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Chemoprevention by Isothiocyanates

Stephen S. Hecht, PhD

American Health Foundation, Naylor Dana Institute for Disease Prevention, Division of Chemical Carcinogenesis, Valhalla, NY 10595

Abstract Naturally occurring and synthetic isothiocyanates are among the most effective chemopreventive agents known. A wide variety of isothiocyanates prevents cancer in the rat lung, mammary gland, esophagus, liver, small intestine, colon, and bladder. Mechanistic studies have shown that this chemopreventive activity is due to favorable modification of phase I and phase II carcinogen metabolism, resulting in increased carcinogen excretion or detoxification and decreased carcinogen DNA interactions. Most studies reported that the isothiocyanate must be present at carcinogen exposure in order to effect tumorigenesis inhibition. Our studies focus on naturally occurring isothiocyanates phenethyl isothiocyanate (PEITC) and benzyl isothiocyanate (BITC) as lung cancer inhibitors. These studies employed the major lung carcinogens in tobacco smoke, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and benzo(*a*)pyrene (B*a*P). Combining chemopreventive agents that inhibit tumorigenesis by NNK and B*a*P in rodents may be effective in addicted smokers. PEITC inhibits lung tumor induction by NNK in F-344 rats and A/J mice, while BITC inhibits B*a*P-induced lung tumorigenesis in A/J mice; combining the two inhibits lung tumorigenesis by combined NNK and B*a*P in A/J mice. PEITC selectively inhibits metabolic activation of NNK in the rodent lung, while inducing glucuronidation of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), one of the major NNK metabolites. Thus, PEITC decreases DNA and hemoglobin adduct formation by NNK while increasing the amounts of NNAL and its glucuronide excreted in the urine. Presently available data indicate that non-toxic doses of PEITC can inhibit the metabolic activation and carcinogenicity of NNK in rat and mouse lung; BITC has similar effects on B*a*P activation and tumorigenicity in mouse lung. Thus, combinations of chemopreventive agents active against different carcinogens in tobacco smoke may be useful in the chemoprevention of lung cancer.

Key words: Benzyl isothiocyanate, chemoprevention, isothiocyanates, lung cancer, phenethyl isothiocyanate

Isothiocyanates occur as thioglycoside conjugates known as glucosinolates in a wide variety of cruciferous vegetables [1]. When vegetable cells are damaged, as in chewing, the enzyme myrosinase is released. Myrosinase catalyzes the hydrolysis of glucosinolates. Isothiocyanates are then formed by a Lossen rearrangement, as illustrated in Figure 1. Isothiocyanates are responsible in part for the pungent taste associated with certain cruciferous vegetables. Consuming normal amounts of vegetables such as watercress or broccoli releases milligram amounts of isothiocyanates [1,2].

Both naturally occurring and synthetic isothiocyanates have been tested as chemopreventive agents. More than twenty compounds have been assessed, as summarized in Table I. Though most are effective, some such as α-naphthyl isothiocyanate would have limited utility due to toxic effects [3]. Isothiocyanates have shown chemopreventive activity in target tissues including rat

Address correspondence to Stephen S. Hecht, PhD, Director of Research, American Health Foundation, Naylor Dana Institute for Disease Prevention, Division of Chemical Carcinogenesis, One Dana Road, Valhalla, NY 10595.

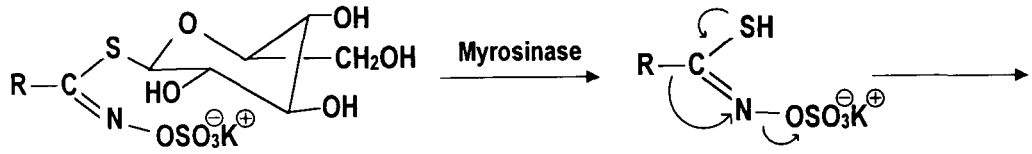


Fig. 1. Formation of isothiocyanates from glucosinolates.

