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Effect of phenethyl isothiocyanate on the metabolism of the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone by cultured rat lung tissue

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The effect of phenethyl isothiocyanate (PEITC), a dietary inhibitor of carcinogenesis, on the metabolism of the tobacco specific nitrosamine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) by cultured rat peripheral lung tissues was investigated. Initially, the metabolism of NNK by the tissues was studied by incubating the lung explants in medium containing 1 and 10 μM [5-^{3}H]NNK for 3, 6, 12, and 24 h. NNK metabolites were analyzed and quantified by HPLC and expressed as nmol/mg DNA. NNK was metabolized by three pathways; alpha-carbon hydroxylation, pyridine *N*-oxidation and carbonyl reduction. The principal metabolic pathway involved the conversion of NNK to the pyridine *N*-oxidized metabolites: 4-(methylnitrosamino)-1-(3-pyridyl-*N*-oxide)-1-butanone (NNK-*N*-oxide) and 4-(methylnitrosamino)-1-(3-pyridyl-*N*-oxide)-1-butanol (NNAL-*N*-oxide). When combined, NNK-*N*-oxide and NNAL-*N*-oxide constituted ~70% of the total metabolites in the medium at 24 h. To determine the effects of PEITC on the metabolism of NNK, lung explants were either treated with both 10 μM [5-^{3}H]NNK and PEITC (10, 50, and 100 μM) for 24 h, or they were pre-treated with these same concentrations of PEITC for 16 h and then co-treated with both PEITC and 10 μM [5-^{3}H]NNK for 24 h. In both treatment series, PEITC inhibited the alpha-carbon hydroxylation and pyridine *N*-oxidation of NNK and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), which is produced from NNK by carbonyl reduction. In general, the inhibition of NNK metabolism was greater when the explants were pre-treated with PEITC. These results suggest that PEITC is an effective inhibitor of the conversion of NNK to metabolites that elicit DNA damage. Our results are in agreement with previously published data in which PEITC was shown to inhibit NNK metabolism and tumorigenesis in the rat lung.

Introduction

The tobacco-specific nitrosamines are the most prevalent class of strong carcinogens found in tobacco and in tobacco smoke (1). They are formed during the curing and processing of tobacco leaves by the nitrosation of nicotine and alkaloids present in tobacco (2). One of these nitrosamines, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK*), induces lung, nasal cavity, liver and pancreatic tumors in F-344 rats (3–5); nasal cavity and lung tumors in hamsters (6); and lung tumors in mice (2,7); supporting the possible role of NNK in the induction of lung cancer in smokers. In addition, the oral swabbing of NNK along with the tobacco-specific nitrosamine, *N'*-nitrosonornicotine, induced oral cavity tumors in rats (8).

The carcinogenicity of NNK is believed to be due to its metabolic conversion to alkyl diazohydroxides which then interact with nucleophiles in DNA to form adducts (9). Metabolites of NNK result from reduction of the carbonyl group, oxidation of the pyridine nitrogen and alpha-carbon hydroxylation of the methylene and methyl carbons adjacent to the *N*-nitroso group (9). The metabolic pathways of NNK are shown in Figure 1. The alpha-carbon hydroxylations are thought to be the important initiating steps in the activation of NNK to DNA-damaging species (9,10).

Recently, interest has increased in identifying synthetic or dietary compounds which may possess anti-carcinogenic properties. Studies have shown that certain fruits and vegetables contain non-nutritive compounds such as phenols and aromatic isothiocyanates that inhibit carcinogenesis in laboratory animals (11,12). Gluconasturtiin, the glucosinolate precursor of phenethyl isothiocyanate (PEITC), is found in certain cruciferous vegetables (13). Recent studies have demonstrated the ability of PEITC to inhibit the metabolism and carcinogenicity of NNK *in vivo* (14,15). The pre-treatment of F-344 rats with a diet containing PEITC resulted in a reduced metabolic demethylation of NNK in hepatic microsomes as well as a decrease in hepatic DNA methylation by NNK (16). In a 2-year bioassay, PEITC caused a 50% reduction in the incidence of NNK-induced lung tumors in F-344 rats (14) and, in a short-term bioassay, PEITC inhibited the number of NNK-induced lung tumors in strain A/J mice (15). PEITC also inhibited 7,12-dimethylbenz[*a*]anthracene-induced mammary tumors in Sprague-Dawley rats, and both forestomach and lung tumors in ICR/Ha mice (17). Recently, PEITC was found to inhibit the induction of esophageal tumors in F-344 rats with *N*-nitrosobenzylmethylamine (18).

