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Effects of dietary indoles and isothiocyanates on N-nitrosodimethylamine and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone α-hydroxylation and DNA methylation in rat liver

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Dietary-related indoles, isothiocyanates, and the allyl isothiocyanate glucosinolate, sinigrin, were administered to F344 rats in the diet for 2 weeks (chronic protocol) or by gavage 2 h before sacrifice (acute protocol) and the effects of these pretreatments on the α-hydroxylation of two carcinogenic nitrosamines, N-nitrosodimethylamine (NDMA) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), were evaluated. α-Hydroxylation was measured *in vitro* by quantitation of formaldehyde formation upon incubation of the nitrosamines with liver microsomes, and *in vivo* by quantitation of levels of 7-methylguanine and O⁶-methylguanine in hepatic DNA, 4 h after nitrosamine treatment. Compounds shown to be inhibitory in the *in vitro* assay were selected to be further evaluated using the *in vivo* assay. The results of the *in vitro* assays showed that indoles were inducers of the demethylation of both nitrosamines. Indole, L-tryptophan and indole-3-carbinol were strong inducers of NDMA and NNK demethylation, respectively. In contrast, isothiocyanates such as phenethyl isothiocyanate and phenyl isothiocyanate demonstrated a wide range of inhibitory activities toward demethylation of these nitrosamines in both the acute and chronic studies. Chronic, but not acute, pretreatment with sinigrin also caused a significant decrease in the demethylation of NDMA and NNK. In view of their promising inhibitory activities, the effects of phenethyl isothiocyanate, phenyl isothiocyanate and sinigrin on the *in vivo* methylation of DNA by NDMA and NNK were evaluated. The results were parallel to those obtained in the *in vitro* assays. Phenethyl isothiocyanate, phenyl isothiocyanate and sinigrin generally inhibited the formation of 7-methylguanine and O⁶-methylguanine in rat hepatic DNA. The results of this study suggest that these compounds could be anticarcinogenic to NDMA and NNK.

Introduction

Recently, a number of dietary-related compounds were evaluated for their ability to inhibit the metabolic α-hydroxylation of N-nitrosopyrrolidine (NPYR)* and N'-nitrosonornicotine (NNN) in target tissues of rats (1). This study was part of an initial screening method designed to identify dietary compounds as potential inhibitors of nitrosamine carcinogenesis. Among those compounds tested, isothiocyanates were shown to significantly inhibit the α-hydroxylation of these structurally-related cyclic nitrosamines. These results prompted us to study the effects of some of these compounds on the metabolic α-hydroxylation of two other environmentally prevalent nitrosamines, N-nitrosodimethylamine (NDMA) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). NDMA and NNK are structurally-related acyclic nitrosamines (Figure 1) and are potent carcinogens. NDMA is one of the most commonly occurring and extensively investigated nitrosamines. It induces primarily liver tumors in rats (2). NNK is the most potent carcinogen among the tobacco-specific nitrosamines. It causes liver, lung, and nasal cavity tumors in rats, lung tumors in mice, and lung, trachea, and nasal cavity tumors in Syrian golden hamsters (3). In the United States, there are estimated to be at least 60 million people voluntarily exposing themselves to this nitrosamine through tobacco usage (4).

In this study, an *in vitro* metabolism assay and an *in vivo* DNA methylation assay were used. In the *in vitro* metabolism assay, the formation of formaldehyde resulting from demethylation of NDMA and NNK upon incubation with rat liver microsomes was measured. In the *in vivo* DNA methylation assay, the formation of 7-methylguanine and O⁶-methylguanine in the target tissue DNA of rats treated by NDMA and NNK was measured. Initially, the effects of dietary compounds on α-hydroxylation were determined using the *in vitro* metabolism assay. The most inhibitory compounds in the *in vitro* assay were further evaluated in the *in vivo* DNA methylation assay.

