetic profile, the particular neoplasms, and the composition of the entire diet.

Although variability exists, fruit and vegetable consumption has often been inversely linked with the incidence of cancer (Gate et al., 1999; Riboli and Norat, 2003; Temple, 1999; Thompson et al., 1999). The reason for variability remains obscure but may relate to oxidative balance (Gate et al., 1999) or other physiological changes as indicated in this chapter. Variations in pro- and anti-oxidant conditions that might arise from the absence or presence of food components is recognized as an influence on several essential cellular functions, including gene expression profiles (Adler et al., 1999; Dalton et al., 1999). This homeostasis is unquestionably complex, as evident by the sensitivity of several kinases and transcription factors to rather subtle shifts in redox status (Torres and Forman, 2003).

The linkages between fruit and vegetable consumption and reduced cancer risk serve as sufficient evidence for the continued examination of individual foods or dietary components as modulators of the initiation, promotion, or progression stages of carcinogenesis. A large number of agents with anti-oxidant properties are found in fruits and vegetables (Table 5), including carotenoids, dithiolthiones, flavonoids, glucosinolates, indoles, isothiocyanates, monoterpenes, phenols, sterols, sulfhydryls, and vitamins (folate, vitamin C, and vitamin E). These dietary components likely have both complementary and overlapping mechanisms of action, including the induction of detoxification enzymes, blockage of carcinogen formation, shifts in hormone homeostasis, slowing of cell division, induction of apoptosis, depression in tumor angiogenesis, and others. Although several macronutrients likely are involved in the cancer process, they do not appear to totally explain the worldwide variance in cancer risk. Furthermore, it is possible that several physiologically important dietary constituents markedly influence their impact. Thus, so-called functional foods (a name based on the ability of selected foods to have health benefits over and beyond the basic nutrition provided) continue to captivate the interest of scientists and legislators and, most importantly throughout the world, the consumer (Palou et al., 2003).

Table 5. Antioxidative activities of fruits and vegetables

Fruit and vegetable	ORAC(μmol)	FRAP(μmol)	TRAP(μmol)	TEAC(μmol)
Apple, raw with skin	2,175.5	562.5	268.5	1,066
Apricot, raw	7901	488	243	153
Asparagus, boiled	1,480	860	874	353
Avocado, raw	2,249	779	324	384
Banana, raw	560	333	117	664
Beans, green boiled	147	149	40	160.5
Beet, red	1,476	1,492	650	623
Blackberries, raw	2,617	4,979	1513	2,709
Blueberries, raw	5,863	5,597	1,349	2,931
Broccoli, raw chopped	424	322	222	351
Cabbage, green raw	264	131	102	106
Cantaloupe, raw	423	576	152	256
Carrots, raw	474	75	115	139
Cauliflower, raw	222	165	81	155
Celery, raw	125	184	19	32
Cherries, raw sweet	2,630	405	284	344
Eggplant (Aubergine), raw	575	113	116	127
Figs, dried	6,326	1,254	385	462
Garlic, raw	143	22	135	163
Grapefruit, pink/red raw	1,209	1,100	497	717
Grapes, white/green	1,202	697	254	2,127
Kiwifruit, raw	472	627	175	467
Lettuce, iceberg, raw	55	58	46	37
Onion, raw	564	416	257	297
Orange, raw	1,746.5	2,094.5	921	1,032
Peach, raw	886	433	130	203
Pear, raw	1,683	565	487	609
Pepper, red/green, raw	312	935	274	413
Pineapple, raw	1,229	1,573	918	1,543
Plum, raw	1,110	986	534	862
Patato, boiled, no skin	399	311	260	365
Radish, raw	430	176	163	100
Raspberries, raw	3,665	5,698	1,289	4,169
Spinach, raw	452	514	256	308
Strawberries, raw	3,264	3,685	1,275	3,125
Tangerine	1,361	806	232	349
Tomato, raw, red	317	357	246	273
Watermelon, raw	194	120	74	135

ORAC, oxygen radical absorbance capacity; FRAP, Ferric reducing ability of plasma; TARP, Total radical trapping parameter assay; and TEAC, Trolox equivalent antioxidant capacity.

NON-NUTRITIVE CHEMOPREVENTIVE COMPOUNDS IN FOODS

To date, more than 500 compounds have been suggested as potential modifiers of experimental carcinogenesis, including colon tumorigenesis (Corpet and Pierre, 2003; Corpet and Tache, 2002; Tanaka, 1992; Tanaka, 1994; Tanaka, 1997a; Tanaka, 1997b; Tanaka et al., 2001a; Tanaka et al., 1993; Tanaka and Mori, 1996). Some of the major anti-oxidant constituents of fruit, vegetables, and beverages are derived from phenolic phytochemicals synthesized through the shikimate pathway from tyrosine and phenylalanine (Shirley, 1996). Many of these exist as *O*-glycosides and *O*-methyl conjugates. Cinnamic acid, widely found in fruits and vegetables, is a transformation product of phenylalanine produced by the action of phenylalanine-ammonia lyase. Isoflavonoids, flavonoids, and lignans are additional plant constituents that make up the three principal classes of phytoestrogens consumed by humans. Soy, a staple for Asians, is a major source of the isoflavonoids daidzein and genistein. Flavonoids are also abundantly present in fruits. Quercetin and kaempferol are two commonly found flavonoids that are particularly profuse in apples, onions, and tealeaves. Plant lignans are present in many cereal grains, fruits, and vegetables and give rise to the mammalian lignans enterodiol and enterolactone. The richest sources of lignan precursors, such as secoisolariciresinol and matairesinol, are linseed (flaxseeds) and other oil seeds. *Allium* foods including garlic, onions, and leeks provide a host of organosulfur compounds that may influence health. Terpenes are a group of hydrocarbons made up of building blocks of isoprene (C_5H_8) units that are widespread in nature. Most occur in plants as constituents of essential oils. Monoterpenes are composed of two units such as limonene, citral, and camphor, whereas sesquiterpenes are made up of three units and include compounds such as humulene, which is a hop aromatic. Carotene is an example of an 8-isoprene or tetraterpene unit.

Fruit and vegetable consumption is not the only dietary factor that can influence cancer risk. Ingestion of green and black tea, herbs, and spices has been reported to be inversely associated with cancer risk (Craig, 1999; Lambert and Yang, 2003; Surh, 1999; Weisburger, 1999). As reviewed by other investigators (Corpet and Pierre, 2003; Corpet and Tache, 2002), numerous non-nutritives in foods (vegetables and fruits) can inhibit colon carcinogenesis in rodents.

