

2

Chemoprevention by Isothiocyanates

Stephen S. Hecht, PhD

CONTENTS

INTRODUCTION

CHEMOPREVENTIVE EFFICACY OF ISOTHIOCYANATES

MECHANISMS OF CHEMOPREVENTION BY ISOTHIOCYANATES

CONCLUSIONS

ACKNOWLEDGMENT

REFERENCES

1. INTRODUCTION

Isothiocyanates are among the most effective known chemopreventive agents (1). Examples of complete inhibition of carcinogenicity in laboratory animals through relatively low doses of isothiocyanates are not unusual. Many isothiocyanates occur naturally as glucosinolate conjugates in frequently consumed cruciferous vegetables (2). Consistent with the animal carcinogenicity data, three recent epidemiologic studies have demonstrated that human consumption of isothiocyanates in vegetables decreases lung cancer risk (3–5). Other studies have shown that consumption of genus *Brassica* vegetables is protective against lung cancer (6). Collectively, these data indicate that isothiocyanates have substantial potential for chemoprevention of human cancers.

More than 100 structurally distinct glucosinolates that are precursors of isothiocyanates have been isolated from plants, and these studies have been extensively reviewed (2,7,8). Significant amounts of glucosinolates are found in commonly consumed cruciferous vegetables such as broccoli, cabbage, cauliflower, turnip, horseradish, watercress, and Brussels sprouts. When the raw vegetables are chewed or otherwise macerated, cells are broken and myrosinase—an enzyme that is normally separated cellularly from the glucosinolates—comes into contact with them and catalyzes hydrolysis, as illustrated in Fig.1. Isothiocyanates are common products of this reaction, although other products such as indole-3-carbinol, in the case of glucobrassicin

(R=3-indolyl-), are also formed. Normal portions of these raw vegetables will release multi-milligram amounts of isothiocyanates, but cooking, which inactivates myrosinase, sharply decreases the isothiocyanate dose. The daily dose of isothiocyanates from raw vegetables greatly exceeds the dose of strong carcinogens from cigarettes (1). Isothiocyanates are plant defense compounds, and are responsible for the sharp taste often associated with these vegetables. Some of these naturally occurring isothiocyanates have received considerable attention as chemopreventive agents. Prominent among these are 2-phenylethyl isothiocyanate (PEITC), which is found in watercress and Chinese cabbage as its conjugate gluconasturtiin ($R = \text{PhCH}_2\text{CH}_2-$), and sulforaphane, which is found in broccoli as its conjugate glucoraphanin ($R = \text{CH}_3\text{S}(\text{O})\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2-$). PEITC has entered human clinical trials. Broccoli sprouts, which are rich in glucoraphanin, are now sold as dietary supplements and can be found on the shelves of upscale grocery stores.

Synthetic isothiocyanates—in many cases structural analogs of naturally occurring isothiocyanates with chemopreventive activity—have also been investigated. Several of these compounds have chemopreventive properties that are many orders of magnitude greater than those of the naturally occurring compounds. However, problems with toxicity—and in some cases, enhancement of carcinogenicity—have hindered the development of these compounds as chemopreventive agents.

This chapter reviews efficacy data for isothiocyanates as inhibitors of carcinogenesis and discusses recent

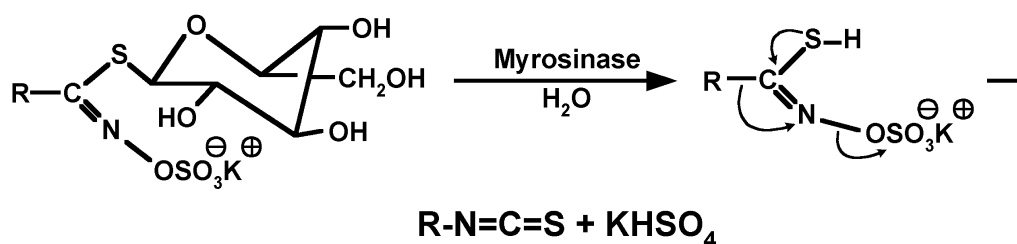


Fig. 1. Conversion of glucosinolates to isothiocyanates, catalyzed by myrosinase.

developments related to our understanding of mechanisms of chemoprevention by isothiocyanates.

2. CHEMOPREVENTIVE EFFICACY OF ISOTHIOCYANATES

Table 1 summarizes the literature on inhibition of carcinogenesis by isothiocyanates. A significant number of isothiocyanates, both naturally occurring and synthetic, have been tested. Naturally occurring isothiocyanates with chemopreventive activity include benzyl isothiocyanate ($R = \text{PhCH}_2$, BITC), PEITC, 3-phenylpropyl isothiocyanate ($R = \text{PhCH}_2\text{CH}_2\text{CH}_2$, PPITC), and sulforaphane. Among these, BITC and PEITC are the most extensively studied.

BITC is an effective inhibitor of rat mammary and mouse lung tumorigenesis by the polycyclic aromatic hydrocarbons (PAH) 7,12-dimethylbenz[*a*]anthracene (DMBA) and benzo[*a*]pyrene (B[*a*]P) (9–12). In mouse lung tumorigenesis experiments, gavage of BITC 15 min prior to treatment with B[*a*]P inhibits lung-tumor multiplicity by as much as 80%, greater than achieved under the same conditions with equimolar doses of either sulforaphane or the well-known antioxidant chemopreventive agent butylated hydroxyanisole (13). Gavaged BITC is also a strikingly potent inhibitor of lung-tumor induction by two other PAH: 5-methylchrysene (5-MeC) and dibenz[*a,h*]anthracene (DB[*a,h*]A) (13). This may be significant because PAH are acknowledged to be important carcinogens in tobacco smoke and certain occupational environments (14,15). BITC appears to be less effective when given in the diet, perhaps because palatability limits the dose. Mixed results have been obtained in studies of BITC as an inhibitor of nitrosamine carcinogenesis. It has no effect on mouse lung tumor induction by the tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) or by *N*-nitrosodiethylamine (DEN), and it has no effect on esophageal tumor

induction in rats by *N*-nitrosobenzylmethylamine (NBMA) (12,16–18). It inhibited rat liver tumor induction by DEN, but enhanced rat bladder tumor induction by a mixture of DEN and *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine (OH-BBN) (19,20).

In contrast to BITC, PEITC has broad inhibitory activity against tumors induced by *N*-nitrosamines. This includes inhibition of lung tumorigenesis in mice and rats by NNK, inhibition of liver tumor induction by DEN in the mouse, inhibition of esophageal-tumor induction by NBMA in the rat, and inhibition of lung and pancreatic tumorigenesis by *N*-nitrosobis(2-oxopropyl)amine (BOP) in the hamster.

Inhibition of NNK-induced pulmonary carcinogenesis by PEITC has been demonstrated in multiple studies in mice and rats (16,21–26). Dietary PEITC is particularly effective. In one study, complete inhibition of lung-tumor induction was achieved in F344 rats treated with NNK in the drinking water and PEITC in the diet (3 $\mu\text{mol/g}$ diet) (23). Dietary PEITC is also an effective inhibitor of lung-tumor induction by NNK in mice (21). PEITC (3 or 6 $\mu\text{mol/g}$ diet) completely inhibited esophageal tumor induction by NBMA in rats (27). When PEITC (100 or 10 μmol) was given by gavage 2 h prior to treatment of hamsters with BOP, virtually complete inhibition of lung tumor induction was observed (28). Pancreatic tumorigenesis was also inhibited at the higher dose of PEITC. These striking results clearly demonstrate the efficacy of PEITC in these models. However, PEITC has limited efficacy against PAH. Both gavaged and dietary PEITC failed to inhibit B[*a*]P-induced lung tumorigenesis in mice (11,21,29). Mixed results have been obtained in the DMBA rat mammary tumor model. Initial studies by Wattenberg, in which PEITC was given by gavage, showed inhibition of mammary tumorigenesis (9). A study by Lubet et al. in which PEITC was given in the diet showed no effect or somewhat enhanced mammary tumorigenesis by DMBA (30). However, another dietary study demonstrated that carcinoma volume, but

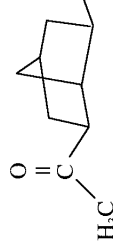
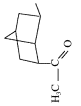
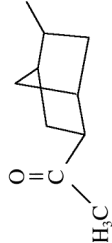
Table 1
Inhibition of Carcinogenesis by Isothiocyanates