lung, mammary gland, esophagus, liver, small intestine, colon, and bladder. Some studies showed a high degree of selectivity with respect to target tissue and isothiocyanate structure. For example, phenethyl isothiocyanate (PEITC) inhibits lung cancer in rats treated with 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), but has no effect on tumor induction in the liver or nasal cavity of these animals [4]. In mice, various isothiocyanates are effective inhibitors of lung and forestomach tumors, but none have shown efficacy against skin carcinogenesis [5]. Chung and co-workers [6,7] have carried out extensive structure-activity studies using isothiocyanates to inhibit mouse lung tumorigenesis induced by NNK. Results indicate that lipophilicity of the isothiocyanate increases chemopreventive efficacy, while reactivity with glutathione decreases efficacy. The rich variety of isothiocyanate inhibitors indicated in Table I suggests that mechanism-based structure-activity studies can lead to the design of highly effective isothiocyanates to inhibit carcinogenesis in a variety of systems.

In studies carried out to date, most isothiocyanates have shown chemopreventive activity in protocols involving administration of the isothiocyanate either before or during exposure to the carcinogen. Only one example of tumor suppression by isothiocyanates has been reported: benzyl isothiocyanate (BITC) given after the carcinogen inhibited 7,12-dimethylbenz[*a*]anthracene (DMBA)-induced rat mammary tumors [8]. These results are consistent with mechanistic studies showing that isothiocyanates are effective in inhibiting cytochrome P-450 enzymes which metabolize carcinogens as well as enhancing certain phase II enzymes such as NAD(P)H:quinone reductase and UDP-glucuronyl transferase involved in carcinogen detoxification. Enzymatic mechanisms involved in inhibition of carcinogenesis by isothiocyanates have been reviewed and will not be discussed here [9,10].

A significant degree of structural specificity has been noted in the effects of isothiocyanates on enzymes involved in carcinogen activation and detoxification. For example, PEITC strongly inhibited NNK metabolic activation and tumorigenicity in mouse lung; BITC had no effect [11, 12]. In contrast, PEITC had no effect on ethoxyresorufin *O*-dealkylase (EROD) activity associated with P-450 1A, while BITC strongly inhibited this enzyme [12]. These observations correlate with the specific chemopreventive activities of PEITC and BITC against mouse lung tumorigenesis by NNK or benzo[*a*]pyrene (B*a*P).

ISOTHIOCYANATES AS INHIBITORS OF LUNG CANCER

Chemoprevention may decrease the risk of lung cancer in both addicted smokers and smokers who have quit but are still at higher risk for lung cancer. The American Cancer Society estimated 153,000 deaths from lung cancer in the United States in 1994 [13], at least 80% of them caused by cigarette smoking [14]. If the use of chemopreventive agents could delay or prevent lung cancer in even a small percentage of addicted smokers, a large number of these deaths could be avoided.

Our approach to developing effective chemopreventive agents for lung cancer has been to focus on inhibition of lung carcinogenesis by tobacco smoke carcinogens. Among the approximately 50 known carcinogens present in tobacco smoke, polynuclear aromatic hydrocarbons (PAH), typified by B*a*P and tobacco-specific

TABLE I. Inhibition of Carcinogenesis by Isothiocyanates

Isothiocyanate R-N=C=S; R=	Carcinogen[a]	Species and Target Organ	Effect	Reference
α-Naphthyl–	3′-Me-DAB	Rat liver	+	20
	Ethionine	Rat liver	+	21
	AAF	Rat liver	+	21
	DAB	Rat liver	+	22
	m-Toluylenediamine	Rat liver	+	23
	NDEA	Rat liver	–	24
	BHBN	Rat bladder	+	25
β-Naphthyl–	DAB	Rat liver	+	22
Ph–	DMBA	Rat mammary	+	26
	NNK	Mouse lung	–	11
$PhCH_2$–	DMBA	Rat mammary	+	8,26
		Mouse forestomach	+	26
		Mouse lung	+	26
	BaP	Mouse lung	+	5,19
		Mouse forestomach	±	5,19
		Mouse skin	–	5
	NNK	Mouse lung	–	11,27

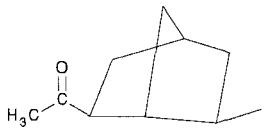
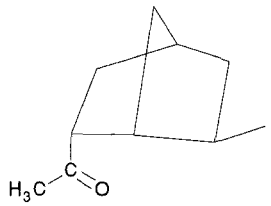
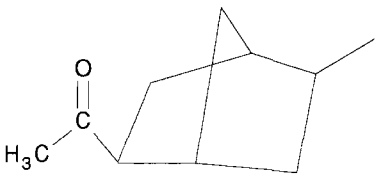
TABLE I. Inhibition of Carcinogenesis by Isothiocyanates (*continued*)