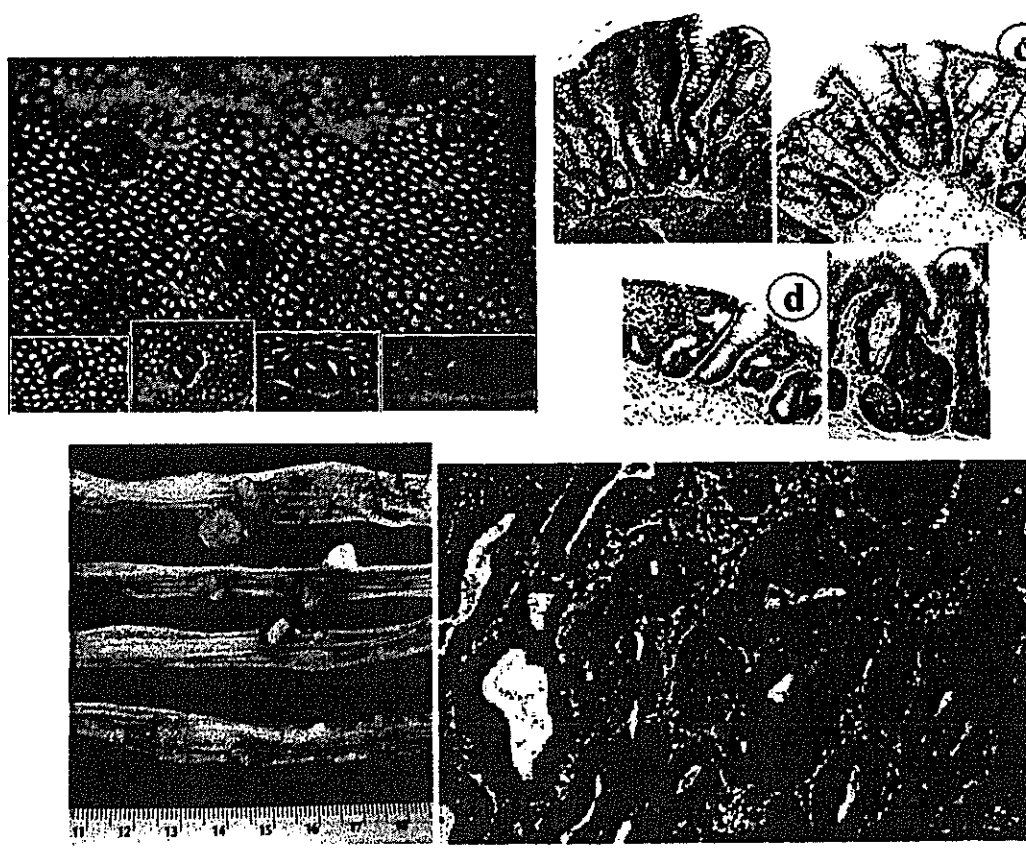

Fig. 1. Colonic lesions induced by a colonic carcinogen azoxymethane (AOM). (a), ACF in the colonic mucosa stained with methylene blue. Inserts are ACF consisted of 1, 2, 3, and 7 aberrant crypts (left to right). (b) Histopathology of ACF stained with ematoxylin and eosin, original magnification x20. (c) PCNA-immunohistochemistry of ACF. The number of PCNA-positive cells in ACF is greater than that in the surrounding normal crypts. Original magnification, x20. (d) PPARγ-immunohistochemistry of ACF. Immunoreactivity of PPARγ in ACF is relatively weak when compared surrounding crypts. Original magnification, x20. (e) BCAC detected by β-catenin-immunohistochemisstry. Original magnification, x20. (f) Macroscopic view of polypoid colonic tumors induced by AOM. (g) Tubular adenocarcinoma induced by AOM. Original magnification, x40.

PRENEOPLASTIC LESIONS FOR COLONIC NEOPLASMS

It has been proposed that aberrant crypt foci (ACF, Fig. 1a-d) being present in carcinogen-treated colons of rodent and in the colons of humans with a high risk for colon cancer could be employed to study modulators of colon carcinogenesis (Tables 2-4) (Bird, 1995; Kawamori et al., 1995; Pereira et al., 1994), since ACF are putative precursor lesions for colon cancer in rodents (Bird, 1995) and humans (Pretlow et al., 1991). ACF possess several biological aberrations including gene mutations and amplification (Bird, 1995). Also, alteration (decreased) of hexosaminidase activity is found in ACF. Tsukamoto *et al.* found down-regulation of both hexosaminidase-α and -β in ACF (Tsukamoto et al., 2001). As shown in Fig. 1c, ACF also have increased cell proliferation activity compared to surrounding normal crypts (Pretlow et al., 1994a; Yamashita et al., 1994). We also have determined hyper-cell proliferation activity of ACF, especially dysplastic ACF (Ochiai et al., 2005) (Table 6). Certain chemopreventive compounds are reported to reduce such hyper-cell proliferation in ACF (Li et al., 1998; Zheng et al., 1997) and to inhibit *c-myc* expression induced by methylazoxymethanol (MAM) acetate (Wang et al., 1993). For demonstrating the inhibitory action of compounds in colon carcinogenesis, we have used two experimental animal bioassays: (1) a 5-week short-term bioassay of ACF for screening natural compounds, which are present in vegetables and fruits, with possible chemopreventive ability (Fig. 2) and (2) a long-term rat colon carcinogenesis model (Fig. 3) for evaluating their inhibitory effects against colon carcinoma development (Figs. 1f, g). In these bioassays, several biochemical and morphologic biomarkers are used (Table 7). Cell proliferation plays an important role in multistage carcinogenesis (Cohen and Ellwein, 1990; Lipkin, 1991; Pegg, 1988; Tanaka, 1992). ODC and polyamines are intimately involved in normal cellular proliferation and are likely to play a role in carcinogenesis including colon tumorigenesis (LaMuraglia et al., 1986; Luk et al., 1986). 5'-Bromodeoxyuridine (BrdU)-labeling index, proliferating cell nuclear antigen (PCNA)-labeling index, and silver-stained nucleolar regions (AgNORs) number are also known to be proliferation biomarkers (Tanaka, 1997a).

Recent data suggest that the balance between the phase I carcinogen-activating enzymes and the phase II detoxifying enzymes is critical to determining an individual's risk for cancer (Wilkinson and Clapper, 1997). Human deficiencies in phase II enzyme activity, specifically glutathione *S*-transferase (GST), have been identified and associated with increased risk for colon cancer (Szarka et al., 1995). Therefore, phase II detoxifying enzymes, such as GST and quinone reductase (QR), might be useful as a biomarker for chemopreventive studies.

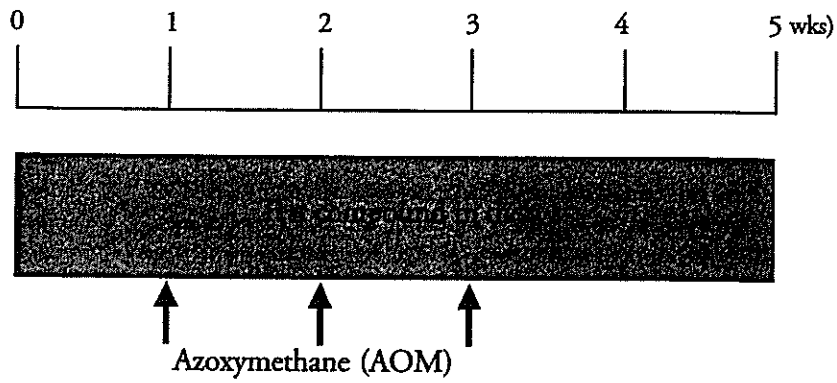

Fig. 2. Experimental protocol (an ACF bioassay) for screening compounds that exert inhibitory activity of ACF formation in colon of rodents. At sacrifice (wk 5), number of ACF and expression of several biomarkers are assayed. Animals are given weekly subcutaneous injections of AOM 2 (20 mg/kg bw) or 3 times (15 mg/kg bw) to induce colonic ACF.