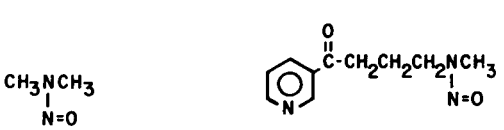
Materials and methods

Chemicals

NDMA was obtained from Sigma Chemical Co., St. Louis, MO. NNK was synthesized by a previously described method (5). Indole, indole-3-carbinol, indole-3-acetonitrile, L-tryptophan, phenyl isothiocyanate, allyl isothiocyanate and its glucosinolate, sinigrin (Figure 2) were all purchased from Aldrich Chemical Co., Milwaukee, WI. Benzyl isothiocyanate was obtained from Fluka, Hauppauge, NY, and phenethyl isothiocyanate was purchased from Eastman Kodak Co., Rochester, NY. NADP and NADPH generating reagents were obtained from Sigma Chemical Co. Reagents for the enriched Nash reagent, ammonium acetate and acetylacetone, were from Sigma Chemical Co. For DNA isolation, the following reagents were used: sodium citrate from Fisher Scientific Co., Fair Lawn, NJ, sodium dodecyl sulfate from Sigma Chemical Co., isoamyl alcohol from Aldrich Chemical Co., and phosphoric acid h.p.l.c. grade, ammonium phosphate (monobasic) and phosphoric acid from Fisher Scientific Co. 7-Methylguanine and guanine were obtained from Sigma Chemical Co. and O⁶-methylguanine was prepared by a published method (6).

Animal treatments

Male F344 rats (200–300 g) were obtained from Charles River Breeding Laboratories, Inc., Wilmington, MA. Rats were housed in groups of 2 or 3 in solid-bottomed polycarbonate cages with hardwood bedding. Animals were kept



NDMA NNK

Fig. 1. Structures of NDMA and NNK.

*Abbreviations: NDMA, N-nitrosodimethylamine; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NPYR, N-nitrosopyrrolidine; NNN, N'-nitrosonornicotine.

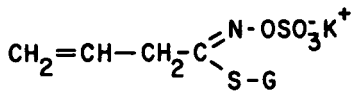


Fig. 2. Structure of sinigrin; G = β-D-glucosyl.

at 20 ± 2°C (S.D.) and 50 ± 10% relative humidity with a 12-h light-dark cycle. Purina lab chow and water were administered *ad libitum*.

For the *in vitro* metabolism assay, in the acute studies, rats were treated by gavage with the appropriate test compound (1 mmol/kg of body weight) in corn oil and sacrificed after 2 h. The control groups received only corn oil, and one control group (three rats) was used for the treated groups (three rats each). In the chronic studies, rats were fed *ad libitum* with NIH-07 diet containing the appropriate test compound (0.03 mmol/g of diet for the indoles or 0.003 mmol/g of diet for the isothiocyanates and sinigrin). It should be noted that NIH-07 diet contains ~0.01 mmol/g diet of L-tryptophan. The appropriate test compound and NIH-07 diet were thoroughly mixed with a mechanical mixer. Diet was prepared weekly and stored at 4°C before use. The control rats were fed NIH-07 diet, and two control groups (four rats each) were used for the nine treated groups (four rats each). After 2 weeks of feeding, the animals were sacrificed by decapitation on day 15.

For the *in vivo* DNA methylation assay, in the acute studies, rats were treated by gavage with 1 mmol/kg body weight of test compound as described above. After 2 h, treated (five rats) and control groups (five rats) were administered NDMA [25 mg/kg (0.34 mmol/kg) in 0.9% w/v NaCl] by i.p. injection (7) or NNK [85 mg/kg (0.41 mmol/kg) in 0.9% w/v NaCl] by tail i.v. injection (8). Four hr after injection of the nitrosamines, rats were sacrificed by decapitation. In these studies at least one control group was used for two treated groups. For the chronic studies, rats in the treated groups (five rats) were fed *ad libitum* with NIH-07 diet containing 0.003 mmol/g of test compound. Rats in the control groups (five rats) were fed with NIH-07 diet only. After 2 weeks, rats of both treated and control groups were treated with either NDMA (25 mg/kg body weight in 0.9% NaCl w/v) by i.p. injection or with NNK (85 mg/kg body weight in 0.9% NaCl w/v) by tail vein injection. Four hours after injection of the nitrosamine, rats were decapitated. At least one control group was used for each two treated groups. In separate experiments, three rats each were also treated with the test compounds alone at the same doses as those used above. These experiments were designed to investigate the possibility of aberrant methylation due to treatment with these compounds.