In this paper, the ability of PEITC to inhibit the metabolism of NNK in cultured explants of rat peripheral lung tissue is described. PEITC was found to be a potent inhibitor of the alpha-carbon hydroxylation of NNK, a pathway that leads to the formation of DNA-damaging metabolites.

Materials and methods

Chemicals and culture media

[5-^{3}H]NNK (2.2 Ci/mmol; purity >98%), was purchased from Chemsyn Science Laboratories, Lenexa, KS. Unlabeled NNK (purity, 99%) was obtained from the National Cancer Institute Chemical Carcinogen Repository, Midwest Research Institute, Kansas City, MO. PEITC was purchased from Aldrich Chemical Company, Milwaukee, WI, and its purity was found to be >99% by HPLC. NNK metabolites used as HPLC reference standards were obtained as described (19). L-15 and CMRL-1066 tissue culture media were purchased from Biofluids, Inc., Rockville, MD.

*Abbreviations: NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; PEITC, phenethyl isothiocyanate; NNK-*N*-oxide, 4-(methylnitrosamino)-1-(3-pyridyl-*N*-oxide)-1-butanone; NNAL-*N*-oxide, 4-(methylnitrosamino)-1-(3-pyridyl-*N*-oxide)-1-butanol; NNAL, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol.

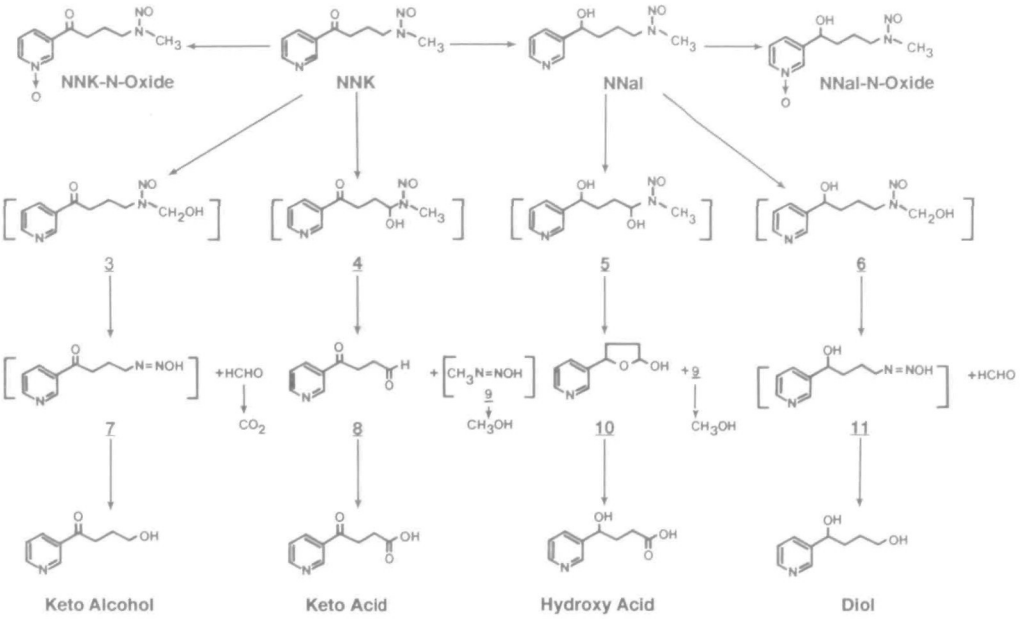

Fig. 1. Pathways for the metabolism of NNK. The metabolites found in brackets are intermediates.

Animals

Male 9- to 10-week-old F-344 rats were purchased from Harlan Sprague Dawley, Indianapolis, IN. The rats were maintained at two or three per cage under standard conditions (20 ± 2°C; 50 ± 10% relative humidity; 12 h light/dark cycle), and fed Wayne lab chow and water *ad libitum*. Animals were killed by i.p. injection of sodium pentobarbitol.