Isothiocyanate R-N=C=S; R=	Carcinogen[a]	Species and Target Organ	Effect	Reference
$PhCH_2$– (*continued*)	NDEA	Mouse forestomach	+	19
		Mouse lung	–	19
		Rat liver	+	28
	MAM	Rat small intestine/colon	+	29
	NBMA	Rat esophagus	–	G.D. Stoner, Unpublished
$Ph(CH_2)_2$–	DMBA	Rat mammary	+	26
		Mouse forestomach	+	26
		Mouse lung	+	26
	NNK	Rat lung	+	4
		Rat nasal cavity, liver	–	4
		Mouse lung	+	6,11,15,30
		Mouse lung	–	27
	NBMA	Rat esophagus	+	31
	B*a*P	Mouse lung	–	5,18
		Mouse skin	–	5
$Ph(CH_2)_3$–	NNK	Mouse lung	+	6,30
	NBMA	Rat esophagus	+	G.D. Stoner, Unpublished

TABLE I. Inhibition of Carcinogenesis by Isothiocyanates (*continued*)

Isothiocyanate R-N=C=S; R=	Carcinogen[a]	Species and Target Organ	Effect	Reference
$Ph(CH_2)_4$–	NNK	Mouse lung	+	6,30
	NBMA	Rat esophagus	+	G.D. Stoner, Unpublished
$Ph(CH_2)_5$–	NNK	Mouse lung	+	6
$Ph(CH_2)_6$–	NNK	Mouse lung	+	6
		Mouse skin	–	5
$Ph(CH_2)_8$–	NNK	Mouse lung	+	7
$Ph(CH_2)_{10}$–	NNK	Mouse lung	+	7
$PhCH(Ph)CH_2$–	NNK	Mouse lung	+	7
$PhCH_2CH(Ph)$–	NNK	Mouse lung	+	7
$CH_2=CHCH_2$–	NNK	Mouse lung	–	7
$CH_3(CH_2)_5$–	NNK	Mouse lung	+	7
$CH_3(CH_2)_3CH(CH_3)$–	NNK	Mouse lung	+	7
$CH_3(CH_2)_{10}CH_2$–	NNK	Mouse lung	+	7
3-Pyr$C(CH_2)_3$– (C=O)	NNK	Mouse lung	–	30
9-Phenanthryl–	B*a*P	Mouse skin	–	5
9-Methylenephenanthryl–	B*a*P	Mouse skin	–	5
6-Chrysenyl–	B*a*P	Mouse skin	–	5

TABLE I. Inhibition of Carcinogenesis by Isothiocyanates (*continued*)

Isothiocyanate R-N=C=S; R=	Carcinogen[a]	Species and Target Organ	Effect	Reference
6-Benzo[*a*]pyrenyl–	B*a*P	Mouse skin	–	5
$CH_3S(CH_2)_4-\overset{\overset{O}{\|}}{}$	DMBA	Rat mammary	+	32
	DMBA	Rat mammary	+	32
	DMBA	Rat mammary	+	32
	DMBA	Rat mammary	+	32

[a] 3′-Me-DAB, 3′-methyl-4-dimethylaminoazobenzene; DAB, 4-dimethylaminoazobenzene; AAF, 2-acetylaminofluorene; DMBA, 7,12-dimethylbenz[*a*]anthracene; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; B*a*P, benzo[*a*]pyrene; NDEA, *N*-nitrosodiethylamine; MAM, methylazoxymethanol acetate; NBMA, *N*-nitrobenzylmethylamine; BHBN, *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine.

NNK, are the most likely causes of lung cancer in smokers. This evaluation is based on tumor induction studies in laboratory animals, biochemical studies with lung tissue and cells from laboratory animals and humans, and detection of DNA adducts in the lungs of smokers. In smokers, exposure to PAH and NNK is chronic, resulting in a steady-state level of various DNA adducts which cause multiple genetic alterations in oncogenes and tumor suppressor genes associated with carcinogenesis. Since these compounds require metabolic activation, agents which decrease formation of the resulting electrophilic DNA binding intermediates should decrease DNA damage and thereby inhibit carcinogenesis.