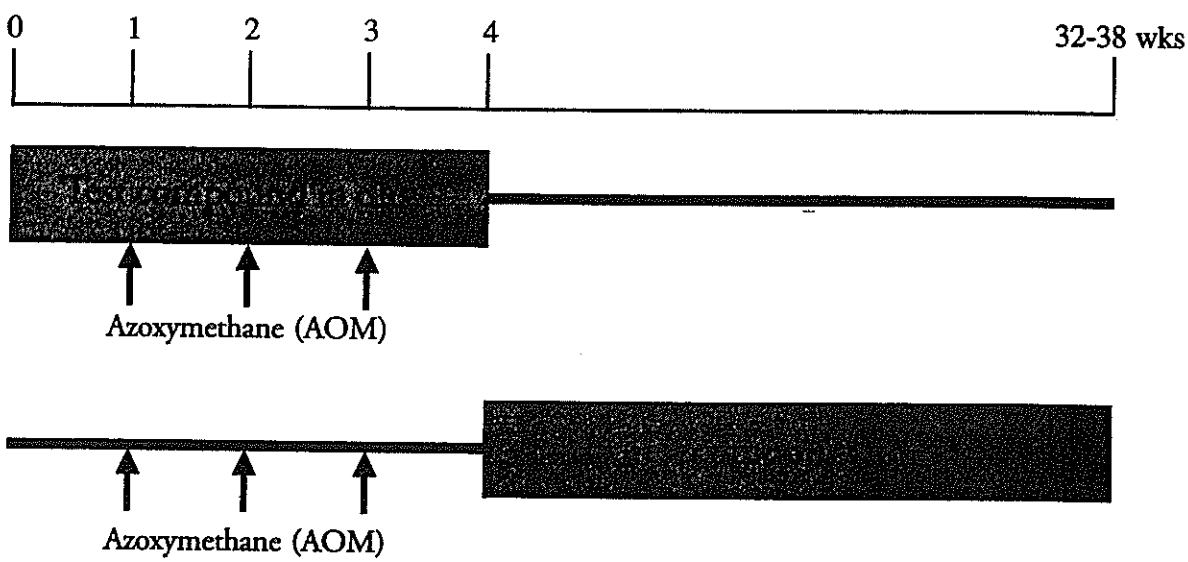

Fig. 3. Experimental protocol (a long-term bioassay) for detecting compounds that can suppress development of colon cancer in rodents. At sacrifice (wks 32-38), incidence and multiplicity of colonic neoplasm and expression of several biomarkers are assayed. Animals receive weekly subcutaneous injections of AOM 2 (20 mg/kg bw) or 3 times (15 mg/kg bw) to induce colonic neoplasms.

Table 6. Proliferative activity of several colonic lesions induced by azoxymethane (AOM) in rats

Lesions		Mean values of:				
		BrdU-labeling index (%)	PCNA-positive nuclei (%)	AgNORs (/nucleus)	Micronuclei (%)	Mitotic index (%)
Normal (without AOM)		5.9	18	2.18	0.24	0.7
Normal (with AOM)		9.7	20	2.68	0.45	1.1
Aberrant crypt foci (ACF)	Hyperplastic ACF	15	28	2.83	0.61	1.6
	Dysplastic ACF	22.2	34	3.11	0.81	2.2
Adenoma		21.1	33	3.07	1.13	2.1
Adenocaracinoma		28.3	58	3.78	1.74	2.5

BrdU, 5' bromodeoxyuridine; PCNA, proliferating cell nuclear antigen; and AgNORs, silver-stained nucleolar regions.

SCREENING OF POSSIBLE CHEMOPREVENTIVE AGENTS AGAINST COLON TUMORIGENESIS ABILITY USING A 5-WEEK SHORT-TERM BIOASSAY OF ACF

As the first bioassay for pilot studies, we investigated the modifying effects on test compounds on the development of ACF. ACF could be induced by weekly subcutaneous injections of azoxymethane (AOM, 15 mg/kg body weight, 3 times; or 20 mg/kg body weight, 2 times) and test chemicals in the basal diet at various dose levels were administered to male F344 rats for 5 weeks, starting 1 week before AOM dosing (Fig. 2). At the end of the study, ACF were counted and expression of several biomarkers was examined. The biomarkers assayed included ODC activity and polyamine level in the colonic mucosa, number of AgNORs protein/nucleus in the colonic crypts, and/or activities of GST and QR in the colonic mucosa (Table 6).

EVALUATION OF CHEMOPREVENTIVE ABILITY OF SELECTED COMPOUNDS USING A LONG-TERM RAT COLON CARCINOGENESIS MODEL

Based on the results in the pilot studies, the second bioassay for evaluating the chemopreventive effects of compounds, which have been screened by a short-term pilot study, on colon carcinogenesis was conducted. Male F344 rats were given subcutaneously injections of AOM (15 mg/kg body weight, weekly, 3 times; or 20 mg/kg body weight, 2 times) to induce colonic adenocarcinoma (Fig. 3). For 'initiation' feeding, oral administration of these compounds in the diets was begun 1 week before the AOM exposure and continued for 4 or 3 weeks, and for 'post-initiation' feeding, experimental diets containing test compounds, beginning 1 week after the last dosing of AOM, were given for 28 or 32 weeks. Several biomarkers (Table 7) were assayed at the termination of the experiment.