<i>Isothiocyanate</i> <i>R-N = C = S; R =</i>	<i>Naturally</i> <i>Occurring:^a</i>	<i>Carcinogen^b</i>	<i>Species and Target Organ</i>	<i>Effect</i>	<i>Reference</i>
α -Naphthyl-	No	3'-Me-DAB	Rat liver	inhibition	112
		Ethionine	Rat liver	inhibition	113
		AAF	Rat liver	inhibition	113
		DAB	Rat liver	inhibition	114
		<i>m</i> -toluylenediamine	Rat liver	inhibition	115
		DEN	Rat liver	no effect	116
		OH-BBN	Rat bladder	inhibition	117
		DAB	Rat liver	inhibition	114
		DMBA	Rat mammary	inhibition	9
		NNK	Mouse lung	no effect	16
β -Naphthyl- Ph-	No	DMBA	Rat mammary	inhibition	9,10
			Mouse forestomach	inhibition	9
			Mouse lung	inhibition	9
			Mouse lung	inhibition	11-13
			Mouse forestomach	inhibition or no effect	11,12
			Mouse lung	inhibition	13
			Mouse lung	inhibition	13
			Mouse lung	no effect	16,17
			Mouse forestomach	inhibition	12
			Mouse lung	no effect	12
Ph(CH ₂) ₂ -	Yes		Rat liver	inhibition	19
			Rat small intestine/colon	inhibition	118
			Rat esophagus	no effect	18
			Rat bladder	enhancement	20
			Mouse lung	no effect	21
			Rat mammary	inhibition or no effect	9,30,31
			Mouse forestomach	inhibition	9
			Mouse lung	inhibition	9
			Rat lung	inhibition	22-24
			Rat nasal cavity, liver	no effect	24
			Mouse lung	inhibition	16,25,26,32,46,119,120

(continued)

Table 1 (continued)

<i>Isothiocyanate</i> <i>R-N = C = S; R=</i>	<i>Naturally</i> <i>Occurring?^a</i>	<i>Carcinogen^b</i>	<i>Species and Target Organ</i>	<i>Effect</i>	<i>Reference</i>	
24	PhCH ₂ - + Ph(CH ₂) ₂ -	Yes	DEN	Mouse lung	no effect	17
			NBMA	Mouse liver	inhibition	120
			BOP	Rat esophagus	inhibition or no effect	18,27,122
				Hamster pancreas and lung	inhibition or no effect	28,123
				Hamster liver	no effect	
			B/a/P	Mouse lung	no effect	11,21,29
				Mouse skin	no effect	11
			DEN + OH-BBN	Rat bladder	enhancement	20
			B/a/P + NNK	Mouse lung	inhibition or no effect	21
			Environmental tobacco smoke, gas phase	Mouse lung	no effect	124
			B/a/P + NNK	Mouse lung	inhibition or no effect	21
			Environmental tobacco smoke, gas phase	Mouse lung	no effect	34
			NNK	Mouse lung	inhibition	25,26
			NBMA	Rat esophagus	inhibition	18
	Ph(CH ₂) ₃ -	Yes	BOP	Hamster lung	inhibition	38
				Hamster pancreas, liver, kidney	no effect	38
			NNN	Rat esophagus	inhibition	36
			B/a/ P + NNK	Mouse lung	inhibition	37
			NNK	Mouse lung	inhibition	25,26
			NBMA	Rat esophagus	inhibition	18
			BOP	Hamster pancreas and lung	inhibition	43
				Hamster liver	enhancement	43
			NNK	Mouse lung	inhibition	26
			NNK	Mouse lung	inhibition	26,46,119
				Rat lung	inhibition	22,125
			B/a/P	Mouse skin	no effect	11
			NBMA	Rat esophagus	enhancement or no effect	50,52
			AOM	Rat colon	enhancement	51
	Ph(CH ₂) ₄ -	Yes				
	Ph(CH ₂) ₅ -	No				
	Ph(CH ₂) ₆ -	No				

Ph(CH ₂) ₈ ⁻	No	NNK	Mouse lung	inhibition	42
Ph(CH ₂) ₁₀ ⁻	No	NNK	Mouse lung	inhibition	42
PhCH(Ph)CH ₂ ⁻	No	NNK	Mouse lung	inhibition	42
PhCH ₂ CH(Ph) ⁻	No	NNK	Mouse lung	inhibition	42
CH ₂ =CHCH ₂ ⁻	Yes	NNK	Mouse lung	no effect	42
CH ₃ (CH ₂) ₅ ⁻	Yes	NNK	Mouse lung	inhibition	42
CH ₃ (CH ₂) ₃ CH(CH ₃) ⁻	?	NNK	Mouse lung	inhibition	42
CH ₃ (CH ₂) ₁₁ ⁻	No	NNK	Mouse lung	inhibition	42,44
3-PyrC(CH ₂) ₃ ⁻	No	NNK	Mouse lung	no effect	25
					
9-Phenanthryl ⁻	No	B[a]P	Mouse skin	no effect	11
9-Methylenepheneanthryl ⁻	No	B[a]P	Mouse skin	no effect	11
6-Chrysenyl ⁻	No	B[a]P	Mouse skin	no effect	11
6-Benzo[a]pyrenyl ⁻	No	B[a]P	Mouse skin	no effect	11
CH ₃ S(CH ₂) ₄ ⁻	Yes	DMBA	Rat mammary	inhibition	39
					
CH ₃ S(CH ₂) ₆ ⁻	Yes	B[a]P	Mouse lung	no effect	13
					
					
					
No	No	NNK	Mouse lung	inhibition	126
No	No	DMBA	Rat mammary	inhibition	39
No	No	DMBA	Rat mammary	inhibition	39
No	No	DMBA	Rat mammary	inhibition	39
No	No	DMBA	Rat mammary	inhibition	39

^aBased on Fenwick et al. (8)

^bAbbreviations: AAF, 2-acetylaminofluorene; AOM, azoxymethane; B[a]P, benzo[a]pyrene; BOP, *N*-nitrosobis(2-oxopropyl)amine; 3'-Me-DAB, 3'-methyl-4-dimethylaminoazobenzene; DAB, 4-dimethylaminoazobenzene; DB[a,h]A, dibenz[a,h]anthracene; DEN, *N*-nitrosodiethylamine; DMBA, 7,12-dimethylbenz[a]anthracene; MAM, methylazoxymethanol acetate; 5-MeC, 5-methylchrysene; NBMA, *N*-nitrosobenzylmethylamine; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; OH-BBN, *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine

not multiplicity or incidence, was decreased by PEITC (31). The effects of PEITC on carcinogenesis by PAH require further investigation.

The contrasting effects of BITC and PEITC on tumorigenesis by PAH and *N*-nitrosamines are interesting and require further study. Gavaged, but not dietary, BITC is a very effective inhibitor of PAH-induced mouse-lung tumorigenesis yet has little effect on tumorigenesis by nitrosamines. Dietary PEITC is a strong inhibitor of tumorigenesis in several *N*-nitrosamine models. In mice, dietary PEITC appears to be more effective than gavaged PEITC as an inhibitor of NNK-induced lung tumorigenesis (21,32). PEITC has little impact on tumorigenesis by PAH in mice. With these results in mind, mixtures of BITC and PEITC have been tested as inhibitors of lung tumor induction in mice by mixtures of NNK and B[a]P (21). Dietary BITC plus PEITC inhibited lung tumor induction by a mixture of NNK and B[a]P. Dietary BITC alone had no effect against NNK and B[a]P, and dietary PEITC did not inhibit lung tumorigenesis by B[a]P. Therefore, it was concluded that inhibition of NNK plus B[a]P-induced lung tumorigenesis by dietary BITC plus PEITC was mainly caused by inhibition of NNK-induced lung tumors by PEITC. Mechanistic studies support this conclusion (33). Gavaged PEITC plus BITC had modest or no effect on lung tumor induction by a mixture of NNK and B[a]P (21).

Recently, a model has been developed in which "environmental tobacco smoke," consisting of 89% sidestream and 11% mainstream cigarette smoke, induces a small but reproducible and significant increase in lung tumor multiplicity in A/J mice (34). Studies have shown that this increase in tumor multiplicity is the result of a component of the gas phase of tobacco smoke, and not B[a]P, NNK, or other well-known carcinogens in the particulate phase. These results appear to conflict with much of the available data on tumor induction by tobacco smoke and its constituents (35). A potential problem with the model is the significant weight loss among the mice treated with smoke. Dietary PEITC and a mixture of dietary BITC and PEITC were tested in this model, but neither had any effect on lung tumor multiplicity (34).