Explant culture

Lungs were collected aseptically from the rats and placed in cold L-15 medium for transport to the tissue culture laboratory The peripheral lung was separated from the trachea and mainstream bronchi and cut into explants (0.2 mm^2). The explants were placed on opposing edges of 60-mm tissue culture dishes (20 explants/dish), and on surfaces of the dish that had been etched with a sterile needle. The explants were allowed to attach to the dish for 10−15 min before the addition of the culture medium. To each dish was added 2 ml of CMRL-1066 medium, supplemented with HEPES buffer (20 mM), L-glutamine (2 mM), insulin (1 μg/ml), hydrocortisone (0.1 μg/ml), penicillin G (100 units/ml) and streptomycin (100 μg/ml). The explants were cultured in an atmosphere of 50% oxygen, 47% nitrogen, and 3% carbon dioxide and on a rocker platform as described previously (20).

To determine non-toxic concentrations of both NNK and PEITC for the metabolism experiments, the explants were treated with NNK (1 and 10 μM, dissolved in dimethyl sulfoxide, with a final concentration of 1% in the culture medium), and PEITC (0, 25, 50, 100 and 200 μM) for 48 h. After the treatment, the tissues were examined by high-resolution light microscopy for PEITC and/or NNK-induced histopathological effects.

Metabolism studies

Initially, the ability of the explants to metabolize NNK was determined. Fresh lung tissues were incubated for 24 h after which the medium was replaced with fresh medium containing either 1 μM [5-^{3}H]NNK (sp. act. = 2.2 Ci/mmol) or 10 μM [5-^{3}H]NNK (sp. act. = 0.22 Ci/mmol). DMSO, the solvent for NNK, was added to the medium at a final concentration of 1%. Medium controls consisted of dishes incubated with solutions of [5-^{3}H]NNK but no tissue. After 3, 6, 12 and 24 h of incubation, the medium was harvested and frozen at −80°C for subsequent analysis of NNK metabolites by HPLC. The explants were washed with HEPES buffered saline and stored at −80°C until analysis for DNA content by the diphenylamine method (21).

Next, the ability of PEITC to inhibit the metabolism of NNK by lung explants was investigated. Two protocols were used. One series of explants was cultured for 24 h in CMRL-1066 medium and then exposed to PEITC (0, 10, 50, 100 μM) plus 10 μM [5-^{3}H]NNK for an additional 24 h (i.e. co-treatment series). A second series of explants was cultured for 24 h, then pre-treated with PEITC (0, 10, 50, 100 μM) for 16 h. After pre-treatment, the explants were incubated for 24 h in fresh medium containing both PEITC (at the same concentrations) and 10 μM [5-^{3}H]NNK (pre-treatment series). The medium and explants from these experiments were harvested as described above.

Medium samples were cleared of cellular debris by filtering with a 0.45-μm Gelman Acro-disc filter and centrifuged. An aliquot of the medium was combined with reference standards and analyzed by HPLC (Waters, Milford, MA) coupled to a FLO-ONE radioflow detector (Radiomatic Instruments, Tampa, FL). The metabolites were separated on an Ultrasphere ODS column (4.6 × 250 mm, Beckman Instruments, Fullerton, CA) with a linear gradient from 100% solvent A (20 mM sodium phosphate buffer, pH 7.0) to 35% solvent A: 65% solvent B (100% methanol) in 75 min at a flow rate of 1 ml/min. The radioactivity (d.p.m.) for each metabolite was converted to nmol and expressed as nmol/mg DNA.

The identities of keto alcohol and keto acid were confirmed by reverse-phase HPLC at pH 4.5, using systems described previously (19,22). The keto alcohol peak was further confirmed by $NaBH_4$ reduction to diol. The identities of 4-(methylnitrosamino)-1-(3-pyridyl-*N*-oxide)-1-butanone (NNK-*N*-oxide) and 4-(methylnitrosamino)-1-(3-pyridyl-*N*-oxide)-1-butanol (NNAL-*N*-oxide) were confirmed by normal phase HPLC on a Lichrosorb Si50 5 μ column with elution by 10% methanol in acetonitrile at flow rates of 1 ml/min for NNK-*N*-oxide and 1.5 ml/min for NNAL-*N*-oxide Retention times of NNK-*N*-oxide and NNAL-*N*-oxide in this system were 9 5 and 10.5 min, respectively.

Results

NNK (1 and 10 μM) was not toxic for explants of rat lung tissue as determined by high resolution light microscopy. Similarly, the concentrations of 10, 25, 50 and 100 μM PEITC were not toxic for the explants. However, at 200 μM, PEITC induced toxic effects largely in the outer portion of the explant that was in direct contact with the medium (data not shown). The nuclei in most cells of the outer portion were either pyknotic or exhibited other signs of necrosis, i.e. karyolysis or karyorrhexis. The cytoplasm showed intense eosinophilic staining indicative of coagulation necrosis. Therefore, the highest concentration of PEITC used in our study was 100 μM.