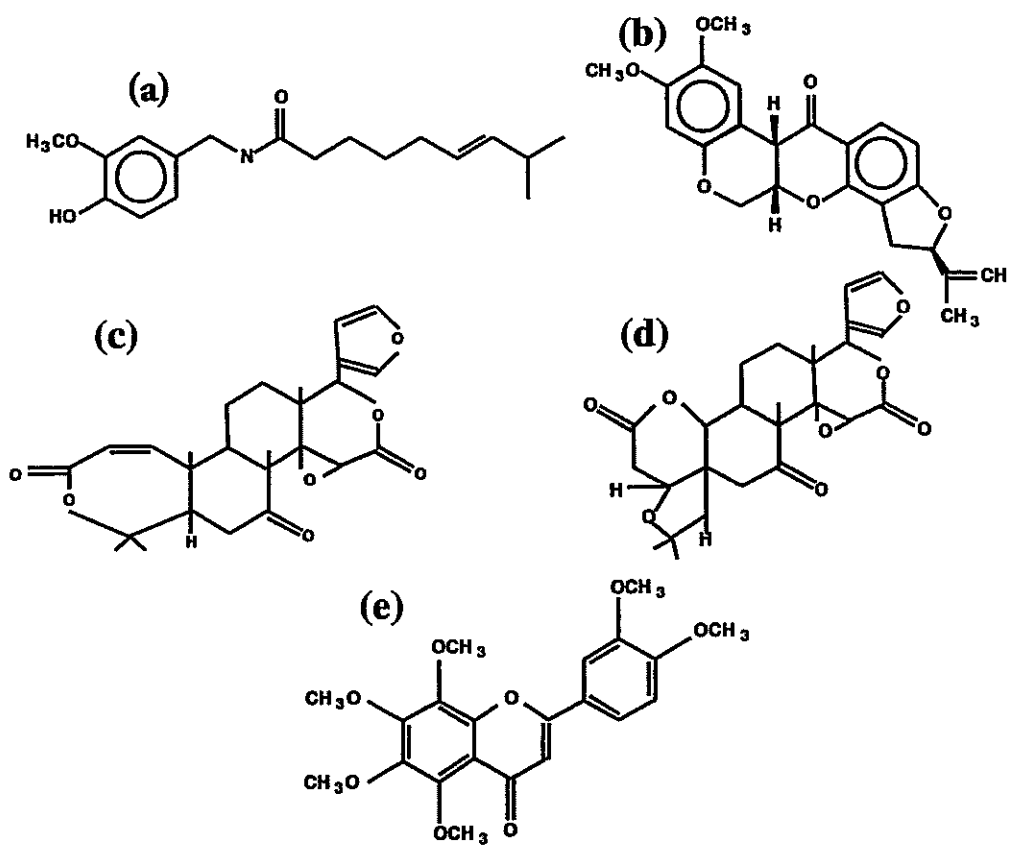

Fig. 4. Chemical structures of (a) capsaicin, (b) rotenone, (c) obacunone, (d) limonin, and (e) nobiletin.

Table 7. Biomarkers used for detection of chemopreventive compounds against colon carcinogenesis

	Biomarkers
Proliferation activity	BrdU-labeling index, PCNA-labeling index, AgNORs number, Apoptotic index, Tumor-angiogenesis, etc.
Biochemistry	ODC activity, Polyamine levels, GST activity, QR activity, MDA, 4-HNE
Histopathology	ACF, BCAC, MDF, Adenoma, Adenocarcinoma

BrdU, 5'-bromodeoxyuridine; PCNA, proliferative nuclear antigen; AgNORs, silver-stained nucleolar regions; ODC, ornithine decarboxylase; GST, glutathione *S*-transferase; QR, quinone reductase; MDA, malondialdehyde; 4-HNE, 4-hydroxy-2(E)-nonenal; ACF, aberrant crypt foci; BCAC, β-catenin accumulated crypts; and MDF, mucin-depleted foci.

Inhibition of colon carcinogenesis by dietary capsaicin and rotenone in rats

Capsaicin (Fig. 4a) is widely consumed as a food additive throughout the world, particularly in South-East Asia and Latin American countries. The Korean people are large consumers of capsicum fruit: average daily per capita consumption of capsaicin may reach 50 mg (Buch and Burks, 1983). The content of capsaicin in capsicum is about 0.02% in fresh fruit and 0.5-1.0% in dried ripe fruit (Yun, 1999). It is currently used as a versatile tool for the study of pain mechanisms and also for pharmacotherapy to treat several pain disorders because of its selective effects on the functions of a defined subpopulation of sensory neurons (Tominaga and Julius, 2000). The intake of excessive hot peppers containing capsaicin has been considered an irritant for the gastric mucosa and may be a risk factor for several gastrointestinal lesions including gastric ulcer and cancer. However, some studies suggested that capsaicin may have a beneficial effect on human peptic ulcer and certain type of cancer (Yun, 1999). In animal carcinogenesis, capsaicin was able to inhibit cancer development in a multiple organ such as stomach, lung, and liver (Yun, 1999).

Rotenone (Fig. 4b) is a naturally occurring pesticide derived from *Derris* and *Lonchorcarpus* species root and back, relatively harmless for mammals, especially after oral administration. Rotenone, deguelin and related compounds (rotenoids) are the active ingredients of botanical insecticides used for at least 150 years to control crop pests. They have been used even longer as fish poisons by native tribes to obtain food in South America and East Africa and more recently in fish management to achieve the desired balance of species. The acute toxicity of rotenone to insects, fish, and mammals is attributable to inhibition of mitochondrial NADH:ubiquinone oxidoreductase activity as the primary target. Rotenoids are known not only as toxicants but also as candidate chemopreventive agents against liver tumors in mice (Cunningham et al., 1995), mammary tumors in rats (Hansen et al., 1965), and skin tumors in mice (Udeani et al., 1997). Also, rotenoids could inhibit cell proliferation induced by peroxisome proliferators in mouse liver (Cunningham et al., 1995), and deguelin and three of its derivatives could inhibit phorbol ester-induced ODC activity as a biomarker of cancer chemopreventive potency (Gerhauser et al., 1997; Gerhauser et al., 1995).

In order to determine, we investigated the modifying effects of dietary feeding with administration of capsaicin and rotenone on AOM-induced colon tumorigenesis were investigated in male F344 rats. Gavage with capsaicin and rotenone significantly elevated phase II enzymes, GST and QR, in the liver and colon. In an ACF bioassay, feeding with capsaicin and rotenone at a dose of 500 ppm for 4 weeks significantly inhibited ACF formation induced by AOM (20 mg/kg body weight, once a week for 2 weeks). In a subsequent long-term study designed to confirm the protective effects of both compounds on ACF development, one group was treated with AOM alone and four other groups received the carcinogen treatment plus diets containing 500 ppm test compounds for 4 weeks (initiation phase) and for 34 weeks (post-initiation phase). Two groups were treated with capsaicin or rotenone alone (500 ppm in diet) and one group was maintained on the basal diet. At the termination of the study, dietary exposure of capsaicin during the initiation phase was found to significantly reduce the incidence of colonic adenocarcinoma (60% vs. 24%, 60% reduction, $P<0.05$). Feeding with rotenone during the post-initiation phase also reduced the frequency of colonic adenocarcinoma (60% vs. 19%, 68% reduction, $P<0.05$).

In this study, both test compounds capsaicin and rotenone elevated phase II enzymes GST and QR in the liver and colon. Dietary feeding with capsaicin and rotenone for 4 weeks during AOM exposure significantly decreased ACF development induced by AOM at weeks 4 and 12. Also, at week 12 the treatments with capsaicin and rotenone significantly suppressed the number of large ACF containing 4 or more crypts, which strongly correlates with tumor formation (Pretlow et al., 1992). These results suggested that the two chemicals could inhibit the growth of colonic ACF and suppress the progression of preneoplasia to malignancy. Subsequent long-term experiments confirmed the results of the pilot study. The data on the incidence of colonic adenocarcinoma indicated that capsaicin could inhibit AOM-induced colon carcinogenesis when fed during the initiation phase, while rotenone exerted its chemopreventive action when fed during the post-initiation phase. This is the first report describing the preventive effects of capsaicin and rotenone in an animal model of colon carcinogenesis. Our data suggest that

capsaicin and rotenone are possible new dietary preventive agents against colon cancer development.

In the study, suppressing effects of rotenone on AOM-induced colon tumorigenesis rather than its blocking effects. The mechanism(s) by which rotenone exert its inhibitory action when fed during the post-initiation are not known, but feeding with rotenone reduced PCNA-labeling index in colonic adenocarcinoma and polyamine level in colonic epithelium. These results may indicate that modulation of cell proliferation by feeding with rotenone accounts in part for its chemopreventive action. Our results suggest possible cancer chemopreventive ability of rotenone. However, Betarbet et al recently reported that chronic exposure of rotenone reproduces several features of human Parkinson's disease in rats (Betarbet et al., 2000), Therefore, chronic toxicity studies of rotenone should be conducted prior to its use as a chemopreventive drug.