PPITC is a very effective inhibitor of carcinogenesis by *N*-nitrosamines. PPITC (0.4, 1.0, or 2.5 $\mu\text{mol/g}$ diet) inhibited NBMA-induced esophageal tumorigenesis in rats by 90–100% (18). It also virtually completely inhibited esophageal tumorigenesis in rats induced by the tobacco-specific *N*-nitrosamine *N'*-nitrosoornico-

tine (NNN) (36). PPITC was more effective than PEITC in the rat NBMA esophageal tumor model, and was also more effective than PEITC as an inhibitor of lung tumorigenesis induced by NNK in mice (18,26). It was also a strong inhibitor of lung tumor igenesis induced by a mixture of B[a]P and NNK in mice (37). PPITC was a very effective inhibitor of lung tumorigenesis induced in hamsters by BOP, but less effective than PEITC in this model (38). It had no effect on pancreatic tumors induced by BOP, in contrast to the inhibitory effects of PEITC.

Limited data are available on chemoprevention by sulforaphane, perhaps because it is expensive and difficult to synthesize in large quantities. It inhibited rat mammary tumor induction by DMBA but was ineffective as an inhibitor of mouse lung tumor induction by B[a]P (13,39). Sulforaphane also had no effect on lung tumor induction by NNK in mice (40). Sulforaphane and PEITC both significantly reduced the formation of colonic aberrant crypt foci (ACF) in F344 rats treated with azoxymethane (41). Reduction was observed in both the initiation and promotion stages of the experiment. The *N*-acetylcysteine (NAC) conjugates of sulforaphane and PEITC were also effective in the post-initiation phase.

Structure-activity studies demonstrate that increased isothiocyanate lipophilicity increases inhibitory potency against NNK-induced lung tumorigenesis in the A/J mouse (42). Thus, single doses of 10-phenyldecyl isothiocyanate or 1-dodecyl isothiocyanate as low as 0.04–1 μmol are sufficient to inhibit mouse-lung tumorigenesis induced by a single dose of 10 μmol NNK. However, different relationships were found in other tumor models. In the rat esophagus, PPITC was a better inhibitor than PEITC, but extension of the chain length to 4-phenylbutyl (PBITC) decreased activity (18). In the hamster lung, PBITC was less effective than either PPITC or PEITC (43). Other studies have shown that the isothiocyanate group, but not the phenyl ring, is necessary for inhibition, and that lower reactivity with glutathione (GSH) leads to better inhibitory potency in the NNK mouse lung-tumor model (42,44). Several isothiocyanates containing a PAH moiety were tested as potential inhibitors of B[a]P-induced mouse-skin tumorigenesis, but no inhibition of tumorigenicity was observed (11). Synthetic rigid analogs of sulforaphane were effective as inhibitors of rat mammary tumorigenesis in the DMBA model (39).

Isothiocyanates are metabolized by conjugation with GSH, and are ultimately excreted as their NAC conjugates (45). GSH and NAC conjugates of PEITC inhibit lung tumorigenesis induced in A/J mice by NNK when given before the carcinogen (46). NAC conjugates of PEITC and PPITC administered in the diet during the period of carcinogen treatment decreased lung tumorigenesis induced in A/J mice by a mixture of B[a]P and NNK, but the NAC conjugate of BITC was ineffective (37). Higher doses of dietary NAC conjugates of BITC and PEITC inhibited B[a]P-induced lung tumorigenesis in A/J mice when given after the carcinogen, suggesting that isothiocyanates and their conjugates may possess a general ability to inhibit carcinogenesis above and beyond modification of carcinogen metabolism (47). The NAC conjugate of PEITC also inhibits the growth rate of prostate-cancer cells and human leukemia cells in culture (48,49)

Enhancement of tumorigenesis has been observed in some studies of isothiocyanates. Both BITC and PEITC promote urinary-bladder carcinogenesis in rats treated with DEN and OH-BBN, although the dose used was higher than that used for chemoprevention (20). 6-Phenylhexyl isothiocyanate ($R = \text{Ph}(\text{CH}_2)_6$, PHITC), which is not known to be naturally occurring, enhances colon and esophageal carcinogenesis in rat-tumor models (50,51). PHITC administered to rats after the esophageal carcinogen NMBA had modest but insignificant promoting activity, and did not have a significant effect on esophageal cell proliferation (52).

3. MECHANISMS OF CHEMOPREVENTION BY ISOTHIOCYANATES

Most isothiocyanates are active as chemopreventive agents when administered before, or concurrently with, the carcinogen. There are relatively few examples of chemoprevention by isothiocyanates given after carcinogen treatment. This indicates that the major effect of isothiocyanates is favorable modulation of carcinogen metabolism—e.g., inhibition of phase 1 enzymes involved in carcinogen activation and induction of phase 2 enzymes involved in carcinogen detoxification. There is now a large body of evidence in support of this concept. The literature on isothiocyanates and related compounds as inhibitors of phase 1 enzymes and inducers of phase 2 enzymes has been extensively reviewed (1,53–56). More recently, it has become apparent that isothiocyanates and their NAC conjugates have a variety of other cellular

effects that may be pertinent to chemoprevention. Important among these is the induction of apoptosis. This chapter examines the conclusions of the pertinent reviews, and discusses studies that are relevant to mechanisms of isothiocyanate chemoprevention, focusing on those published since 2000.

3.1. Effects on Cytochrome P450 Enzymes

Cytochrome P450 enzymes (P450s) play a critical role in the activation of carcinogens to electrophiles that bind to DNA, producing DNA adducts. The formation of DNA adducts is a necessary step for cancer induction by many carcinogens. Inhibition of P450s involved in carcinogen activation to DNA adducts frequently results in inhibition of tumor formation. Isothiocyanates can selectively inhibit P450s by binding to the apoprotein or heme moiety, or by serving as competitive inhibitors. Inhibition depends both on the structure of the isothiocyanate and the particular cytochrome P450. P450s 1A2, 2B1, and 2E1 are among those that are inhibited by isothiocyanates.

Nakajima et al. studied the inhibition and inactivation of human P450s by PEITC using microsomes from baculovirus-infected insect cells expressing specific human P450s (57). PEITC competitively inhibited P450 1A2 and, to a lesser extent, P450 2A6. PEITC was found to be a very strong noncompetitive inhibitor of P450 2B6. PEITC was also a noncompetitive inhibitor of P450 2C9, and was a mechanism-based inactivator of P450 2E1.

Nakajima's results are generally consistent with other studies. Smith et al. demonstrated that PEITC inhibited P450 1A2-catalyzed metabolism of NNK (58). We found that consumption of watercress, an abundant source of PEITC, altered NNK metabolism in smokers, consistent with inhibition of P450 1A2 (59,60). However, our results do not support an effect of watercress consumption on P450 2A6 activity in humans. Watercress consumption failed to alter metabolism of nicotine to cotinine, and did not significantly affect 7-hydroxylation of coumarin (61,62). Both of these reactions are catalyzed by P450 2A6. Others have observed the inhibitory effects of watercress consumption on P450 2E1-catalyzed reactions in humans (63,64).

The strong inhibitory effect of PEITC on P450 2B6 observed by Nakajima et al. is potentially interesting because this enzyme is a good catalyst of NNK activation (Murphy SE, unpublished data). Conaway et al. also observed that PEITC inhibited P450 2B1-related activities in rat liver microsomes (65).

The effects of BITC on rat and human P450s were studied by Hollenberg's group. BITC is a potent mechanism-based inactivator of rat P450 2B1; inactivation occurs primarily through protein modification (66). BITC is also a mechanism-based inactivator of rat P450s 1A1, 1A2, and 2E1 and human P450s 2B6 and 2D6. It was most effective in inactivating P450s 2B1, 2B6, 1A1, and 2E1. Analysis of BITC metabolites in the P450 2B1 reactions indicated that benzylamine was the major metabolite, suggesting conversion of BITC to benzyl isocyanate, which modified the P450 apoprotein or was hydrolyzed to benzylamine (67). In related work, the inactivation of P450 2E1 by *t*-butyl isothiocyanate was explored (68). The results suggested that *t*-butyl isothiocyanate inactivated P450 2E1 by binding to a critical active-site amino acid residue, which may have acted as the sixth ligand to heme, thus interfering with oxygen and substrate binding. PEITC inactivated P450 2E1, as well as a mutant in which the conserved threonine at position 303 was replaced by alanine (69). BITC did not inactivate the mutant, but inhibited it in a competitive manner. These results indicate differences in the mechanisms by which PEITC and BITC interact with P450 2E1.

Conaway et al. investigated the inhibition of P450-mediated reactions by thiol conjugates of isothiocyanates, which have chemopreventive properties that are analogous to those of the parent isothiocyanates (70). Inhibition of pentoxifyresorufin *O*-dealkylation, for P450 2B1, and ethoxyresorufin *O*-dealkylation, for P450 1A1, roughly paralleled the extent of decomposition of the conjugates to their parent isothiocyanates, suggesting that the parent compounds were responsible for the observed inhibition.