In vitro metabolism assay

After the rats were sacrificed, the livers were removed. Liver microsomes were prepared by a procedure previously described (9). Microsomes were assayed for protein concentration by the method of Lowry *et al.* (10). The assay for formaldehyde formation by metabolic α-hydroxylation was carried out essentially as described previously for NDMA (11). In the procedure, 0.1 mM NDMA or 2.8 mM NNK was preincubated at 37°C and pH 7.4 for 2 min in 1 ml of a medium containing 70 mM Tris-HCl, pH 7.4, 14 mM $MgCl_2$, 215 mM KCl, 10 mM potassium isocitrate, 0.25 mg NADPH, and 0.3 units of isocitrate dehydrogenase. Microsomes (0.2 – 1.7 mg protein) were then added to the incubation mixture and the reactions were carried out in duplicate in polypropylene test tubes by shaking at 37°C for 30 min. The reactions were terminated by adding 0.1 ml of 25% $ZnSO_4$ and 0.1 ml of saturated $Ba(OH)_2$. The resulting mixture was centrifuged at 5°C at 2000 *g* for 10 min and 0.5 ml of the supernatant was mixed with 108 μl of Nash reagent (15 g ammonium acetate, 0.2 ml acetylacetone in 18 ml of 3% acetic acid) and incubated at 50°C for 30 min. Finally, the absorbance of the mixture was measured at 412 nm. Both blanks and standards for the formaldehyde assay were carried out by incubation with microsomes, but in the absence of the NADPH-generating system. The results were expressed as nmol/mg protein/min. Inhibition or induction was expressed as an index obtained by dividing the mean values of the nmol/mg protein/min of formaldehyde measured in the experiments with microsomes from treated rats by the mean values in the corresponding control experiments. Therefore, the index value was >1 for induction and <1 for inhibition of demethylation of NDMA and NNK.

In vivo methylation assay.

Rat hepatic DNA was isolated by the modified Marmur method (12). The isolated DNA was dissolved in 10 mM sodium cacodylate at pH 7.0 (200 μl/mg DNA). The DNA solution was heated at 100°C for 30 min to thermally release 7-methylguanine. The solution was then cooled in an ice bath. To the cold DNA solution was added 1 N HCl (20 μl/mg DNA) to precipitate the DNA. The DNA was collected by centrifugation at 2000 *g* for 10 min. The supernatant was used for the analysis of 7-methylguanine and the re-precipitated DNA was hydrolyzed in 0.1 N HCl at 80°C for 30 min. The resulting hydrolysate was used for O^6-methylguanine analysis (13). The quantitative analyses were performed with a h.p.l.c.-fluorescence system composed of a Waters Associates Model U6K septumless injector and Model 510 solvent delivery system coupled to a Whatman Partisil 10 SCX column (Whatman Inc., Clifton, NJ) and a Perkin-Elmer 650-10S fluorescence spectrophotometer (Perkin-Elmer Corp., Norwalk, CT). The column was eluted with 0.02 M ammonium phosphate buffer, pH 2.0, at 2 ml/min. The fluorescence detector settings were as follows: excitation wavelength, 286 nm; emission wavelength, 366 nm. The levels of methylated guanines were expressed as μmol/mol guanine. These values were converted into the index of inhibition or induction as described above. The statistical significance of the index was calculated by the 2-sample t-test.