Inhibition of rat colon carcinogenesis by dietary feeding with citrus limonoids, obacunone and limonin

Limonoids are a group of triterpene derivatives present in the *Rutaceae* and *Maliaceae* families. Limonoids, including obacunone (Fig. 4c) and limonin (Fig. 4d), are also found in citrus seeds, commercial citrus juice and *Philodendron amurense* (Kihada). For example, commercial orange juice contains an average of 320 ppm limonoid glucosides (Fong et al., 1989). These glucosides are responsible for delayed bitterness in citrus juices and processed products (Miller et al., 2004). Obacunone and limonin have been reported to enhance GST activity in various organs of mice (Miller et al., 2004). Limonin and nomilin are reported to inhibit forestomach, buccal pouch, lung, and skin carcinogenesis in rodents (Miller et al., 2004). However, the modifying effects of the citrus limonoids obacunone and limonin on large bowel carcinogenesis have not been reported. Therefore, we investigated the modifying effects of dietary administration of the citrus limonoids obacunone and limonin on AOM-induced colon tumorigenesis were investigated in two experiments in male F344 rats. In a pilot study, we examined the modifying effects of obacunone and limonin on AOM-induced (20 mg/kg body wt, once a week for 2 weeks) formation of ACF. Dietary feeding with both compounds at dose levels of 200 and 500 ppm during AOM exposure for 4 weeks ('initiation' feeding) or after AOM treatment for 4 weeks ('post-initiation' feeding) significantly inhibited ACF formation (55-65% reduction by 'initiation' feeding, $P<0.001$; 28-42% reduction by 'post-initiation' feeding, $P<0.05$). In a long-term study designed to confirm the protective effects of obacunone and limonin on ACF development, one group was treated with AOM alone and another four groups received the carcinogen treatment plus diets containing 500 ppm of test compounds for 3 weeks (initiation phase) or 29 weeks (post-initiation phase). Two groups were treated with obacunone or limonin alone (500 ppm in diet) and one group was maintained on the basal diet. At the termination of the study, dietary exposure to obacunone or limonin during the initiation phase was found to have significantly reduced the incidence of colonic adenocarcinoma (72% vs. 25% or 6%, $P<0.005$). Feeding with obacunone or limonin during the post-initiation phase also reduced the frequency of colonic adenocarcinoma (72 versus 13%, $P<0.001$).

In the pilot study, dietary feeding with obacunone and limonin for 4 weeks both during or after AOM exposure significantly decreased development of ACF, suggesting that the two chemicals tested could inhibit the growth of colonic ACF and suppresses the progression of preneoplasia to malignancy. Subsequent long-term experiments confirmed the results of the pilot study and indicated that the suppressing effects of both compounds fed to rats during either the initiation or post-initiation phase were significant. It should be noted, however, that since commercial orange juice contains 320 ppm limonoid glucosides (Fong et al., 1989), the concentrations necessary to achieve the effects observed in our study would be ~12- to 30-fold higher than those obtained from normal dietary ingestion of these limonoids. Our data suggest that obacunone and limonin are possible new dietary preventive agents against colon cancer development.

One possible mechanism for the suppression of colonic tumor development might be through the control of cell proliferation in ACF and/or 'normal appearing' crypts of rats exposed to AOM. Increased cell proliferation is suggested to play an important role in multistage carcinogenesis (Dictor et al., 1999), including colon tumorigenesis (Lipkin, 1988). There is a greater correlation between ACF and reduction in PCNA labeling index in ACF than between ACF and reduction in size of the proliferative component of ACF in rats (Zheng et al., 1997). Over-expression of cyclin D1 has been reported in ACF and adenocarcinoma in the mouse colon (Wang et al., 1998). Over-expression of cyclin D1 plays an important role and is an early event in colon tumorigenesis. Therefore, we suspect that dietary administration of obacunone and limonin post-AOM injections might lower cell proliferation activity in ACF and/or colonic tumors.

The results of our study clearly demonstrate the inhibitory effects of dietary obacunone and limonin on AOM-induced colon tumorigenesis. Further experiments, including pre-clinical efficacy and mechanistic studies, are warranted to fully evaluate these natural compounds for their cancer preventive properties and to understand their mode of action. Additional toxicity studies, such as genotoxicity, reproduction toxicity, acute oral toxicity, and 2-year carcinogenicity trials should also be conducted prior to their use as chemopreventive drugs. One advantage of these compounds as chemopreventive agents in human trials is that, unlike synthetic chemopreventive agents, they are naturally occurring compounds that are produced endogenously in edible plants and are present in human foods.

Inhibition of rat colon carcinogenesis by dietary feeding with a citrus polymethoxy flavonoid, nobiletin

Citrus fruit is a rich source of cancer inhibiting agents (Tanaka et al., 2001a). Nobiletin (5,6,7,8,3',4'-hexamethoxyflavone) is a polymethoxy flavonoid extracted from citrus fruits (Fig. 4e) (Montanari et al., 1998). The compound is reported to inhibit proliferation of human cancer cells (Kandaswami et al., 1991) and exert anti-mutagenic activity (Wall et al., 1988). These

findings suggest a possible inhibitory effect of nobiletin on colon carcinogenesis. In the current study, the possible modifying effect of nobiletin on AOM-induced rat colon tumorigenesis was investigated. Also, several biomarkers for cancer chemoprevention studies were assayed for mechanistic investigation.

Firstly, we conducted the ACF bioassay to determine the modifying effects of dietary feeding with a polymethoxyflavonoid nobiletin isolated from *Citrus unshiu* on the development of AOM-induced colonic ACF in male F344 rats. We also assessed the effects of nobiletin on cell proliferation activity of ACF using a monoclonal antibody MIB-5. Rats were given subcutaneous injections of AOM (15 mg/kg body weight) once a week for 3 weeks to induce ACF. They also received the experimental diet containing 100 ppm or 500 ppm nobiletin for 5 weeks, starting one week before the first dosing of AOM. AOM exposure produced 139 ± 35 ACF/rat at the end of the study (week 5). Dietary administration of nobiletin caused significant reduction in the frequency of ACF: 70 ± 15 (50% reduction, $P<0.001$) at a dose of 100 ppm and 63 ± 10 (55% reduction, $P<0.001$) at a dose of 500 ppm. Feeding with nobiletin significantly lowered MIB-5-index in ACF. Also, dietary administration of nobiletin significantly reduced prostaglandin (PG) E_2 content in the colonic mucosa. These findings might suggest possible chemopreventive ability of nobiletin, through suppression of cell proliferating activity of ACF, in the development of ACF.