Isothiocyanates, both naturally occurring and synthetic, can inhibit the metabolic activation and carcinogenicity of PAH and NNK, making them prime candidates for development as chemopreventive agents. Tobacco smoke also contains tumor promoters; in the classical initiation-promotion paradigm, inhibitors of tumor promotion should also be effective chemopreventive agents. Therefore, we believe combinations of chemopreventive agents, including those that inhibit metabolic activation as well as those that inhibit oxidative damage and tumor promotion, will eventually be necessary for maximum prevention of lung cancer in smokers.

PEITC has received the most attention in our studies to date. PEITC is a naturally occurring isothiocyanate, found as its glucosinolate conjugate gluconasturtiin in several vegetables including watercress. PEITC is released from watercress upon chewing by the action of myrosinase. Consumption of approximately 50 g of watercress entails the release of 10–15 mg of PEITC [2].

When PEITC was added to NIH-07 diet at a concentration of 498 ppm (3 μmol/g diet) before and during NNK treatment of male F-344 rats, it caused a significant and selective 50% reduction in the incidence of adenocarcinoma of the lung [4] (see Table II). A single 5 μmol dose of PEITC administered to A/J mice 2 hr prior to treatment with 10 μmol NNK resulted in a significant 62% reduction in lung tumor multiplicity [15]. Several other studies using multiple doses of PEITC have shown similar results in A/J mice (Table I). Thus, PEITC has been firmly established as an effective inhibitor of lung tumorigenesis induced by NNK in both rats and mice.

The potential toxicity of PEITC has been examined in 13-week studies in male and female

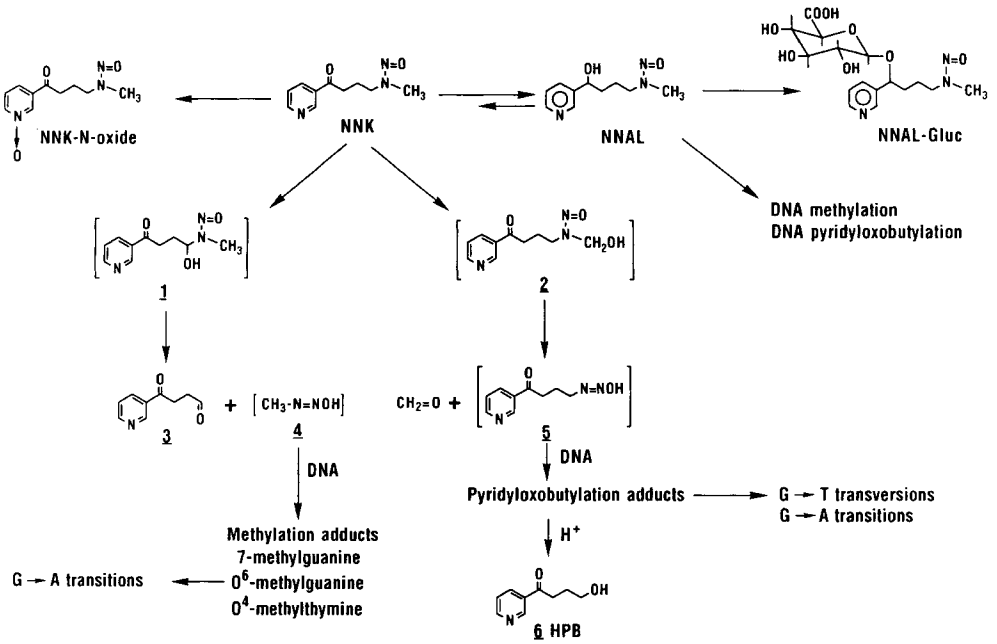


Fig. 2. Metabolism of NNK: An overview.