Table I shows the production of NNK metabolites into the culture medium. The percent recovery of the initial radioactivity in the medium from time zero to the termination of culture was ~90% (with the remainder bound to tissue). After HPLC analysis, the NNK and metabolites accounted for ~85% of the initial radioactivity. At both 1 and 10 μM, the majority of the NNK was metabolized to NNAL-*N*-oxide and NNK-*N*-oxide by pyridine *N*-oxidation. For example, in cultures incubated for 12 and 24 h in 10 μM [5-^{3}H]NNK, NNK-*N*-oxide and NNAL-*N*-oxide represented from 60 to 69% of the total metabolites, respectively. The rate of formation of NNK-*N*-oxide exceeded that of NNAL-*N*-oxide as indicated by the larger quantities of NNK-*N*-oxide in the medium after 3 and 6 h. In these same cultures,

Table I. Metabolism of NNK by rat lung explants[a]

NNK (μM)	Time (h)	NNK and metabolites in nmol/mg DNA (mean ± SE)[b] Hydroxy acid	Keto acid	NNAL-*N*-oxide	Diol	NNK-*N*-oxide	Keto alcohol	NNAL	NNK
1	0	–	–	–	–	–	–	–	13.99
	3	n.d[c]	0.29 ± 0.00	0.57 ± 0.01	0.09 ± 0.00	4.28 ± 0.13	1.61 ± 0.06	1.00 ± 0.13	3.28 ± 1.26
	6	0.09 ± 0.01	0.75 ± 0.13	1.09 ± 0.17	2.26 ± 0.05	4.84 ± 0.91	1.20 ± 0.26	0.59 ± 0.13	0.07 ± 0.03
	12	0.18 ± 0.03	1 36 ± 0.28	1.88 ± 0.30	0.27 ± 0.10	4.30 ± 0.91	0 48 ± 0.17	0.32 ± 0.03	0.02 ± 0.01
	24	0 41 ± 0.05	2.33 ± 0.48	4.00 ± 0 73	0.68 ± 0.17	6.27 ± 1.18	0.48 ± 0.17	0.27 ± 0.04	0.05 ± 0.01
10	0	–	–	–	–	–	–	–	139.86
	3	0.02 ± 0.01	1.04 ± 0.08	2.26 ± 0.05	0 41 ± 0.01	16.17 ± 0.12	7.49 ± 0.10	14.48 ± 0.29	47.95 ± 5.39
	6	0.41 ± 0.04	2.99 ± 0.22	7.24 ± 0.14	1.29 ± 0.01	33.95 ± 2.68	15.95 ± 1.56	21.44 ± 3.39	24.43 ± 6.99
	12	1.92 ± 0.39	7.01 ± 1.83	16.29 ± 3.53	3.80 ± 1.71	31.19 ± 7.32	9.52 ± 2.09	9.50 ± 2.26	0.66 ± 0.19
	24	5.23 ± 0.52	13.35 ± 1.51	32.46 ± 4.10	3.33 ± 1.08	35.65 ± 4.58	4.98 ± 1.00	3.73 ± 1.37	0.23 ± 0.03

[a]Rat lung explants were cultured in medium containing either 1 or 10 μM [5-^{3}H]NNK for 3, 6, 12, and 24 h. NNK and metabolites were quantified by HPLC as described in Materials and methods. The quantity of DNA per culture dish (with 20 explants) averaged 0.20 mg.
[b]Data are mean ± SE from three experiments. In each experiment, three explant cultures were used per variable (i.e. conc. NNK and time point). Therefore, each data point represents the calculated amount of either NNK or its metabolites in the medium from a total of nine explant cultures.
[c]n.d. = not detected.

the production of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) by carbonyl reduction reached a peak at 6 h after which some of the NNAL was metabolized. At 6 h, NNAL represented 26% of the total metabolites whereas, at 12 and 24 h, it represented only 12 and 4%, respectively. The products of alpha-carbon hydroxylation of NNK (keto alcohol, keto acid and diol) and of NNAL (i.e. hydroxy acid and diol, although these products have not yet been demonstrated to be produced from NNAL in rat lung *in vivo*) represented 28 and 27% of the total metabolites at 12 and 24 h, respectively. The release of hydroxy acid and keto acid into the medium was linear during 24 h, whereas the peak level of keto alcohol occurred at 6 h and that of diol at 12 h. The increased production of hydroxy acid at 12 and 24 h and of diol at 12 h corresponded with the depletion of NNAL from the medium. NNK was nearly depleted from the medium after 12 h of incubation. A similar profile was observed for the production of NNK metabolites by explants cultured in 1 μM NNK. However, when added at only 1 μM, the NNK was nearly depleted from the medium after only 6 h of incubation.