Subsequently the experiment was conducted to investigate the inhibitory effects of dietary feeding with citrus nobiletin on AOM-induced rat colon carcinogenesis using a long-term bioassay. Five-week old male F344 rats were initiated with two weekly subcutaneous injections of AOM (20 mg/kg bw) to induce colonic tumors. They were also given the diets containing 100 ppm or 500 ppm nobiletin for 34 weeks, starting one week after the last dosing of AOM. At the end of the study, the incidence of colonic adenocarcinoma were 67% in the AOM alone group, 55% in the AOM→100 ppm nobiletin group, 35% ($P<0.05$) in the AOM→500 ppm nobiletin group. Also, feeding with nobiletin reduced the cell-proliferation activity, increased the apoptotic index, and decreased the PGE_2 content in colonic adenocarcinoma and/or colonic mucosa. These findings might suggest that citrus nobiletin has chemopreventive ability against AOM-induced rat colon carcinogenesis.

The results indicate that dietary feeding with nobiletin effectively suppresses AOM-induced large bowel carcinogenesis in rats. Nobiletin was reported to inhibit increased cell-proliferation activity (Kandaswami et al., 1991). In this study, feeding with nobiletin caused reduction in expression of cell proliferation biomarkers such as PCNA-labeling index in colonic tumors and polyamine level in non-lesional colonic mucosa. In addition, dietary nobiletin increased apoptotic index in the colonic adenocarcinoma, as found in an in vitro study (Zheng et al., 2002). Thus, it likely that the inhibition of AOM-induced colonic adenocarcinoma formation for animals consuming nobiletin is due in part to the alteration of cell proliferating activity in the colonic mucosa and neoplasms.

In this study, administration of nobiletin reduced biosynthesis of PGE_2 in colonic adenocarcinoma and in their surrounding mucosa. Eicosanoids including PGE_2, the metabolites of arachidonic acid (AA) through the lipoxygenase (LOX) and cyclooxygenase (COX) pathways, have a variety of biological activities. AA products synthesized via these pathways could modulate colon carcinogenesis (Bennett et al., 1987) and some inhibitors of the AA cascade possess chemopreventive activity in colon carcinogenesis (Cuendet and Pezzuto, 2000; Steele et al., 1999). Although, we did not investigate expression of COX and LOX in colonic mucosa in the current study, nobiletin, reported to suppress the COX-2 expression in RAW 264.7 cells treated with lipopolysaccharide (Murakami et al., 2000) and interferon (IFN)-γ, suggesting that nobiletin may affect both pathways of AA. The results of this study suggest that dietary nobiletin has a beneficial effect on chemically induced rat colon carcinogenesis. Our findings and recent studies on possible anti-metastatic ability of nobiletin (Minagawa et al., 2001; Sato et al., 2002) may suggest need for further investigations of biological functions and its mechanisms of nobiletin for fighting cancer development. In this context, our recent study indicating that nobiletin has anti-genotoxic effects against tobacco-specific nitrosamine-induced mouse lung tumorigenesis is of interest (Ikeda M, et al., submitted).

Suppression of colon carcinogenesis by feeding with a polyphenolic anti-oxidant flavonoid, silymarin in rats

Silymarin, the collective name for an extract from milk thistle [*Silybum marianum* (L.) Gaertneri] is a naturally occurring polyphenolic flavonoid anti-oxidant (Valenzuela et al., 1986). It is composed mainly (~80%, w/w) of silybin (also called silybinin, silibin or silibinin), with smaller amounts of other stereoisomers, such as isosilybin, dihydrosilybin, silydianin and silychristin. Silymarin protects experimental animals against the hepatotoxin a-amanitin and has a strong anti-oxidant property. Other biologic properties of silymarin and its components have been reported, including inhibition of LOX (Fiebrich and Koch, 1979a) and PG syntheses (Fiebrich and Koch, 1979b). For over 20 years, silymarin has been used clinically in Europe for the treatment of alcoholic liver disease and as an anti-hepatotoxic agent. As a therapeutic agent, it is well tolerated and largely free of adverse effects (Comoglio et al., 1990). It might be a potent anti-carcinogen against in vitro and in vivo carcinogenesis. However, animal chemopreventive studies have been mainly limited to skin (Katiyar et al., 1997; Lahiri-Chatterjee et al., 1999) and only few studies have involved the digestive organs, including colon. The silymarin group of flavonoids (silybin, silychristin and silydianin) inhibits xanthine oxidase (Sheu et al., 1998). Silymarin induces G1 arrest in human prostate carcinoma DU 145 cell and causes growth inhibition by inactivation of erbB1-SHC signaling pathway leading to up-regulation of Kip1/p27 followed by its increased binding with CDK causing a decrease in CDK- and cyclin-associated kinase activity (Zi et al., 1998b). These findings led us to evaluate the possible suppressing effects of dietary silymarin on the development of ACF, and early biomarker of colorectal carcinogenesis and colorectal tumors in rats.

The modifying effect of dietary administration of the

polyphenolic anti-oxidant flavonoid silymarin, isolated from milk thistle [*Silybum marianum* (L) Gaertnerf], on AOM-induced colon carcinogenesis was investigated in male F344 rats. In the short-term study, the effects of silymarin on the development of AOM-induced colonic ACF, being putative precursor lesions for colonic adenocarcinoma, were assayed to predict the modifying effects of dietary silymarin on colon tumorigenesis. Also, the activity of detoxifying enzymes, GST and QR, in liver and colonic mucosa was determined in rats that were gavaged with silymarin. Subsequently, the possible inhibitory effects of dietary feeding with silymarin on AOM-induced colon carcinogenesis were evaluated using a long-term animal experiment. In the short-term study, dietary administration of silymarin (100, 500 and 1,000 ppm in diet), either during or after carcinogen exposure, for 4 weeks caused significant reduction in the frequency of colonic ACF in a dose-dependent manner. Silymarin had given by gavage elevated the activity of detoxifying enzymes in both organs. In the long-term experiment, dietary feeding with silymarin (100 and 500 ppm) during the initiation or post-initiation phase of AOM-induced colon carcinogenesis reduced the incidence and multiplicity of colonic adenocarcinoma. The inhibition by feeding with 500 ppm silymarin was significant ($P<0.05$ by 'initiation' feeding and $P<0.01$ by 'post-initiation' feeding). Also, silymarin administration in the diet lowered the PCNA labeling index and increased the number of apoptotic cells in adenocarcinoma. β–Glucuronidase activity, PGE_2 level and polyamines content were decreased in colonic mucosa. These results clearly indicate a chemopreventive ability of dietary silymarin against chemically induced colon tumorigenesis and will provide a scientific basis for progression to clinical trials of the chemoprevention of human colon cancer.

These results clearly indicate that dietary feeding with silymarin effectively suppresses the occurrence of colonic ACF and adenocarcinoma induced by AOM when administered during or after the carcinogen treatment. The results described here are basically in agreement with those of Gershbein (Gershbein, 1994), who found that dietary feeding with silymarin (1,000 ppm) during the entire period of 1,2-dimethylhydrazine (DMH)-induced rat intestinal carcinogenesis significantly inhibited the development of large and small intestinal adenocarcinomas. We did not observe the inhibitory effect of silymarin on the incidence of small intestinal neoplasms. This may due to their low incidence and the use of a different carcinogen. Silymarin inhibited the growth of human breast (Zi et al., 1998a) and prostate (Zi et al., 1998b) cancer cell lines. A chemopreventive effect of silymarin on mouse bladder carcinogenesis (Vinh et al., 2002), rat tongue carcinogenesis, and rat prostate tumorigenesis (Kohno et al., 2005) has been found. Thus, silymarin may possess cancer chemopreventive ability in multiple organs.