TABLE II. Incidence of Lung, Liver, and Nasal Cavity Tumors After Treatment with NNK, NNK+PEITC, and PEITC [4]

		Number of Rats with Tumors (%)								
		Lung			Liver			Nasal Cavity		
Treatment	No. of Rats	Adenoma	Carcinoma	Total	Adenoma	Hepatocellular Carcinoma	Total	Benign[a]	Malignant[b]	Total
NNK	40	8	24	32 (80)	12	3	15 (38)	8	3	11 (28)
NNK + PEITC	40	5	12[c]	17 (43)[d]	9	5	14 (35)	6	1	7 (18)
PEITC	20	0	0	0 (0)	4	2	6 (30)	0	1	1 (5)
Control	20	1	0	1 (5)	3	1	4 (20)	0	1	1 (5)

[a] Squamous cell papillomas, transitional cell papillomas, polyps; [b]squamous cell carcinoma; [c]one squamous cell carcinoma, 11 adenocarcinoma; [d] $p < 0.05$ compared to NNK group.

F-344 rats. PEITC was added to NIH-07 diet at concentrations of 500, 1,500, and 2,500 ppm. Although analysis of the data from this study is not complete, the results to date demonstrate no significant toxic effects, with the exception of some thickening of the keratinized layer of the forestomach at the 2,500 ppm dose. No toxic effects were observed in the rats treated with 500 ppm, which is the dose employed in the NNK chemoprevention studies.

An overview of the major metabolic activation and detoxification pathways of NNK are illustrated in Figure 2 [16]. In laboratory animals and humans, NNK is rapidly converted to 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) by carbonyl reductase enzymes. NNAL, a potent pulmonary carcinogen, is partially converted to its diastereomeric glucuronides, NNAL-Gluc. These glucuronides are believed to be detoxification products of NNK, although this has not been definitively established. Pyridine *N*-oxidation of NNK and NNAL gives the corresponding *N*-oxides which are detoxification products. Metabolic activation of NNK proceeds by α-hydroxylation of the methylene and methyl carbons, producing unstable intermediates 1 and 2. These spontaneously decompose with formation of aldehydes and the electrophilic diazohydroxides 4 and 5. Diazohydroxide 4 methylates DNA of NNK target tissues, producing permanent mutations, mainly of the G→A type. Diazohydroxide 5 alkylates DNA, producing both G→A and G→T mutations. It also reacts with hemoglobin to form ester adducts. Hydrolysis of DNA or hemoglobin obtained from animals treated with NNK or from smokers produces 4-hydroxy-1-(3-pyridyl)-1-butanone (HPB), a biomarker of the metabolic activation of NNK [17]. Smokers' urine contains quantifiable amounts of NNAL and NNAL-Gluc as biomarkers; the ratio of NNAL-Gluc to NNAL may be useful as an index of NNK detoxification [17].

The mechanism of inhibition of NNK carcinogenesis by PEITC has been examined. Initial studies demonstrated that PEITC inhibited NNK's metabolic activation to electrophiles which methylate and pyridyloxobutylate pulmonary DNA in rats; inhibition of hemoglobin adduct formation was also observed. Subsequently, detailed investigations of the effects of PEITC on NNK metabolism in mouse and rat liver and lung, as well as studies of other enzyme activities, have clearly demonstrated that PEITC's effect on NNK carcinogenesis is due to inhibition of NNK metabolic activation to methylating and pyridyloxobutylating electrophiles and enhancement of its detoxification [9,12]. In rats treated with PEITC by gavage or by addition to the diet, a persistent inhibition of NNK metabolic activation is observed in lung microsomes, resulting from inhibition of cytochrome P-450 enzymes. In contrast, a persistent inhibition in liver microsomes is not observed; rather, there is induction after initial inhibition. Experiments *in vitro* have shown that PEITC is a competitive inhibitor of NNK metabolic activation in rat lung microsomes, with IC_{50}s ranging from 150–210 nM, and in explants of rat lung.

The effects of PEITC on NNK metabolism have also been examined *in vivo*. In these experiments, the goal was to determine whether the observed inhibition of tumorigenesis was due to specific inhibition of NNK metabolic activation, or whether treatment with PEITC might have caused a change in NNK distribution, resulting in diminished amounts of the carcinogen reaching extrahepatic tissues. Experiments using a protocol essentially identical to that employed in the carcinogenicity study showed that levels of NNK and its primary metabolite NNAL were not markedly different in tissues of PEITC-treated and control rats. However, the data clearly indicated a decrease in the levels of NNK metabolic activation in the PEITC-treated rats in almost all tissues examined.