3.2. Effects on Phase 2 Enzymes

Isothiocyanates accumulate in high concentrations in cultured cells, and the resulting levels are related to their abilities to induce phase 2 enzymes such as NAD(P)H:quinone reductase (QR) (71). The intracellular forms of sulforaphane and BITC were found to be dithiocarbamates resulting from GSH conjugation, suggesting that conjugation with GSH is responsible for accumulation of isothiocyanates in murine hepatoma cells (71). Initial uptake rates of four isothiocyanates in MCF-7 human breast cancer cells correlated with GSH conjugation, but not lipophilicity. It was concluded that GSH conjugation is the dominant mechanism affecting isothiocyanate uptake in cells (72). Intracellular accumulation of BITC, PEITC, sulforaphane and allyl

isothiocyanate in mouse-skin papilloma cells correlated with elevations in GSH content, QR activity, and glutathione-*S*-transferase (GST) activity. The elevations were mediated by the DNA regulatory antioxidant/electrophile-response element (ARE/EpRE) (73).

Isothiocyanates are good inducers of QR. The 5'-promoter region of the human QR gene contains the *cis*-acting AP-1 and NF κ B transcription factor-binding sites. Exposure of HT29 human colon cells to BITC caused an increase in AP-1 and NF κ B binding, and activation of c-Jun N-terminal kinase (JNK), which phosphorylates c-Jun, a component of AP-1. These results suggest that JNK is involved in QR induction as an initial event that precedes an increase in transcription-factor binding (74). 6-(Methylsulfinyl)hexyl isothiocyanate, an active principal of wasabi, induced QR in Hepa 1c1c7 cells. Induction of QR transcription involved activation of ARE/EpRE (75). The related compounds 7-(methylsulfinyl)heptyl and 8-(methylsulfinyl)octyl isothiocyanates were also shown to be potent inducers of QR. These isothiocyanates are found in watercress at far lower concentrations than PEITC, but are stronger inducers of QR (76). Several other methyl(sulfinylalkyl) isothiocyanates were also shown to be inducers of QR (77). Sulforaphane was found to be a potent inducer of QR activity in human prostate-cancer-cell lines (78).

BITC is an inducer of GST activity. Nakamura et al. examined the induction of GST by BITC in rat liver epithelial RL34 cells (79). BITC specifically enhanced GSTP1. Addition of BITC to cells resulted in an immediate increase in reactive oxygen intermediates. With different isothiocyanates, the induction of *GSTP1* closely correlated with production of reactive oxygen intermediates. The *GSTP1* enhancer I-containing region was essential for induction of the *GSTP1* gene. These data suggest that production of reactive oxygen intermediates is involved in the induction of *GSTP1* by BITC. Expression of multidrug resistance-associated protein 2 (MRP2), which is an efflux pump that contributes to biliary secretion of xenobiotics, was increased in primary rat and human hepatocytes treated with sulforaphane. Sulforaphane-related formation of reactive oxygen intermediates may have contributed to the MRP2 induction (80).

Kong et al. and Kwak et al. have reviewed the activation of the ARE/EpRE present in many phase 2 genes (54,55). Basic leucine-zipper transcription factors, including nuclear factor-erythroid 2 (NF-E2)-related factor-1 (Nrf1), Nrf2, and small Maf, have been impli-

cated in the binding and transcriptional activation of ARE/EpRE sequences. Activation of the mitogen-activated protein kinase (MAPK) pathway by various isothiocyanates—including PEITC, BITC, and sulforaphane—has been observed, ultimately leading to phase 2 enzyme induction via ARE/EpRE.

The favorable effects of isothiocyanates on phase 1 and phase 2 enzymes should be reflected in decreased DNA binding of carcinogens that are metabolized by these enzymes. Previous studies of isothiocyanate effects on NNK and NBMA provide support for this hypothesis (reviewed in ref. 1). Decreased DNA binding of NNK in the mouse and rat lung is consistent with decreased lung tumorigenesis in animals treated with PEITC. This has been attributed to inhibition by PEITC of specific pulmonary P450s involved in the metabolic activation of NNK (1). In a recent study, we investigated the effects of dietary BITC and PEITC on DNA adduct formation in A/J mice treated with a mixture of B[a]P and NNK (33). Dietary PEITC, or dietary BITC plus PEITC, inhibited the formation of pyridyloxobutyl-DNA adducts of NNK. There were no effects of dietary isothiocyanates on levels of *O*⁶-methylguanine (from NNK) or *N*²-(7,8,9-trihydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene-10-yl)deoxyguanosine (from B[a]P). These results were consistent with our previous studies of the effects of PEITC on NNK-DNA binding in rats, supporting a role for inhibition of pyridyloxobutyl-DNA adducts as a mechanism of inhibition of tumorigenesis by dietary PEITC or BITC plus PEITC. However, the observed inhibition was modest, suggesting that other effects of isothiocyanates were involved.

We also investigated the role of DNA adduct modification in the contrasting effects of BITC and PEITC on lung tumorigenesis in A/J mice treated with B[a]P (81). As discussed previously, BITC but not PEITC inhibits B[a]P-induced tumorigenesis. DNA adducts were measured under conditions closely similar to those used in the tumor studies. Both BITC and PEITC inhibited B[a]P-DNA adduct formation in the lung. Inhibition was modest, and there was no difference between adduct levels in the mice treated with BITC and B[a]P vs PEITC and B[a]P. These results suggest that other effects of BITC may be involved in inhibition of B[a]P-induced lung tumorigenesis in the A/J mouse.

3.3. Other Cellular Effects

These results suggest that isothiocyanates have effects other than modification of DNA adduct formation that are important in chemoprevention. Prominent

among these is induction of apoptosis. A considerable body of evidence now indicates that isothiocyanates induce apoptosis in various systems. BITC and PEITC both induce sustained activation of JNK, and this is associated with induction of apoptosis in various cell types (82). Treatment with isothiocyanates under conditions of apoptosis induction causes rapid and transient induction of caspase-3/CPP32-like activity (83). PEITC induces apoptosis in mouse epidermal JB6 cells through a *p*53-dependent pathway (84). PEITC induces apoptosis in human leukemia cells and inhibits cell growth in this system (48,85). Further studies have shown that the formation of GSH conjugates of PEITC is important during the induction of apoptosis, and that this may lead to depletion of cellular GSH (86). The caspase pathway plays an essential role, and the JNK pathway a supporting role in the induction of apoptosis in HL60 cells by PEITC and allyl isothiocyanate (87). A recent study demonstrates that PEITC further increases apoptosis induced in the respiratory tract of rats by cigarette smoke (88). BITC and sulforaphane induce apoptosis in human colon cancer cells (89–91).

Yang et al. investigated the effects of the NAC conjugates of BITC and PEITC on molecular events associated with apoptosis in the A/J mouse lung (47). Both compounds inhibited B[a]P-induced lung tumorigenesis when administered after the carcinogen. There was a significant increase in apoptosis in the lung. The MAPK pathway was activated in animals treated with the NAC conjugates. The phosphorylation of *p*38 and extracellular signal-regulated kinases (ErKs) 1 and 2 was also induced, and AP-1 activity was increased. Phosphorylation of *p*53 was also higher in the groups treated with the NAC conjugates of BITC and PEITC.

Sulforaphane has antiinflammatory properties. It decreased lipopolysaccharide-induced secretion of pro-inflammatory and pro-carcinogenic signaling factors in cultured Raw 264.7 macrophages. It caused reduction of inducible nitric oxide synthase (iNOS) and cyclooxygenase (COX-2) protein expression. NFκB was identified as the key mediator of these responses (92).

It is apparent that isothiocyanates affect a number of signal-transduction pathways. Kong et al. hypothesize that at low concentrations, isothiocyanates and other chemopreventive agents may activate the MAPK pathway, leading to induction of gene expression such as phase 2 enzymes resulting in protection or survival mechanisms (93). At higher concentrations, they will activate the MAPK pathway and the caspase pathway

will also be activated, leading to apoptotic cell death. This intriguing hypothesis merits further investigation.

3.4. Isothiocyanate Metabolism and its Relationship to Epidemiologic Results

Conjugation of isothiocyanates with GSH and excretion in the urine as NAC conjugates is a well-established metabolic pathway for BITC, PEITC, and allyl isothiocyanate in rats and humans (45,94–99). The disposition and pharmacokinetics of PEITC and PHITC were compared in rats with the goal of gaining insight into the higher efficacy of PHITC as a chemopreventive agent against lung cancer (100). In contrast to PEITC, a relatively small proportion of PHITC metabolites was excreted in urine, and higher effective doses of PHITC were found in the lung and other tissues, which may in part explain its higher activity.