Several mechanisms by which chemopreventive agents exert their inhibitory effects on tumorigenesis could be considered. AOM is an intermediate of the colonic carcinogen DMH and is metabolized by cytochrome P-450 2E1 and, possibly, P450 1A, as well as by the phase II carcinogen-detoxifying enzyme GST (Sohn et al., 1991). However, silymarin has no influence in liver P4502E1 (Miguez et al., 1994). We (Tanaka et al., 2001a) and others (Reddy et al., 1993) have reported that certain chemopreventive agents inhibit the development of ACF and carcinoma induced by AOM through induction of GST and QR. Also, epidemiologic observations suggest that consumption of certain cruciferous vegetables reduces the risk of colon cancer in individuals with GSTM1 null type (Lin et al., 1998). Our results on GST and QR activities in liver and colon could explain the decrease in ACF formation and the colon cancer development in rats given silymarin during the initiation phase. In conclusion, dietary administration of silymarin significantly suppressed the development of AOM-induced rat colonic carcinoma, in conjunction with modulation of cell growth in the colonic adenocarcinoma and induction of the phase II enzymes QR and GST in the liver and large intestine, and reduced the levels of β–glucuronidase and PGE_2 in the colorectal mucosa. Further studies to assess the chemopreventive ability of silymarin are needed in different carcinogenesis models. The results described and those reported by others suggest that silymarin has cancer chemopreventive effects in several organs through several mechanisms.

DISCUSSION

As described above, our recent data on the chemopreventive effects of naturally occurring compounds, capsaicin, rotenone, obacunone, limonin, nobiletin, and silymarin, present in edible plants against AOM-induced colon tumorigenesis are described. All these compounds are anti-oxidants. In general, plants are complicated mixtures of numerous chemicals, and interactions with their components may affect the effectiveness of the anti-oxidant. The effectiveness of tested compounds as *in vivo* anti-oxidants has been reported, but the metabolic pathway and action of naturally occurring anti-oxidative compounds is not clear. Flavonoids compounds, which are widely distributed in the plant kingdom and occur in considerable quantities, show a wide range of pharmacological activities other than their anti-oxidative properties. These compounds have been used to treat various pathological conditions including allergies, inflammation, and diabetes. Experimental data including this report showing their anti-tumor activities is accumulating; their chemopreventive potential, however, has not been fully proven clinically. Their behavior and fate should be investigated *in vivo*.

As reported, commonly consumed foods contain non-nutritive compounds capable to inhibit colon cancer in an animal model. The diet provides a rich abundance of these compounds that have the ability to intervene in all phases of carcinogenesis. Mechanisms of action include effects of Phase I and Phase II enzymes activities, interception of DNA mutating agents, and influences on cell proliferation and oncogene activation. Each of these mechanisms has been study in isolation. For explanation of reduced risk for cancer in populations with a greater reliance on fruits and vegetables in the daily diet, future research should focus on potential combinations of foods and the protective components within them.

The association of certain malignancies with chronic inflammation has been recognized for many years (Gordon and Weitzman, 1993). The link between inflammation and subsequent malignancy in visceral sites is known. Examples include large bowel cancer after ulcerative colitis or Crohn's disease (Collins et al., 1987; Gordon and Weitzman, 1993). Central to the concept of inflammation and cancer is the finding that chronic irritation of squamous or glandular epithelium will result in migration of inflammatory cells to the injured site by a mechanism dependent on neutrophils adhesion molecules. These cells, stimulated to produce reactive oxygen species (including superoxide radicals, NO and/or hydroxy radicals) via the respiratory burst and NADPH activation, can function as facilitators in the process of carcinogenesis. There is convincing evidence from animal model systems that prolonged exposure of cells to these products of activated oxygen can result in cell injury and play a role in several stages of carcinogenesis (Tanaka et al., 2005a; Tanaka et al., 2003; Tanaka et al., 2005b). Recently, up-regulation of COX-2, but not COX-1, gene expression was reported in human colorectal neoplasms (Eberhart et al., 1994). New drugs, specific for inhibition of COX-2, may provide effective tumor prevention with reduced side effects (Oshima et al., 1996; Reddy et al., 1996; Sheng et al., 1997). The elevation of COX-2 expression can protect intestinal epithelial cells from apoptosis (Tsuji and DuBois, 1995). Certain COX-2 inhibitors can induce apoptosis (Hara et al., 1997) and inhibit tumor angiogenesis (Tsuji et al., 1998). Elegant review on chemopreventive ability of NSAIDs including COX-2 inhibitors against colon tumorigenesis has been published in this journal (Wakabayashi, 2000). More recently, synthetic anti-oxidants have reported to reduce COX-2 expression PG production, and cell proliferation of colorectal cancer cells (Chinery et al., 1998). This may suggest that COX-2 may provide a new chemopreventive target in colorectal malignancies (Rustgi, 1998), if there are the natural products being a specific inhibitor of COX-2 expression in edible plants.

From the evidence mentioned above, our search for chemopreventives against colon cancer focuses on several flavonoids and some other compounds possessing certain biological activities including anti-inflammatory and/or anti-oxidative properties present in foods. Approximately 2,000 individual members of the flavonoid class have been described and the flavonoids are consumed in rather large amounts through dietary vegetables and fruits.

OTHER NON-NUTRITIVES THAT MAY EXERT SUPPRESSIVE EFFECTS ON RAT COLON TUMORIGENESIS

Our recent studies demonstrated that juices rich in hesperidin and β-cryptoxanthin could inhibit AOM-induced rat colon tumorigenesis (Kohno et al., 1999; Tanaka et al., 2000a). Juices rich in hesperidin and β-cryptoxanthin also inhibit lung tumorigenesis in mice (Kohno et al., 2001a). Thus, citrus fruit is a rich source of cancer inhibiting agents (Tanaka et al., 2001a). The rhizomes of *Zingiber zerumbet* Smith are used for anti-inflammatory folk medicine in Indonesia (Elliott and Brimacombe, 1987). A sesquiterpene zerumbone isolated from the rhizome is a potent inhibitor of 12-*O*-tetradecanoyl-13-acetate-induced Epstein-Barr virus activation (Murakami et al., 1999), and expression of inducible nitric oxide synthase (iNOS) and COX-2 expression in RAW 264.7 macrophages treated with lipopolysaccharide and interferon-γ and NO/O_2^- generation in leukocytes (Murakami et al., 2002). We demonstrated that dietary feeding with zerumbone is able to suppress AOM-induced ACF formation in rats (Tanaka et al., 2001d). A polyisoprenylated benzophenone, garcinol (also named camboginol) is present in *Guttiferae*. Dried rind of *G. indica* ('Kokum') containing garcinol (2-3%, w/w) is used as a garnish for curry and in traditional medicine in India. We have recently found the inhibitory effects of garcinol on AOM-induced ACF (Tanaka et al., 2000b). On-going long-term experiments will provide the data showing that these compounds could modify (possibly inhibit) colonic carcinoma development. Ferulic acid (FA), widely found in bran from rice, wheat and barley, vegetables, and other edible plants, is able to inhibit chemically-induced colon carcinogenesis (Kawabata et al., 2000). Recently, Tsuda's group synthesized a new chemical, 3-(4'-geranyloxy-3-methoxyphenyl)-2-propenoate (EGMP), from the parent compound FA by adding a geranyl chain. They tested the chemopreventive efficacy of EGMP and FA in AOM-induced ACF, since the compound is more potent anti-oxidant than FA. They concluded that both compounds are effective in reducing ACF formation and the effect of EGMP is more potent than FA (Han et al., 2001).