Ongoing rat studies examine the effects of chronic PEITC treatment on hemoglobin adducts and urinary metabolites of NNK. Results of the urinary metabolite analyses show that chronic PEITC treatment caused significant 4–6-fold increases in the levels of NNAL and NNAL-Gluc in urine; this most likely results from a decrease in NNK metabolic activation since hemoglobin adducts of NNK also decreased (data not shown). The ratio of NNAL-Gluc to NNAL, a potential biomarker of NNK detoxification, increased upon PEITC treatment, consistent with previous observations that PEITC induced UDP-glucuronosyl transferase activity [9]. Collectively, the results of these studies clearly show that PEITC exerts a specific inhibitory effect on the metabolic activation of NNK while enhancing detoxification, without causing any apparent toxic effects in rats.

TABLE III. Effects of BITC and PEITC on BaP-Induced Lung and Forestomach Tumorigenesis in A/J Mice[a] [5]

Group	% Mice with Tumors		Tumors per Mouse	
	Lung	Forestomach	Lung	Forestomach
BaP only	95	95	4.8	4.8
BITC/BaP	80	95	2.6[b]	4.9
PEITC/BaP	90	90	4.0	2.5[b]

[a] Female A/J mice given 6.7 μmol isothiocyanate ig 15 min prior to 7.9 μmol BaP, 3 times at 2 week intervals, and sacrificed 26 weeks after first dose, [b]$p < 0.001$.

EFFECTS OF PEITC AND BITC ON BaP-INDUCED LUNG CARCINOGENESIS

While PEITC is an effective inhibitor of NNK-induced lung carcinogenesis, studies to date have not demonstrated efficacy with respect to BaP. In one study in A/J mice, PEITC was administered by gavage prior to intraperitoneal (ip) injection of BaP. No inhibition of BaP-induced lung tumorigenesis was observed over a range of PEITC doses [18]. In a second study, a single dose of 6.7 μmol PEITC was given by gavage to A/J mice, 15 min prior to gavage of 7.9 μmol of BaP. No inhibition of lung tumorigenesis was observed, although PEITC did inhibit BaP-induced forestomach tumors. In contrast, a 7.9 μmol dose of BITC given by the same protocol achieved a statistically significant 50% reduction of BaP-induced lung tumor multiplicity in the A/J mouse model (Table III) [5]. These results agree with previously reported data on inhibition of BaP-induced lung tumorigenesis by BITC [19]. The contrasting effects of PEITC and BITC on lung tumorigenesis by BaP in A/J mice are consistent with mechanistic studies showing that BITC but not PEITC significantly inhibited ethoxyresorufin *O*-dealkylase activity in A/J mouse lung microsomes, indicating inhibition of cytochrome P-450 1A, which may be involved in the metabolic activation of BaP [12]. In ongoing studies, we are examining the effects of BITC and PEITC on metabolic activation and DNA binding of BaP in A/J mouse lung and liver.

A MODEL FOR EVALUATING CHEMOPREVENTIVE AGENTS AGAINST NNK AND BaP

Since both NNK and BaP are likely to play significant roles in the induction of lung cancer in smokers, we have developed an A/J mouse model for evaluating chemopreventive agents against these two carcinogens in combination.

Groups of female A/J mice were treated by either intragastric gavage (ig) or by ip injection with various doses of NNK and/or BaP for eight consecutive weeks. The mice were sacrificed either eight or eighteen weeks later and tumors of the lung and forestomach were counted. The ig route of administration proved to be more satisfactory than ip administration because it avoided complications due to tumor formation at the injection site and associated mortality. A dose-response relationship for lung tumor induction by ig administration of NNK and BaP in combination was established in the mice sacrificed eight or eighteen weeks after completion of carcinogen treatment (Table IV). Forestomach tumors were also observed in all mice treated ig with BaP, or NNK and BaP. The highest total doses of NNK and BaP (total of 24 μmol of each) induced more lung tumors than would have been expected by extrapolation from the lower doses. Comparisons of NNK and BaP given individually showed that BaP was more tumorigenic to the lung than NNK when given by the ig route; the ip administrations of BaP were complicated by local tu-

TABLE IV. Incidence and Multiplicity of Lung Adenomas in A/J Mice Treated with NNK, B*a*P, or Both[a] [33]