Chung and colleagues have developed a urinary biomarker for total isothiocyanates in human urine (101). Results of analyses of human urine for this biomarker correlated well with levels of NAC conjugates of PEITC or allyl isothiocyanate that were independently analyzed. Application of this assay to urine samples from Singapore residents demonstrated a highly significant positive association between dietary intake and urinary excretion levels of total isothiocyanates (102). Levels of total isothiocyanates in the urine of individuals who consumed fresh or cooked watercress, or fresh or steamed broccoli, were compared. The results demonstrate that, because of the inactivation of myrosinase during cooking or steaming, the total isothiocyanate dose is considerably less upon consumption of cooked or steamed vegetables compared to raw vegetables (103,104). Broccoli sprouts were shown to be a good source of glucosinolates and isothiocyanates, based on analysis of their urinary NAC conjugates (105).

GSH conjugation is important in isothiocyanate metabolism. *GSTM1*, *GSTP1*, *GSTA1*, and *GSTA2* are involved to varying extents in the catalysis of isothiocyanate conjugation with GSH (106). These genes are polymorphic in the human population (107–109). It is proposed that individuals with low or null activity would have higher levels of free circulating isothiocyanates, and could potentially be protected against cancer more effectively than those in whom the isothiocyanates were conjugated (110). Lin et al. demonstrated that individuals exposed to dietary broccoli and who have the *GSTM1*-null phenotype are less at risk for

colon cancer than those who are *GSTM1*-positive (111). This is consistent with a lower conjugation of isothiocyanates in the *GSTM1*-null individuals. These results demonstrate the potential confounding effects of *GST* genotype, since *GSTM1*-null individuals would also be expected to detoxify carcinogens less readily, and therefore would be at higher risk. There is a balance between *GST* catalysis of isothiocyanate conjugation vs carcinogen detoxification.

Three recent epidemiologic studies have examined the relationship between isothiocyanate intake, *GST* genotype, and lung cancer. London et al. examined the relationship between total isothiocyanate concentrations in urine, collected before diagnosis, and the subsequent risk of lung cancer among 232 incident cases of lung cancer and 710 matched controls from a cohort of 18,244 men in Shanghai, China (3). Individuals with detectable isothiocyanates in urine were at decreased risk of lung cancer, and the protective effect was seen primarily among individuals with homozygous deletion of *GSTM1*, and particularly with deletion of both *GSTM1* and *GSTT1*. Spitz et al. examined the relationship of isothiocyanate consumption and *GST* status to lung cancer in 503 newly diagnosed lung cancer cases and 465 controls (4). Cases reported significantly lower isothiocyanate intake per day than controls. Low isothiocyanate intake and *GSTM1*- and *GSTT1*-null genotypes were associated with increased lung cancer risk in current smokers. Zhao et al. evaluated the link between dietary isothiocyanate intake, *GSTM1* and *GSTT1* polymorphisms, and lung cancer risk in 420 Chinese women (5). Higher weekly intake of isothiocyanates reduced the risk of lung cancer to a greater extent in smokers than in nonsmokers. The inverse association was stronger among subjects with homozygous deletion of *GSTM1* and/or *GSTT1*. These results illustrate the complexity of gene-carcinogen and gene-chemopreventive agent interactions in molecular epidemiology. However, there generally was a consistent relationship between isothiocyanate intake and decreased cancer risk.

4. CONCLUSIONS

Isothiocyanates are now firmly established as effective chemopreventive agents in a wide range of animal models. Inhibition is observed in many different tissues and in animals treated with a variety of carcinogens. Complete inhibition of tumor formation is not an unusual occurrence in these studies. Although some isothiocyanates can enhance carcinogenesis in certain

models, the overwhelming proportion of evidence is toward protection. A large body of evidence clearly demonstrates that isothiocyanates can inhibit specific cytochrome P450 enzymes responsible for carcinogen activation, and can induce a variety of phase 2 enzymes responsible for carcinogen detoxification. The mechanisms of inhibition and induction are now very well understood. These combined properties can explain much of the observed tumor-inhibitory effects of isothiocyanates. However, a rapidly increasing number of studies now indicate that isothiocyanates as well as their NAC conjugates have cellular effects above and beyond the alteration of carcinogen metabolism. Isothiocyanates induce a cascade of signal-transduction events that lead to apoptosis. Other potentially beneficial effects have also been observed. Humans receive large doses of isothiocyanates upon consumption of normal amounts of cruciferous vegetables. Robust biomarkers for quantifying these doses are now available. The application of these biomarkers in epidemiologic studies and consideration of gene-isothiocyanate interactions have produced encouraging results indicating that isothiocyanates protect against cancer in humans.

ACKNOWLEDGMENT

Research in the author's laboratory on isothiocyanates is supported by NCI grant CA-46535 from the National Cancer Institute.