Results

In this study, dietary indoles, isothiocyanates and sinigrin were tested for their effects on the metabolic α-hydroxylation of NDMA and NNK. Doses used for these dietary-related test compounds were the same as previously described (1). Initially, the effects of the test compounds on the *in vitro* rat hepatic microsomal metabolism of NDMA and NNK to formaldehyde were measured. The concentrations of NDMA and NNK used in this assay were 0.1 mM and 2.8 mM, respectively. At these concentrations, both NDMA and NNK yielded ~0.8 nmol/mg protein/min of formaldehyde upon incubation with hepatic microsomes of control rats. Table I shows the level of formaldehyde formation from NDMA and NNK in rat liver microsomes in control and treated groups. Table II summarizes the effects of the test compounds on the demethylation of NDMA and NNK in the *in vitro* assays. Chronic feeding of indole and its derivatives to rats increased significantly the hepatic demethylase activities for both NDMA and NNK. Indole-3-carbinol was the most potent inducer of NNK demethylation. Because of their activities as inducers, indoles were not further evaluated. In contrast to the indoles, phenethyl isothiocyanate and phenyl isothiocyanate caused a marked decrease in NDMA and NNK demethylation in both the chronic and acute studies. Interestingly, benzyl isothiocyanate had little effect on NDMA demethylation and induced NNK demethylation in the chronic protocol. Sinigrin demonstrated significant inhibitory effects on NDMA and NNK demethylation in the chronic studies but was inactive in the acute studies.

Because of their promising inhibitory activities, phenethyl isothiocyanate, phenyl isothiocyanate, and sinigrin were further evaluated in the *in vivo* DNA methylation assay. In this assay, the influence of the test compounds on the formation of 7-methylguanine and O^6-methylguanine in hepatic DNA of rats treated by NDMA or NNK was measured. Doses of test compounds used in this assay were identical to those used in the *in vitro* assays. No aberrant DNA methylation was detected when rats were treated only with the test compounds. Typical h.p.l.c. profiles from the assays of 7-methylguanine and O^6-methylguanine in hepatic DNA of rats treated with NNK are shown in Figure 3. Table III shows the levels of NDMA and NNK methylation of hepatic DNA in control and treated groups. These data were converted into an index of inhibition as summarized in Table IV. The formation of 7-methylguanine and O^6-methylguanine in hepatic DNA of rats treated with NDMA and NNK was inhibited almost by all treatments studied. These results are parallel to those obtained from the *in vitro* demethylation assay.

Discussion

NDMA and NNK are acyclic nitrosamines which possess at least one methyl group adjacent to the nitroso functionality. Upon α-hydroxylation, which is believed to be a key metabolic pathway for the activation of these nitrosamines, NDMA and NNK release formaldehyde and/or form a reactive intermediate which is

Table I. Demethylation of NDMA and NNK in rat liver microsomes in control and treated rats. Rats were treated with the appropriate test compounds by either the acute or chronic protocol described in Materials and methods. After sacrifice liver microsomes were isolated and used for the metabolism of NDMA or NNK to formaldehyde. Incubations were carried out at least in duplicate for each rat liver microsomal preparation. The data are expressed as nmol/mg protein/min and are the mean ± S.D. of experiments using 3–4 rats/group