Compound(s)	Route	Total Dose (μmol)	Effective No. of Mice[b]		No. of Mice at Termination		Weight at Termination (g±S.D.)		% Lung Adenoma-Bearing Mice at		Lung Adenomas/ Mouse±S.D. at	
			16 wk	26 wk	16 wk	26 wk	16 wk	26 wk	16 wk	26 wk	16 wk	26 wk
NNK[c]	ig	8	–	15	–	15	–	24.3±2.09	–	64	–	0.93±0.96
NNK	ig	16	–	15	–	14	–	26.0±2.94	–	100	–	4.6±3.3[d]
NNK	ig	24	–	14	–	15	–	24.3±2.03	–	100	–	10.0±6.3[d]
B*a*P	ig	8	–	15	–	15	–	24.8±3.43	–	93	–	3.3±4.1[d,e]
B*a*P	ig	16	–	15	–	15	–	23.9±1.64	–	100	–	7.2±3.9[d,e]
B*a*P	ig	24	–	15	15	15	–	25.1±2.72	–	100	–	14.0±3.9[d,e]
NNK+B*a*P	ig	8+8	–	15	15	15	23.7±2.06	24.8±2.88	80	87	1.7±1.2[d]	3.3±3.0[d]
NNK+B*a*P	ig	16+16	15	15	15	15	23.2±2.76	26.0±2.07	100	100	4.2±2.5[d]	7.3±4.0[d]
NNK+B*a*P	ig	24+24	15	15	15	15	22.5±2.97	23.9±2.46	100	100	10.5±4.4[d]	22.7±10.0[d]
NNK+B*a*P	ig	8+24	15	15	15	15	25.1±3.43	25.1±2.79	100	100	8.4±4.1[d]	19.7±10.5[d]
Vehicle Control	ig	–	15	15	15	15	19.1±1.64	24.5±2.70	20	0	0.2±0.4	0

TABLE IV. Incidence and Multiplicity of Lung Adenomasin A/J Mice Treated with NNK, B*a*P, or Both[a] (*continued*)

Compound(s)	Route	Total Dose (μmol)	Effective No. of Mice[b]		No. of Mice at Termination		Weight at Termination (g±S.D.)		% Lung Adenoma-Bearing Mice at		Lung Adenomas/ Mouse±S.D. at	
			16 wk	26 wk	16 wk	26 wk	16 wk	26 wk	16 wk	26 wk	16 wk	26 wk
NNK[c]	ip	8	–	15	–	15	–	24.3±2.02	–	93	–	2.5±1.5[d,g]
NNK	ip	16	–	15	–	15	–	24.3±2.50	–	100	–	14.5±5.8[d,f,g]
NNK	ip	24	–	15	–	15	–	24.8±2.83	–	100	–	28.9±6.1[d,f,g]
B*a*P	ip	8	–	14	–	14	–	22.1±1.73[i]	–	79	–	4.9±4.1[d,e,h]
B*a*P	ip	16	–	15	–	10	–	22.5±5.58[i]	–	80	–	6.2±5.2[d]
B*a*P	ip	24	–	13	–	4	–	23.0±2.55[i]	–	69	–	7.2±8.2[d]
NNK+B*a*P	ip	8+8	15	15	15	13	24.5±3.81	23.5±2.75	93	100	5.1±3.5[d,g]	12.7±4.7[d,g]
NNK+B*a*P	ip	16+16	15	15	15	4	20.6±2.56	22.8±1.86[i]	93	100	12.6±6.9[d,g]	23.9±17.7[d,g]
NNK+B*a*P	ip	24+24	14	15	12	2	20.8±1.29[i]	24.0±7.07[i]	86	87	18.1±9.4[d,g]	9.5±10.5[d,g]
NNK+B*a*P	ip	8+24	10	15	10	1	21.9±2.18[i]	22.6±2.18[i]	83	73	8.4±7.8[d]	7.5±8.4[d]
Vehicle Control	ip	–	15	15	15	15	20.3±1.35	22.9±2.07	6.7	6.7	0.1±0.5	0.1±0.3