FUTURE PROSPECTIVE

An important component of the chemopreventive agent development research is the identification and characterization of intermediate biomarkers (Armstrong and Taylor, 2005; Tanaka, 1997a) that may serve as surrogate end points for cancer incidence reduction in chemoprevention clinical trials. Such effort is critical to the progress of chemoprevention and potential for cost-effective development of chemopreventive research.

ACF were first reported in rodents injected with AOM by Bird in 1987 (Bird, 1987) and similar lesions were characterized in humans in 1991 (Pretlow et al., 1991) and 1994 (Pretlow et al., 1994b) by Pretlow; since then, the AOM-induced ACF model has been the most widely used animal model system for evaluating naturally occurring compounds (flavonoids, carotenoids, green tea, etc.) as well as synthetic chemicals COX-2 inhibitors, iNOS inhibitors and peroxisome proliferators-activator receptor (PPAR)-γ agonists for their colon cancer chemopreventive efficacy (Corpet and Pierre, 2003; Corpet and Tache, 2002; Tanaka et al., 2001a). The growth dynamics, morphological and molecular features of ACF support the contention that ACF are putative preneoplastic lesions. For instance, ACF have a hyper-proliferative epithelium and their size increases with time (Dashwood et al., 2001; McLellan et al., 1991a; McLellan et al., 1991b; Roncucci et al., 1993). The nuclear atypia observed in some ACF are similar to those seen in the crypts of adenocarcinomas in colons (McLellan et al., 1991a). Furthermore, identification of dysplasia and monoclonality strongly Inks this lesion to neoplastic progression

(Siu et al., 1999). Recently, two new types of lesion have been described in the AOM-induced ACF model. Yamada *et al.*, identified new possible precursor lesions β-catenin accumulated crypts (BCAC, Fig. 1e) for colon carcinoma in the whole-mount preparations of the colon in rats exposed to AOM by using immunohistochemical method (Yamada et al., 2000; Yamada et al., 2001). The lesions are different in their morphology and location from ACF. In the lesions, accumulation are different is more prominent than did not present a ACF-like appearance (Yamada et al., 2000). Cell proliferation activity estimated by counting the number of AgNORs/nucleus in the lesions is also greater than in ACF (Yamada et al., 2001). In addition, Cademi et al. (Cademi et al., 2003; Femia et al., 2005; Femia et al., 2004) have identified mucin depleted foci (MDF) in unsectioned colon stained with high-iron diamine alcian blue (HID-AB). These newly described lesions are not yet well characterized and we do not know if BCAC and MDF are related lesions. It is interesting to note that BCAC, like MDF, have a low production of mucins and are thought to be premalignant lesions rather than preneoplastic lesions. A recent review article described significance of these three lesions (ACF, BCAC, and MDF) in colon carcinogenesis (Mori et al., 2005). Since, ACF are widely accepted as a reliable end point in experimental colon carcinogenesis, this study reports the effects of herbal supplements on the 'classical' ACF. We should thus estimate chemopreventive efficacy of non-nutritives in edible plants reported using both ACF and these new lesions as biological markers for colon carcinogenesis in future studies. Since ligands for PPARs can inhibit AOM-induced ACF, which weakly express PPARγ (Fig. 1d) (Kohno et al., 2001b; Tanaka et al., 2001b), we are now searching natural compounds acting as a ligand for PPARs (Kohno et al., 2002b; Kohno et al., 2004a; Kohno et al., 2004b). In the near future, we would like to provide promising non-nutritive compounds (including citrus compounds, auraptene and nobiletin) with less toxicity in Asian edible plants (Tanaka, 1976; Yun, 1999) for use in clinical colon cancer chemoprevention trials. Also, new compounds with more effective chemopreventive effects can be synthesized from the non-nutritive compounds, including collinin in edible plants (Kohno et al., 2006), when a small amount of the parent compound can be isolated (Curini et al., 2004). Known non-nutritive chemopreventive agents with low dose in combination can be considered to obtain a pronounced chemopreventive effect against colon cancer development in future.

Epidemiological studies showed that obesity or diabetes might be one of the risk factors for colon cancer development (McTiernan, 2005). An animal study using *db/db* mice, which have obese and diabetic phenotypes because of disruption of the leptin receptor, demonstrated that they have high susceptibility of colon carcinogenesis (Hirose et al., 2004). *Citrus unshiu* segment membrane (CUSM) contains fiber, flavonoids, and pectin, but its biological activity is unknown. Therefore, we conducted a short-term experiment to determine whether dietary CUSM affects the development of AOM-induced ACF and BCAC in the colon in C57BL/KsJ-*db/db* mice (Suzuki et al. 2006). Male db/db mice were given subcutaneous injections of AOM (15 mg/kg body weight) once a week for 5 weeks. From one week after the last dosing AOM, they received the diet containing 200, 1,000, or 5,000 ppm CUSM for 7 weeks. At week 12, dietary administration of CUSM caused a reduction in the frequency of ACF (53-59% reduction). In addition, the number of BCAC was lowered by the treatment with CUSM (29-62% reduction). Also, pathological alterations (fibrosis) in liver, which were resembled a metabolic disorder, non-alcoholic steatohepatitis (NASH) (Pessayre and Fromenty, 2005) were reduced by feeding with CUSM. NASH may in some cause fibrosis, cirrhosis, and premature death resulting from liver failure. Its prevalence is increasing, and it is probably underestimated as a cause for cirrhosis and/or liver cell cancer. The need for an effective treatment is clear and urgent using an animal model of NASH (Sahai et al., 2004). Our data may indicate that CUSM is useful for reducing the risk for colon carcinogenesis in obesity or diabetes and that for NASH.

CONCLUSION

In conclusion, certain flavonoids and other substances with biological activity including anti-oxidative and/or anti-inflammatory properties, which are present in edible plants including vegetables and fruits, could exert chemopreventive action in rat colon carcinogenesis as shown here. However, more work needs to be done to better understand the underlying mechanism(s) of action and to confirm their safety for use in humans. Since plants are complex mixtures of chemicals, the potential for finding new chemopreventive agents in plants is high. Studies are underway to identify new compounds in edible plants with chemopreventive potential. For screening chemopreventive agents based on different mechanisms, new *in vitro* co-culture model might be useful (Mace et al., 1998) and microarray analysis (Hokaiwado et al., 2004). The effects of these agents on colon carcinogenesis should be carefully studies to assist the discovery and development of new chemopreventive agents, and to understand carcinogenesis mechanisms. Our goal is to develop chemopreventive agents that could be effective in decreasing the risk of colon cancers in general and/or high-risk populations. The strategy was only partially successful; it could give a significant impact on reduction of colon cancer mortality.

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