Test compound	NDMA		NNK	
	Acute	Chronic	Acute	Chronic
Control	0.80 ± 0.18	0.80 ± 0.14[a]	0.79 ± 0.15	0.82 ± 0.17[a]
Indole	N.D.[b]	3.88 ± 0.68	N.D.	2.14 ± 0.46
Indole-3-carbinol	N.D.	2.12 ± 0.16	N.D.	4.38 ± 0.47
Indole-3-acetonitrile	N.D.	1.45 ± 0.39	N.D.	2.78 ± 0.43
L-tryptophan	N.D.	3.85 ± 0.70	N.D.	2.33 ± 0.38
Benzyl isothiocyanate	0.68 ± 0.14	1.16 ± 0.34	0.78 ± 0.14	1.92 ± 0.17
Allyl isothiocyanate	0.44 ± 0.13	0.83 ± 0.27	0.59 ± 0.19	0.56 ± 0.11
Phenethyl isothiocyanate	0.53 ± 0.12	0.51 ± 0.17	0.43 ± 0.04	0.58 ± 0.10
Phenyl isothiocyanate	0.31 ± 0.09	0.51 ± 0.10	0.25 ± 0.08	0.61 ± 0.07
Sinigrin	0.91 ± 0.19	0.37 ± 0.07	0.91 ± 0.24	0.56 ± 0.12

[a]Mean of the formaldehyde values from the two control groups used.
[b]N.D., not determined.

Table II. Effects of some indoles and isothiocyanates on the demethylation of NDMA and NNK in rat liver microsomes. Rats were treated with the appropriate test compound by either the acute or chronic protocol described in Materials and methods. After sacrifice liver microsomes were isolated and used for the metabolism of NDMA or NNK to formaldehyde

Index[a]

Test compounds	NDMA		NNK	
	Acute	Chronic	Acute	Chronic
Indole	N.D.[b]	4.9[c]	N.D.	2.6[c]
Indole-3-carbinol	N.D.	2.6[c]	N.D.	5.3[c]
Indole-3-acetonitrile	N.D.	1.8[d]	N.D.	3.4[c]
L-tryptophan	N.D.	4.8[c]	N.D.	2.8[c]
Benzyl isothiocyanate	0.9	1.4[c]	1.0	2.3[c]
Allyl isothiocyanate	0.6[c]	1.0	0.7[d]	0.7[d]
Phenethyl isothiocyanate	0.7[d]	0.6[c]	0.5[c]	0.7[c]
Phenyl isothiocyanate	0.4[c]	0.6[c]	0.3[c]	0.7[d]
Sinigrin	1.1	0.5[c]	1.1	0.7[c]

[a]Mean of the treated rat formaldehyde values, expressed as nmol/mg protein/min (see Table I) divided by the mean of the control rat values.
[b]N.D., not determined.
[c]$p < 0.01$.
[d]$p < 0.05$.

Table III. Methylation of hepatic DNA by NDMA or NNK in control and treated rats. Rats were treated with phenethyl isothiocyanate, phenyl isothiocyanate or sinigrin by either the acute or chronic protocol described in Materials and methods, and were then given either NDMA (25 mg/kg i.p.) or NNK (85 mg/kg i.v.) 4 h prior to sacrifice. Hepatic DNA was isolated and assayed for 7-methylguanine and O⁶-methylguanine by h.p.l.c.-fluorescence. The data are expressed as μmol 7-methylguanine (7-mG)/mol of guanine or μmol O⁶-methylguanine (O⁶-mG)/mol of guanine and are the mean ± S.D. of experiments using 5 rats/group

Test compound	NDMA						NNK					
	Acute			Chronic			Acute			Chronic		
	7-mG	O⁶-mG	O⁶-mG/7-mG	7-mG	O⁶-mG	O⁶-mG/7-mG	7-mG	O⁶-mG	O⁶mG/7-mG	7-mG	O⁶-mG	O⁶-mG/7-mG
Control (isothiocyanates)	5500 ± 422	610 ± 41	0.11	6400 ± 167	670 ± 95	0.11	760 ± 109	74 ± 11	0.10	470 ± 40	47 ± 6	0.10
Phenethyl isothiocyanate	2300 ± 129	320 ± 17	0.14	3000 ± 307	250 ± 31	0.08	330 ± 82	38 ± 10	0.12	320 ± 20	35 ± 3	0.11
Phenyl isothiocyanate	990 ± 195	100 ± 25	0.10	2500 ± 819	290 ± 84	0.12	220 ± 23	17 ± 3	0.08	320 ± 14	28 ± 1	0.09
Control (sinigrin)		N.D.[a]		5200 ± 267	640 ± 20	0.12		N.D.		350 ± 37	34 ± 5	0.10
Sinigrin		N.D.		4400 ± 1400	560 ± 190	0.12		N.D.		250 ± 39	20 ± 6	0.08