REFERENCES

1. Hecht SS. Inhibition of carcinogenesis by isothiocyanates. *Drug Metabol Rev* 2000; 32:395–411.
2. Fahey JW, Zalcman AT, Talalay P. The chemical diversity and distribution of glucosinolates and isothiocyanates among plants. *Phytochemistry* 2001;56:5–51.
3. London SJ, Yuan JM, Chung FL, et al. Isothiocyanates, glutathione S-transferase M1 and T1 polymorphisms, and lung-cancer risk: a prospective study of men in Shanghai, China. *Lancet* 2000;356:724–729.
4. Spitz MR, Duphorne CM, Detry MA, et al. Dietary intake of isothiocyanates: evidence of a joint effect with glutathione S-transferase polymorphisms in lung cancer risk. *Cancer Epidemiol Biomarkers Prev* 2000;9:1017–1020.
5. Zhao B, Seow A, Lee EJ, et al. Dietary isothiocyanates, glutathione S-transferase -M1, -T1 polymorphisms and lung cancer risk among Chinese women in Singapore. *Cancer Epidemiol Biomark Prev* 2001;10:1063–1067.
6. Verhoeven DTH, Goldbohm RA, van Poppel G, et al. Epidemiological studies on *Brassica* vegetables and cancer risk. *Cancer Epidemiol Biomark Prev* 1996;5:733–748.
7. Tookey HL, VanEtten CH, Daxenbichler ME. Glucosinolates, in *Toxic Constituents of Plant Stuffs*. Liener IE, ed. Academic Press, New York, 1980, pp.103–142.
8. Fenwick GR, Heaney RK, Mawson R. Glucosinolates, in *Toxicants of Plant Origin, vol. II: Glycosides*. Cheeke PR, ed. CRC Press, Inc., Boca Raton, FL 1989, pp. 2–41.
9. Wattenberg LW. Inhibition of carcinogenic effects of polycyclic hydrocarbons by benzyl isothiocyanate and related compounds. *J Natl Cancer Inst* 1977;58:395–398.
10. Wattenberg LW. Inhibition of carcinogen-induced neoplasia by sodium cyanate, tert-butyl isocyanate, and benzyl isothiocyanate administered subsequent to carcinogen exposure. *Cancer Res* 1981;41:2991–2994.
11. Lin JM, Amin S, Trushin N, Hecht SS. Effects of isothiocyanates on tumorigenesis by benzo[a]pyrene in murine tumor models. *Cancer Lett* 1993;74:151–159.
12. Wattenberg LW. Inhibitory effects of benzyl isothiocyanate administered shortly before diethylnitrosamine or benzo[a]pyrene on pulmonary and forestomach neoplasia in A/J mice. *Carcinogenesis* 1987;8:1971–1973.
13. Hecht SS, Kenney PMJ, Wang M, Upadhyaya P. Benzyl isothiocyanate: an effective inhibitor of polycyclic aromatic hydrocarbon tumorigenesis in A/J mouse lung. *Cancer Lett* 2002;187:87–94.
14. Hecht SS. Tobacco smoke carcinogens and lung cancer. *J Natl Cancer Inst* 1999;91:1194–1210.
15. International Agency for Research on Cancer. Polynuclear Aromatic Compounds, Part 1, Chemical, Environmental, and Experimental Data. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Lyon, France, IARC, 1983, pp. 62–66.
16. Morse MA, Amin SG, Hecht SS, Chung FL. Effects of aromatic isothiocyanates on tumorigenicity, O⁶-methylguanine formation, and metabolism of the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in A/J mouse lung. *Cancer Res* 1989;49:2894–2897.
17. Morse MA, Reinhardt JC, Amin SG, et al. Effect of dietary aromatic isothiocyanates fed subsequent to the administration of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone on lung tumorigenicity in mice. *Cancer Lett* 1990;49:225–230.
18. Wilkinson JT, Morse MA, Kresty LA, Stoner GD. Effect of alkyl chain length on inhibition of *N*-nitrosomethylbenzylamine-induced esophageal tumorigenesis and DNA methylation by isothiocyanates. *Carcinogenesis* 1995;16:1011–1015.
19. Sugie S, Okumura A, Tanaka T, Mori H. Inhibitory effects of benzyl isothiocyanate and benzyl thiocyanate on diethylnitrosamine-induced hepatocarcinogenesis in rats. *Jpn J Cancer Res* 1993;84:865–870.
20. Hirose M, Yamaguchi T, Kimoto N, et al. Strong promoting activity of phenylethyl isothiocyanate and benzyl isothiocyanate on urinary bladder carcinogenesis in F344 male rats. *Int J Cancer* 1998;77:773–777.
21. Hecht SS, Kenney PMJ, Wang W, et al. Effects of phenethyl isothiocyanate and benzyl isothiocyanate, individually and in combination, on lung tumorigenesis induced in A/J mice by benzo[a]pyrene and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone. *Cancer Lett* 1999;150:49–56.
22. Chung FL, Kelloff G, Steele V, et al. Chemopreventive efficacy of arylalkyl isothiocyanates and N-acetylcysteine for lung tumorigenesis in Fischer rats. *Cancer Res* 1996;56:772–778.
23. Hecht SS, Trushin N, Rigotty J, et al. 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.
24. Morse MA, Wang CX, Stoner GD, et al. Inhibition of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced DNA adduct formation and tumorigenicity in lung of F344 rats by dietary phenethyl isothiocyanate. *Cancer Res* 1989;49:549–553.
 25. Morse MA, Eklind KI, Amin SG, et al. Effects of alkyl chain length on the inhibition of NNK-induced lung neoplasia in A/J mice by arylalkyl isothiocyanates. *Carcinogenesis* 1989;10:1757–1759.
 26. Morse MA, Eklind KI, Hecht SS, et al. Structure-activity relationships for inhibition of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone lung tumorigenesis by arylalkyl isothiocyanates in A/J mice. *Cancer Res* 1991;51:1846–1850.
 27. Stoner GD, Morrissey D, Heur YH, et al. Inhibitory effects of phenethyl isothiocyanate on *N*-nitrosobenzylmethylamine carcinogenesis in the rat esophagus. *Cancer Res* 1991;51:2063–2068.
 28. Nishikawa A, Furukawa F, Uneyama C, et al. Chemopreventive effects of phenethyl isothiocyanate on lung and pancreatic tumorigenesis in *N*-nitrosobis(2-oxopropyl)amine-treated hamsters. *Carcinogenesis* 1996;17:1381–1384.
 29. Adam-Rodwell G, Morse MA, Stoner GD. The effects of phenethyl isothiocyanate on benzo[*a*]pyrene-induced tumors and DNA adducts in A/J mouse lung. *Cancer Lett* 1993;71:35–42.
 30. Lubet RA, Steele VE, Eto I, et al. Chemopreventive efficacy of anethole trithione, *N*-acetyl-L-cysteine, miconazole and phenethylisothiocyanate in the DMBA-induced rat mammary cancer model. *Int J Cancer* 1997;72:95–101.
 31. Futakuchi M, Hirose M, Miki T, et al. Inhibition of DMBA-initiated rat mammary tumour development by 1-*O*-hexyl-2,3,5-trimethylhydroquinone, phenylethyl isothiocyanate, and novel synthetic ascorbic acid derivatives. *Eur J Cancer Prev* 1998;7:153–159.
 32. El Bayoumy K, Upadhyaya P, Desai DH, et al. Effects of 1,4-phenylenebis(methylene)selenocyanate, phenethyl isothiocyanate, indole-3-carbinol, and *d*-limonene individually and in combination on the tumorigenicity of the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in A/J mouse lung. *Anticancer Res* 1996;16:2709–2712.
 33. Sticha KRK, Kenney PMJ, Boysen G, et al. Effects of benzyl isothiocyanate and phenethyl isothiocyanate on DNA adduct formation by a mixture of benzo[*a*]pyrene and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in A/J mouse lung. *Carcinogenesis* 2002;23:1433–1439.
 34. Witschi H. Successful and not so successful chemoprevention of tobacco smoke-induced lung tumors. *Exp Lung Res* 2000;26:743–755.
 35. International Agency for Research on Cancer. Tobacco Smoking. IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Lyon, FR: IARC, 1986:37–375.
 36. Stoner GD, Adams C, Kresty LA, et al. Inhibition of *N*'-nitrososornicotine-induced esophageal tumorigenesis by 3-phenylpropyl isothiocyanate. *Carcinogenesis* 1998;19:2139–2143.
 37. Hecht SS, Upadhyaya P, Wang M, et al. Inhibition of lung tumorigenesis in A/J mice by *N*-acetyl-*S*-(*N*-2-phenethylthiocarbamoyl)-L-cysteine and *myo*-inositol, individually and in combination. *Carcinogenesis* 2002;23:1455–1461.
 38. Nishikawa A, Furukawa F, Ikezaki S, et al. Chemopreventive effects of 3-phenylpropyl isothiocyanate on hamster lung tumorigenesis initiated with *N*-nitrosobis(2-oxopropyl)amine. *Jpn J Cancer Res* 1996;87:122–126.
 39. Zhang Y, Kensler TW, Cho CG, et al. Anticarcinogenic activities of sulforaphane and structurally related synthetic norbornyl isothiocyanates. *Proc Natl Acad Sci USA* 1994;91:3147–3150.
 40. Chung FL, Jiao D, Conaway CC, et al. Chemopreventive potential of thiol conjugates of isothiocyanates for lung cancer and a urinary biomarker of dietary isothiocyanates. *J Cell Biochem [suppl]* 1997;27:76–85.
 41. Chung FL, Conaway CC, Rao CV, Reddy BS. Chemoprevention of colonic aberrant crypt foci in Fischer rats by sulforaphane and phenethyl isothiocyanate. *Carcinogenesis* 2000;21:2287–2291.
 42. Jiao D, Eklind KI, Choi CI, et al. Structure-activity relationships of isothiocyanates as mechanism-based inhibitors of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced lung tumorigenesis in A/J mice. *Cancer Res* 1994;54:4327–4333.
 43. Son HY, Nishikawa A, Furukawa F, et al. Modifying effects of 4-phenylbutyl isothiocyanate on *N*-nitrosobis(2-oxopropyl)amine-induced tumorigenesis in hamsters. *Cancer Lett* 2000;160:141–147.
 44. Jiao D, Smith TJ, Kim S, et al. The essential role of the functional group in alkyl isothiocyanates for inhibition of tobacco-nitrosamine-induced lung tumorigenesis. *Carcinogenesis* 1996;17:755–759.
 45. Brüsewitz G, Cameron BD, Chasseaud LF, et al. The metabolism of benzyl isothiocyanate and its cysteine conjugate. *Biochem J* 1977;162:99–107.
 46. Jiao D, Smith TJ, Yang CS, et al. Chemopreventive activity of thiol conjugates of isothiocyanates for lung tumorigenesis. *Carcinogenesis* 1997;18:2143–2147.
 47. Yang Y-M, Conaway CC, Chiao JW, et al. Inhibition of benzo[*a*]pyrene-induced lung tumorigenesis in A/J mice by dietary *N*-acetylcysteine conjugates of benzyl and phenethyl isothiocyanates during the post-initiation phase is associated with activation of MAP kinases and p53 activity and induction of apoptosis. *Cancer Res* 2002;62:2–7.
 48. Chiao JW, Chung FL, Krzeminski J, et al. Modulation of growth of human prostate cancer cells by the *N*-acetylcysteine conjugate of phenethyl isothiocyanate. *Int J Oncol* 2000;16:1215–1219.
 49. Xu K, Thornalley PJ. Studies on the mechanism of the inhibition of human leukaemia cell growth by dietary isothiocyanates and their cysteine adducts in vitro. *Biochem Pharmacol* 2000;60:221–231.
 50. Stoner GD, Siglin JC, Morse MA, et al. Enhancement of esophageal carcinogenesis in male F344 rats by dietary phenylhexyl isothiocyanate. *Carcinogenesis* 1995;16:2473–2476.
 51. Rao CV, Rivenson A, Simi B, et al. Enhancement of experimental colon carcinogenesis by dietary 6-phenylhexyl isothiocyanate. *Cancer Res* 1995;55:4311–4318.
 52. Hudson TS, Carlton PS, Gupta A, et al. Investigation of the enhancement of *N*-nitrosomethylbenzylamine-induced esophageal tumorigenesis by 6-phenylhexyl isothiocyanate. *Cancer Lett* 2001;162:19–26.