The effect on the metabolism of NNK (10 μM) by rat lung tissues treated concurrently with NNK and PEITC (cotreatment series) is shown in Figure 2a and b. The data represent an incubation time of 24 h. Figure 2a shows that as the concentration of PEITC increased, the amount of NNK and NNAL in the medium also increased, indicating an inhibition in the metabolism of both NNK and NNAL by PEITC. Figure 2b shows that PEITC inhibited the conversion of NNK and NNAL to their respective *N*-oxides by pyridine *N*-oxidation. In addition, the isothiocyanate inhibited the conversion of NNK and NNAL to their respective alpha-carbon hydroxylated metabolites; keto acid, diol, and hydroxy acid. In contrast, at 10 and 50 μM, PEITC appeared to stimulate the conversion of NNK to keto alcohol and/or to inhibit the conversion of keto alcohol to other products.

The effect on the metabolism of NNK (10 μM) by explants that had been pre-treated with PEITC for 16 h, then treated concurrently with PEITC and NNK for 24 h (pre-treatment series) is shown in Figure 3a and b. As was the case in the co-treatment series, PEITC inhibited the metabolism of NNAL as indicated by the accumulation of NNAL in the medium (Figure 3a). In addition, the conversion of NNK to its metabolites was inhibited. The isothiocyanate inhibited the conversion of both NNK and

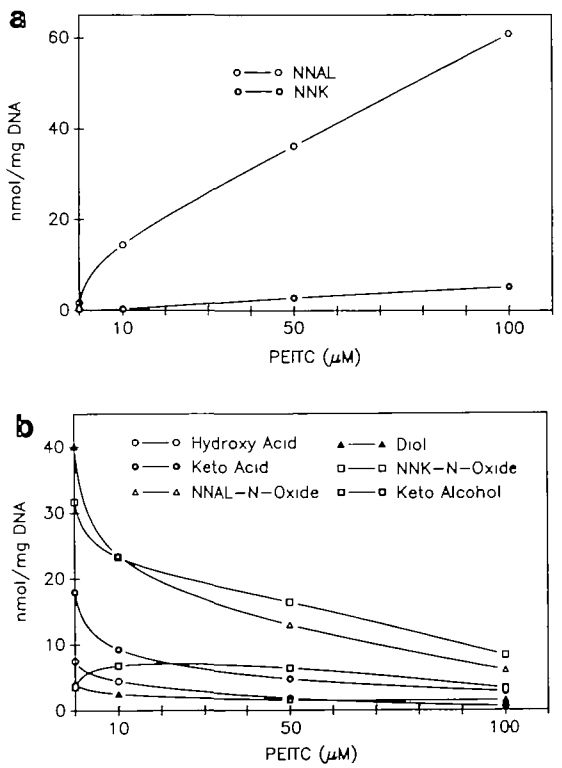

Fig. 2. (a) Effect of PEITC on the metabolism of NNK and production of NNAL by rat lung explants (co-treatment series). Explants were co-cultured with PEITC and NNK for a period of 24 h. Data are average values from three experiments. (b) Effect of PEITC on the production of NNK metabolites by rat lung explants (co-treatment series). Explants were cultured with PEITC and NNK for a period of 24 h. Data are average values from three experiments.

NNAL to their respective pyridine *N*-oxidized and alpha-carbon hydroxylated products in a dose-dependent manner (Figure 3b).

A summary of the effects of PEITC on the metabolism of NNK by cultured rat lung tissues is given in Table II. As indicated, with the exception of the concentration of diol in the medium of explants treated with 10 μM PEITC, the percent inhibition of formation of all NNK metabolites in the pre-treatment series exceeds that of the co-treatment series.