[a]N.D., not determined.

capable of methylating DNA. The formation of 7-methylguanine and O⁶-methylguanine in tissue DNA of animals treated with NDMA has been extensively studied (2). Recently, both methylated guanines have also been identified in lung and liver DNA of rats treated by NNK (8). Therefore, quantitation of formaldehyde formation in rat liver microsomal incubations and of 7-methylguanine or O⁶-methylguanine in hepatic DNA are methods for assessing the extents of NDMA and NNK α-hydroxylation *in vitro* and *in vivo*, respectively.

Numerous studies have shown that agents which inhibit the metabolism of carcinogens and decrease their binding to DNA are also inhibitors of carcinogenicity. For example, disulfiram,

Table IV. Effects of phenethyl isothiocyanate, phenyl isothiocyanate, and sinigrin on methylation of hepatic DNA by NDMA and NNK. Rats were treated with the appropriate test compound by either the acute or chronic protocol described in Materials and methods, and were then given either NDMA (25 mg/kg i.p.) or NNK (85 mg/kg i.v.) 4 h prior to sacrifice. Hepatic DNA was isolated and assayed for 7-methylguanine (7-mG) and O^6-methylguanine (O^6-mG) by h.p.l.c.-fluorescence

Test compounds	Index[a]							
	NDMA				NNK			
	Acute		Chronic		Acute		Chronic	
	7-mG	O^6-mG	7-mG	O^6-mG	7-mG	O^6-mG	7-mG	O^6-mG
Phenethyl isothiocyanate	0.4[b]	0.5[b]	0.5[b]	0.4[b]	0.4[b]	0.5[b]	0.7[b]	0.5[b]
Phenyl isothiocyanate	0.2[b]	0.2[b]	0.4[b]	0.4[b]	0.3[b]	0.2[b]	0.7	0.6[b]
Sinigrin	N.D.[c]		0.9	0.9	N.D.		0.7[b]	0.6[b]

[a]Mean of methylated guanine values, expressed as μmol per mol of guanine, in the treated rats divided by the mean of the values in the control rats. These data are shown in Table III. Five rats were used for each test compound, and in each control group.
[b]$p < 0.01$ compared to control.
[c]N.D., not determined.

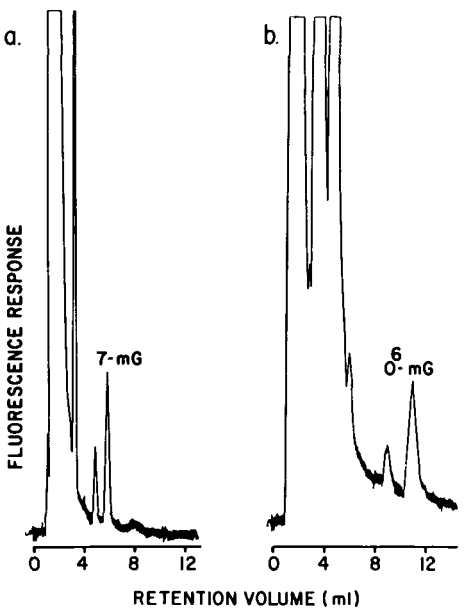


Fig. 3. Typical h.p.l.c.-fluorescence profiles of 50 μl of DNA hydrolysates (see Materials and methods) from the assays of **a**. 7-methylguanine (7-mG) and **b**. O^6-methylguanine (O^6-mG) in hepatic DNA of a rat treated with NNK.