85. Xu K, Thornalley PJ. Inhibition of leukaemia growth and apoptosis induced by phenethyl isothiocyanate and cysteine conjugate. *Br J Cancer* 1999;81:574–575.
86. Xu K, Thornalley PJ. Involvement of glutathione metabolism in the cytotoxicity of the phenethyl isothiocyanate and its cysteine conjugate to human leukaemia cells in vitro. *Biochem Pharmacol* 2001;61:165–177.
87. Xu K, Thornalley PJ. Signal transduction activated by the cancer chemopreventive isothiocyanates: cleavage of BID protein, tyrosine phosphorylation and activation of JNK. *Br J Cancer* 2001;84:670–673.
88. D'Agostini F, Balansky RM, Izzotti A, et al. Modulation of apoptosis by cigarette smoke and cancer chemopreventive agents in the respiratory tract of rats. *Carcinogenesis* 2001;22:375–380.
89. Kirlin WG, Cai J, DeLong M, et al. Dietary compounds that induce cancer preventive phase 2 enzymes activate apoptosis at comparable doses in HT29 colon carcinoma cells. *J Nutr* 1999;129:1827–1835.
90. Bonnesen C, Eggleston IM, Hayes JD. Dietary indoles and isothiocyanates that are generated from cruciferous vegetables can both stimulate apoptosis and confer protection against DNA damage in human colon cell lines. *Cancer Res* 2001;61:6120–6130.
91. Gamet-Payrastré L, Li P, Lumeau S, et al. Sulforaphane, a naturally occurring isothiocyanate, induces cell cycle arrest and apoptosis in HT29 human colon cancer cells. *Cancer Res* 2000; 60:1426–1433.
92. Heiss E, Herhaus C, Klimo K, et al. Nuclear factor kappa B is a molecular target for sulforaphane-mediated anti-inflammatory mechanisms. *J Biol Chem* 2001;276:32,008–32,015.
93. Kong AN, Yu R, Hebbar V, et al. Signal transduction events elicited by cancer prevention compounds. *Mutat Res* 2001;480–481:231–241.
94. Gorler K, Krumbiegel G, Mennicke WH. The metabolism of benzyl isothiocyanate and its cysteine conjugate in guinea-pigs and rabbits. *Xenobiotica* 1982;12:535–542.
95. Mennicke WH, Gorler K, Krumbiegel G. Metabolism of some naturally occurring isothiocyanates in the rat. *Xenobiotica* 1983;13:203–207.
96. Mennicke WH, Görler K, Krumbiegel G, et al. Studies on the metabolism and excretion of benzyl isothiocyanate in man. *Xenobiotica* 1988;18:441–447.
97. Ioannou YM, Burka LT, Matthews HB. Allyl isothiocyanate: comparative disposition in rats and mice. *Toxicol Appl Pharmacol* 1984;75:173–181.
98. Eklind KI, Morse MA, Chung FL. Distribution and metabolism of the natural anticarcinogen phenethyl isothiocyanate in A/J mice. *Carcinogenesis* 1990;11:2033–2036.
99. Chung FL, Morse MA, Eklind KI, Lewis J. Quantitation of human uptake of the anticarcinogen phenethyl isothiocyanate after a watercress meal. *Cancer Epidemiol Biomark Prev* 1992;1:383–388.
100. Conaway CC, Jiao D, Kohri T, et al. Disposition and pharmacokinetics of phenethyl isothiocyanate and 6-phenylhexyl isothiocyanate in F344 rats. *Drug Metabol Dispos* 1999;27:13–20.
101. Chung FL, Jiao D, Getahun SM, Yu MC. A urinary biomarker for uptake of dietary isothiocyanates in humans. *Cancer Epidemiol Biomark Prev* 1998;7:103–108.
102. Seow A, Shi CY, Chung FL, et al. Urinary total isothiocyanate (ITC) in a population-based sample of middle-aged and older Chinese in Singapore: relationship with dietary total ITC and glutathione S-transferase M1/T1/P1 genotypes. *Cancer Epidemiol Biomark Prev* 1998;7:775–781.
103. Getahun SM, Chung FL. Conversion of glucosinolates to isothiocyanates in humans after ingestion of cooked watercress. *Cancer Epidemiol Biomark Prev* 1999;8:447–451.
104. Conaway CC, Getahun SM, Liebes LL, et al. Disposition of glucosinolates and sulforaphane in humans after ingestion of steamed and fresh broccoli. *Nutr Cancer* 2000;38:168–178.
105. Shapiro TA, Fahey JW, Wade KL, et al. Chemoprotective glucosinolates and isothiocyanates of broccoli sprouts: metabolism and excretion in humans. *Cancer Epidemiol Biomark Prev* 2001;10:501–508.
106. Meyer DJ, Crease DJ, Ketterer B. Forward and reverse catalysis and product sequestration by human glutathione S-transferases in the reaction of GSH with dietary aralkyl isothiocyanates. *Biochem J* 1995;306:565–569.
107. Spivack SD, Fasco MJ, Walker VE, Kaminsky LS. The molecular epidemiology of lung cancer. *Crit Rev Toxicol* 1997;27:319–365.
108. Watson MA, Stewart RK, Smith GBJ, et al. Human glutathione S-transferase P1 polymorphisms: relationship to lung tissue enzyme activity and population frequency distribution. *Carcinogenesis* 1998;19:275–280.
109. Coles BF, Morel F, Rauch C, et al. Effect of polymorphism in the human glutathione S-transferase A1 promoter on hepatic GSTA1 and GSTA2 expression. *Pharmacogenetics* 2001;11:663–669.
110. Ketterer B. Dietary isothiocyanates as confounding factors in the molecular epidemiology of colon cancer. *Cancer Epidemiol Biomark Prev* 1998;7:645–646.
111. Lin HJ, Probst-Hensch NM, Louie AD et al. Glutathione transferase null genotype, broccoli, and lower prevalence of colorectal adenomas. *Cancer Epidemiol Biomark Prev* 1998;7:647–652.
112. Sasaki S. Inhibitory effects by α -naphthyl-isothiocyanate on liver tumorigenesis in rats treated with 3'-methyl-4-dimethyl-aminoazobenzene. *J Nara Med Assoc* 1963;14:101–2115.
113. Sidransky H, Ito N, Verney E. Influence of α -naphthyl-isothiocyanate on liver tumorigenesis in rats ingesting ethionine and N-2-fluorenylacetamide. *J Natl Cancer Inst* 1966;37:677–686.
114. Lacassagne A, Hurst L, Xuong MD. Inhibition, par deux naphthylisothiocyanates, de l'hépatocarcinogénèse produit, chez le rat, par le p-diméthylaminoazobenzène (DAB). *C R Séances Société Biol Fil* 1970;164:230–233.
115. Ito N, Hiasa Y, Konishi Y, Marugami M. The development of carcinoma in liver of rats treated with m-toluylenediamine and the synergistic and antagonistic effects with other chemicals. *Cancer Res* 1969;29:1137–1145.
116. Makiura S, Kamamoto Y, Sugihara S, et al. Effect of 1-naphthyl isothiocyanate and 3-methylcholanthrene on hepatocarcinogenesis in rats treated with diethylnitrosamine. *Jpn J Cancer Res* 1973;64:101–104.
117. Ito N, Matayoshi K, Matsumura K, et al. Effect of various carcinogenic and non-carcinogenic substances on development of bladder tumors in rats induced by N-butyl-N-(4-