Discussion

In the present report, the metabolism of NNK in cultured rat lung tissues and the effect of PEITC on this metabolism is described. Analysis of NNK metabolites in the culture medium at different time periods indicated that all three pathways of NNK metabolism, i.e. pyridine *N*-oxidation, alpha-carbon hydroxylation and carbonyl reduction, are functional in rat lung tissues. The predominant metabolites (~70%) produced from NNK by pyridine *N*-oxidation were the two *N*-oxides, NNK-*N*-oxide and NNAL-*N*-oxide. This contrasts to results obtained with explants of A/J mouse or Syrian golden hamster lung, in which alpha-carbon hydroxylation was the predominant pathway (23,24). However, caution should be used in comparing the results of the present study with those of previous studies involving A/J mouse and hamster lung tissues since the experimental conditions (i.e. type of culture medium, concentration of NNK, incubation time before addition of the NNK etc.) in the present study were different from those in the previous studies. Our observations suggest that rat lung tissue can actively detoxify NNK since both *N*-oxides are more polar than the parent compounds, NNK and NNAL. In addition, NNK-*N*-oxide is significantly less tumorigenic than NNK in A/J mice (24).

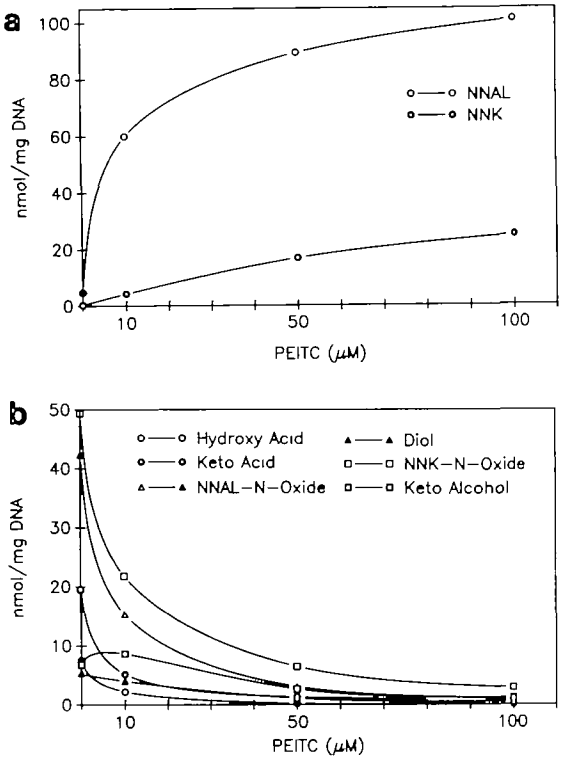

Fig. 3. (a) Effect of PEITC on the metabolism of NNK and production of NNAL by rat lung explants (pre-treatment series). Explants were pre-treated with PEITC for 16 h and then co-treated with PEITC and NNK for 24 h. Data are average values from three experiments. (b) Effect of PEITC on the production of NNK metabolites by rat lung explants (pre-treatment series). Explants were pre-treated with PEITC for 16 h and then co-treated with PEITC and NNK for 24 h. Data are average values from three experiments.

The conversion of NNK to hydroxy acid, keto acid, keto alcohol and diol by alpha-carbon hydroxylation is consistent with the observation that NNK methylates and pyridyloxobutylates rat lung DNA *in vivo* (10). Several lines of evidence indicate that the alpha-carbon hydroxylation of nitrosamines, including NNK, is catalyzed, at least in part, by cytochrome P450 enzymes (25). The metabolism of NNK by rat liver microsomes requires NADPH (9). The metabolism of NNK by rat lung microsomes was inhibited by antibodies to P450-2 (26) and its metabolism by cultured hamster lung was inhibited by SKF-525A, an inhibitor of P450 monooxygenases (23). The conversion of NNK to NNAL by carbonyl reduction is not completely understood, but studies with rat liver microsomes and 9000 *g* supernatant indicates that this occurs through an enzymatic mechanism involving carbonyl reductase (9).

PEITC inhibited the pyridine *N*-oxidation of NNK and NNAL to their respective *N*-oxides in explants of rat lung. This was responsible, in part, for the higher concentrations of both NNK and NNAL in the medium of PEITC-treated tissues. If a similar inhibitory effect of PEITC were to occur *in vivo* in lung and in liver tissues, then both NNK and NNAL could accumulate in these tissues. Since both NNK and NNAL are carcinogens (4,24), this could result in an elevated tumor response in PEITC-treated animals. However, the fact that PEITC has been shown to inhibit the induction of lung tumors by NNK in rats (14) and in mice (15,27) indicates that any accumulation of NNK and NNAL in tissues may be offset by the ability of PEITC to inhibit the alpha-carbon hydroxylation of NNK to DNA damaging metabolites. In addition, it is possible that any excess of NNK and NNAL in tissues may be eliminated by excretion in the urine (9).