pregnenolone-16α-carbonitrile, and aminoacetonitrile inhibited metabolism of NDMA and have been shown to reduce the hepatotoxicity and/or hepatocarcinogenicity of NDMA in rats (14–17). However, a change in the target organ of NDMA upon treatment with disulfiram has also been noted (18). Inhibitors of 1,2-dimethylhydrazine colon tumorigenesis such as disulfiram and pyrazole have been shown to inhibit the metabolic activation of 1,2-dimethylhydrazine, and to reduce the formation of O^6-methylguanine in colon DNA of rats treated by these agents (19). Due to its miscoding properties and persistence in target tissues, O^6-methylguanine has been widely recognized as a critical lesion involved in the tumorigenicity of these methylating agents (20). Thus, compounds which decrease the formation of formaldehyde in the *in vitro* assay and of the methylated guanines in the *in vivo* assay, are considered to be potential inhibitors of NDMA and NNK tumorigenesis.

In a previous study, we have demonstrated that certain isothiocyanates which occur in cruciferous vegetables are inhibitors of the metabolic α-hydroxylation of NPYR and NNN in rat target tissues. Indole and its derivatives, on the other hand, are relatively potent inducers of NPYR metabolism (1). Indoles are known to be good inducers of microsomal enzyme systems (21,22). Feeding indoles and L-tryptophan to rats increased NDMA demethylase activity (23). We have also observed this induction in the present study using a lower substrate concentration of NDMA. Induction of NNK metabolism by indoles, especially indole-3-carbinol, is also consistent with these earlier studies. As in our studies on NPYR and NNN metabolism, certain isothiocyanates such as allyl isothiocyanate, phenethyl isothiocyanate and phenyl isothiocyanate were clearly demonstrated to have inhibitory effects on the metabolism of NDMA and NNK. Phenethyl isothiocyanate and phenyl isothiocyanate showed a broad range of inhibitory activities toward NDMA and NNK metabolism. These inhibitory activities were demonstrated consistently in both the *in vitro* and *in vivo* assays. Very little is known about the metabolic fate and pharmacokinetics of these isothiocyanates or their corresponding glucosinolates. The mechanism(s) of inhibition by treatment with these compounds is presently not clear. Nonetheless, it is plausible that inhibition of metabolizing enzymes, possibly the cytochrome P-450 mixed function oxidase system, may be involved.

Indoles and isothiocyanates are two major classes of compounds which occur as glucosinolates in a variety of cruciferous vegetables (24). Glucosinolates can be hydrolyzed by myrosinase, an enzyme found in these vegetables. After hydrolysis, aglycons are released as isothiocyanates, thiocyanates or nitriles (25). It has been estimated that the average daily intake of total glucosinolates through consumption of cooked vegetables in the United Kingdom is ~30 mg per person (26). Sinigrin, used in this study, is the corresponding glucosinolate of allyl isothiocyanate and one of the major glucosinolates which is found most abundantly in cabbage, brussels sprouts, and cauliflower (26). Considering the extent of human exposure to sinigrin, it may be important that our results show that chronic feeding of sinigrin to rats resulted in a significant decrease in the metabolism of NDMA and NNK to formaldehyde as well as a decrease in methylation of hepatic DNA by NNK.

This study has shown that certain dietary-related isothiocyanates and a glucosinolate are good inhibitors of NDMA and NNK α-hydroxylation. Inhibition was demonstrated in both the *in vitro* metabolism assay and the *in vivo* DNA methylation assay. The results suggest that these compounds might inhibit NDMA and NNK carcinogenesis. The inhibitory activities demonstrated by these compounds appear to be non-specific since they have now been observed with four structurally different nitrosamines. The broad spectrum of inhibitory activities on nitrosamine metabolism observed upon treatment with these dietary-related compounds

requires that bioassays be performed to determine whether they inhibit nitrosamine carcinogenesis.

Acknowledgements

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