85. Xu K, Thornalley PJ. Inhibition of leukaemia growth and apoptosis induced by phenethyl isothiocyanate and cysteine conjugate. *Br J Cancer* 1999;81:574–575.
86. Xu K, Thornalley PJ. Involvement of glutathione metabolism in the cytotoxicity of the phenethyl isothiocyanate and its cysteine conjugate to human leukaemia cells in vitro. *Biochem Pharmacol* 2001;61:165–177.
87. Xu K, Thornalley PJ. Signal transduction activated by the cancer chemopreventive isothiocyanates: cleavage of BID protein, tyrosine phosphorylation and activation of JNK. *Br J Cancer* 2001;84:670–673.
88. D'Agostini F, Balansky RM, Izzotti A, et al. Modulation of apoptosis by cigarette smoke and cancer chemopreventive agents in the respiratory tract of rats. *Carcinogenesis* 2001;22:375–380.
89. Kiriln WG, Cai J, DeLong M, et al. Dietary compounds that induce cancer preventive phase 2 enzymes activate apoptosis at comparable doses in HT29 colon carcinoma cells. *J Nutr* 1999;129:1827–1835.
90. Bonnesen C, Eggleston IM, Hayes JD. Dietary indoles and isothiocyanates that are generated from cruciferous vegetables can both stimulate apoptosis and confer protection against DNA damage in human colon cell lines. *Cancer Res* 2001;61:6120–6130.
91. Gamet-Payastre L, Li P, Lumeau S, et al. Sulforaphane, a naturally occurring isothiocyanate, induces cell cycle arrest and apoptosis in HT29 human colon cancer cells. *Cancer Res* 2000; 60:1426–1433.
92. Heiss E, Herhaus C, Klimo K, et al. Nuclear factor kappa B is a molecular target for sulforaphane-mediated anti-inflammatory mechanisms. *J Biol Chem* 2001;276:32,008–32,015.
93. Kong AN, Yu R, Hebbar V, et al. Signal transduction events elicited by cancer prevention compounds. *Mutat Res* 2001;480–481:231–241.
94. Gorler K, Krumbiegel G, Mennicke WH. The metabolism of benzyl isothiocyanate and its cysteine conjugate in guinea-pigs and rabbits. *Xenobiotica* 1982;12:535–542.
95. Mennicke WH, Gorler K, Krumbiegel G. Metabolism of some naturally occurring isothiocyanates in the rat. *Xenobiotica* 1983;13:203–207.
96. Mennicke WH, Görler K, Krumbiegel G, et al. Studies on the metabolism and excretion of benzyl isothiocyanate in man. *Xenobiotica* 1988;18:441–447.
97. Ioannou YM, Burka LT, Matthews HB. Allyl isothiocyanate: comparative disposition in rats and mice. *Toxicol Appl Pharmacol* 1984;75:173–181.
98. Eklind KI, Morse MA, Chung FL. Distribution and metabolism of the natural anticarcinogen phenethyl isothiocyanate in A/J mice. *Carcinogenesis* 1990;11:2033–2036.
99. Chung FL, Morse MA, Eklind KI, Lewis J. Quantitation of human uptake of the anticarcinogen phenethyl isothiocyanate after a watercress meal. *Cancer Epidemiol Biomark Prev* 1992;1:383–388.
100. Conaway CC, Jiao D, Kohri T, et al. Disposition and pharmacokinetics of phenethyl isothiocyanate and 6-phenylhexyl isothiocyanate in F344 rats. *Drug Metabol Dispos* 1999;27:13–20.
101. Chung FL, Jiao D, Getahun SM, Yu MC. A urinary biomarker for uptake of dietary isothiocyanates in humans. *Cancer Epidemiol Biomark Prev* 1998;7:103–108.
102. Seow A, Shi CY, Chung FL, et al. Urinary total isothiocyanate (ITC) in a population-based sample of middle-aged and older Chinese in Singapore: relationship with dietary total ITC and glutathione S-transferase M1/T1/P1 genotypes. *Cancer Epidemiol Biomark Prev* 1998;7:775–781.
103. Getahun SM, Chung FL. Conversion of glucosinolates to isothiocyanates in humans after ingestion of cooked watercress. *Cancer Epidemiol Biomark Prev* 1999;8:447–451.
104. Conaway CC, Getahun SM, Liebes LL, et al. Disposition of glucosinolates and sulforaphane in humans after ingestion of steamed and fresh broccoli. *Nutr Cancer* 2000;38:168–178.
105. Shapiro TA, Fahey JW, Wade KL, et al. Chemoprotective glucosinolates and isothiocyanates of broccoli sprouts: metabolism and excretion in humans. *Cancer Epidemiol Biomark Prev* 2001;10:501–508.
106. Meyer DJ, Crease DJ, Ketterer B. Forward and reverse catalysis and product sequestration by human glutathione S-transferases in the reaction of GSH with dietary aralkyl isothiocyanates. *Biochem J* 1995;306:565–569.
107. Spivack SD, Fasco MJ, Walker VE, Kaminsky LS. The molecular epidemiology of lung cancer. *Crit Rev Toxicol* 1997;27:319–365.
108. Watson MA, Stewart RK, Smith GBJ, et al. Human glutathione S-transferase P1 polymorphisms: relationship to lung tissue enzyme activity and population frequency distribution. *Carcinogenesis* 1998;19:275–280.
109. Coles BF, Morel F, Rauch C, et al. Effect of polymorphism in the human glutathione S-transferase A1 promoter on hepatic GSTA1 and GSTA2 expression. *Pharmacogenetics* 2001;11:663–669.
110. Ketterer B. Dietary isothiocyanates as confounding factors in the molecular epidemiology of colon cancer. *Cancer Epidemiol Biomark Prev* 1998;7:645–646.
111. Lin HJ, Probst-Hensch NM, Louie AD et al. Glutathione transferase null genotype, broccoli, and lower prevalence of colorectal adenomas. *Cancer Epidemiol Biomark Prev* 1998;7:647–652.
112. Sasaki S. Inhibitory effects by α -naphthyl-isothiocyanate on liver tumorigenesis in rats treated with 3'-methyl-4-dimethyl-aminoazobenzene. *J Nara Med Assoc* 1963;14:101–2115.
113. Sidransky H, Ito N, Verney E. Influence of α -naphthyl-isothiocyanate on liver tumorigenesis in rats ingesting ethionine and N-2-fluorenylacetamide. *J Natl Cancer Inst* 1966;37:677–686.
114. Lacassagne A, Hurst L, Xuong MD. Inhibition, par deux naphthylisothiocyanates, de l'hépatocarcérogénèse produit, chez le rat, par le p-diméthylaminoazobenzène (DAB). *C R Séances Society Biol Fil* 1970;164:230–233.
115. Ito N, Hiasa Y, Konishi Y, Marugami M. The development of carcinoma in liver of rats treated with m-toluylenediamine and the synergistic and antagonistic effects with other chemicals. *Cancer Res* 1969;29:1137–1145.
116. Makiura S, Kamamoto Y, Sugihara S, et al. Effect of 1-naphthyl isothiocyanate and 3-methylcholanthrene on hepatocarcinogenesis in rats treated with diethylnitrosamine. *Jpn J Cancer Res* 1973;64:101–104.
117. Ito N, Matayoshi K, Matsumura K, et al. Effect of various carcinogenic and non-carcinogenic substances on development of bladder tumors in rats induced by N-butyl-N-(4-

- hydroxybutyl)nitrosoamine. *Jpn J Cancer Res* 1974; 65:123–130.
118. Sugie S, Okamoto K, Okumura A, et al. Inhibitory effects of benzyl thiocyanate and benzyl isothiocyanate on methylazoxymethanol acetate-induced intestinal carcinogenesis in rats. *Carcinogenesis* 1994;15:1555–1560.
 119. Morse MA, Eklind KI, Amin SG, Chung FL. Effect of frequency of isothiocyanate administration on inhibition of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced pulmonary adenoma formation in A/J mice. *Cancer Lett* 1992;62:77–81.
 120. Matzinger SA, Crist KA, Stoner GD, et al. K-ras mutations in lung tumors from A/J and A/JxTSG-p53 F₁ mice treated with 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and phenethyl isothiocyanate. *Carcinogenesis* 1995;16:2487–2492.
 121. Pereira MA. Chemoprevention of diethylnitrosamine-induced liver foci and hepatocellular adenomas in C3H mice. *Anticancer Res* 1995;15:1953–1956.
 122. Siglin JC, Barch DH, Stoner GD. Effects of dietary phenethyl isothiocyanate, ellagic acid, sulindac and calcium on the induction and progression of *N*-nitrosomethylbenzylamine-induced esophageal carcinogenesis in rats. *Carcinogenesis* 1995;16:1101–1106.
 123. Nishikawa A, Furukawa F, Kasahara K, et al. Failure of phenethyl isothiocyanate to inhibit hamster tumorigenesis induced by *N*-nitrosobis(2-oxopropyl)amine when given during the post-initiation phase. *Cancer Lett* 1999;141:109–115.
 124. Witschi H, Espiritu I, Yu M, Willits NH. The effects of phenethyl isothiocyanate, *N*-acetylcysteine and green tea on tobacco smoke-induced lung tumors in strain A/J mice. *Carcinogenesis* 1998;19:1789–1794.
 125. Hecht SS, Trushin N, Rigotty J, et al. Inhibitory effects of 6-phenylhexyl isothiocyanate on 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone metabolic activation and lung tumorigenesis in rats. *Carcinogenesis* 1996;17:2061–2067.
 126. Yano T, Yajima S, Virgona N, et al. The effect of 6-methylthiohexyl isothiocyanate isolated from *Wasabia japonica* (wasabi) on 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced lung tumorigenesis in mice. *Cancer Lett* 2000;155:115–120.

Cancer Chemoprevention

Volume 1: Promising Cancer Chemopreventive Agents

Kelloff, G.J.; Hawk, E.T.; Sigman, C.C. (Eds.)

2004, XVI, 697 p. 59 illus., 1 illus. in color., Hardcover

ISBN: 978-1-58829-076-2

A product of Humana Press