PEITC was found to inhibit the alpha-carbon hydroxylation of NNK in rat lung explants. The mechanism(s) of this inhibition

Table II. Percent inhibition in formation of NNK metabolites by rat lung explants treated with NNK and PEITC

Treatment	PEITC (μM)	Hydroxy acid	Keto acid	NNAL-*N*-oxide	Diol	NNK-*N*-oxide	Keto alcohol
Co-treatment series[a]	control	–[b]	–	–	–	–	–[c]
	10	41	48	41	39	27	–
	50	76	74	67	62	48	–
	100	92	84	85	62	74	–
Pre-treatment series[a]	control	–	–	–	–	–	–
	10	71	73	64	25	56	0
	50	97	93	93	80	87	61
	100	99	96	97	91	94	84

[a]See Materials and methods for protocol for the treatment of lung explants with 10 μM NNK and PEITC.
[b]Data are average values from three experiments. In each experiment, three explant cultures were used per variable (i.e. conc. NNK and time point). Therefore, each data point represents the percent inhibition in metabolite formation calculated from a total of nine explant cultures.
[c]In the co-treatment series, keto alcohol was not inhibited; rather, its production was increased.

is (are) unknown, but presumably occurred via an alteration in the activity of cytochrome P450. The observation that PEITC was more inhibitory when the explants were pre-treated with PEITC before exposure to NNK is consistent with the hypothesis that PEITC acts via direct interaction with P450, thus inhibiting its expression. In addition, these results suggest that PEITC may be a more effective inhibitor of NNK carcinogenesis in animals if administered prior to the carcinogen, as well as during carcinogen treatment. Indeed, in animal studies in which PEITC was shown to inhibit NNK-induced lung tumorigenesis, the isothiocyanate was given either prior to and during NNK treatment (14), or prior to NNK treatment (15,27). In a recent study, PEITC was not effective as an inhibitor of NNK lung tumorigenesis in strain A/J mice when administered 1 week after the carcinogen (28). Based upon this observation, one could predict that PEITC will have little, if any, effect on the metabolism of NNK in rat lung explant cultures when administered after the NNK. Our results on the ability of PEITC to inhibit the alpha-carbon hydroxylation of NNK in rat lung explants are in agreement with a previous study in F344 rats. When the rats were administered PEITC in the diet for 2 weeks and then given four injections of [^{3}H]NNK, the methylation and pyridyloxobutylation of NNK in rat lung were inhibited (14). Additional studies are required for a further understanding of the mechanism of this inhibitory effect of PEITC.

In the present experiments, the inhibition of NNK metabolism by PEITC was highest under conditions in which the concentration of PEITC (100 μM) was 10-fold higher than that of the NNK (10 μM). This suggests that PEITC must be present in at least a 10-fold excess of the carcinogen in order to elicit the maximum inhibitory effect. However, in preliminary experiments, we observed that 85–90% of the PEITC in the culture medium becomes strongly bound to the plastic in the tissue culture dish within a short period after its addition to the medium. Attempts were made to reduce the binding of the PEITC to the surface of the dish by pre-coating the dishes with either serum or albumin, but these procedures did not reduce the binding. Therefore, the binding of PEITC to the dish in our experiments reduced the effective concentration of the isothiocyanate in the medium from 100 μM to ~10–15 μM. Considering that the NNK was added at 10 μM, PEITC appeared to be active in inhibiting NNK metabolism on an approximate mole/mole basis. These results suggest that PEITC is a potent inhibitor of NNK metabolism.

In a recent study, other isothiocyanates with increased alkyl chain lengths were shown to be more active than PEITC as inhibitors of NNK-induced lung tumors in strain A/J mice (27). For example, 3-phenylpropyl isothiocyanate and 4-phenylbutyl isothiocyanate were more potent inhibitors than PEITC. If these effects are associated with the ability of these compounds to inhibit the metabolism of NNK, then these isothiocyanates may be more effective inhibitors of NNK carcinogenesis in the rat lung than PEITC.

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