[a] Beginning at age 7 weeks (mean weight 18.0±0.29 g), groups of 15 female A/J mice were given 8 consecutive weeks of treatments of NNK, B*a*P, or NNK+B*a*P in 0.1 ml cottonseed oil by the routes indicated. Each weekly dose was 1/8 of the total indicated. Mice were sacrificed 16 or 26 weeks after the first treatment, and lung adenomas were counted. Results for these sacrifice times are indicated in the "16 wk" and "26 wk" colums; [b]no. of mice alive at 12 weeks; [c]mice given either NNK or B*a*P were sacrificed at 26 weeks only; [d]significantly greater than vehicle control, $p < 0.001$; [e]significantly greater than NNK group, $p < 0.005$; [f]significantly greater than B*a*P group, $p < 0.001$; [g]significantly greater than ig treated group, $p < 0.001$; [h]significantly greater than ig treated group, $p < 0.05$; [i]mean ±S.D. based on mice alive at 26 weeks.

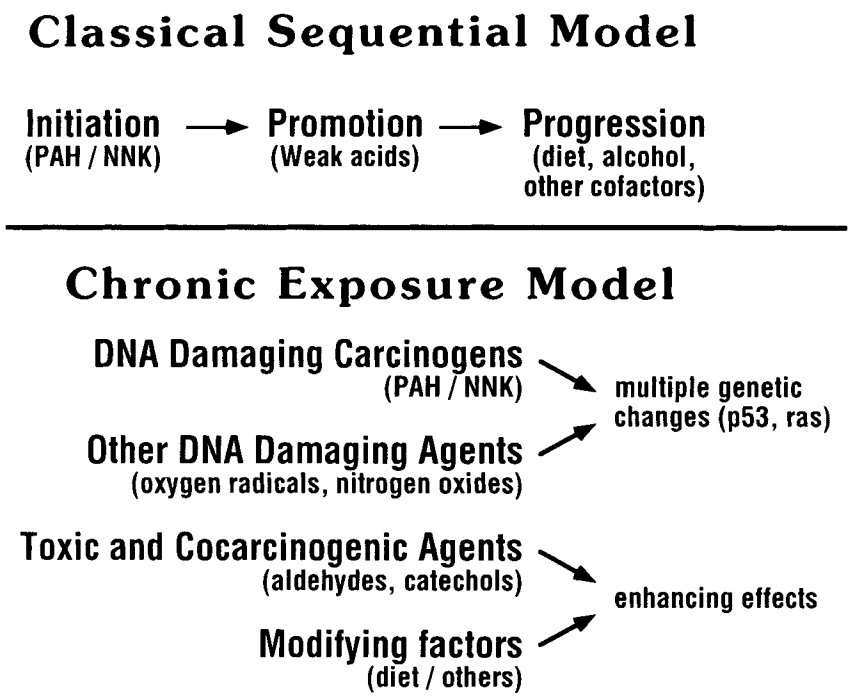


Fig. 3. Two models of tobacco-induced lung cancer. In the classical sequential model, exposure to a DNA-damaging initiator is followed by exposure to agents which cause promotion and progression. In the chronic exposure model, continual simultaneous exposure to all compounds in tobacco smoke leads to multiple genetic changes and other effects associated with the multistage carcinogenic process.

mor formation and mortality. The most favorable dosing regimen of NNK and B*a*P to evaluate chemopreventive agents against lung tumor formation appears to be a total dose of 24 μmol of each, administered in eight weekly ig subdoses, with sacrifice eight weeks after completion of dosing. This regimen induced 10.5 ± 4.4 lung adenomas per mouse. In view of the data described above, we evaluated the effects of BITC and PEITC in combination (6 μmol of each), given 2 hr prior to each of eight ig treatments with NNK and B*a*P (3 μmol of each; 24 μmol total). The mixture of BITC and PEITC was an effective inhibitor of lung tumor formation, reducing the tumor multiplicity from 10.5 ± 4.4 to 5.9 ± 5.7 lung adenomas per mouse ($p < 0.001$) and completely inhibiting forestomach tumor development. These results for BITC and PEITC in combination are consistent with their individual effects as inhibitors of B*a*P and NNK carcinogenesis in the A/J mouse lung.

STRATEGIES FOR CHEMOPREVENTION OF LUNG CANCER

Since smoking causes up to 80% of all lung cancer, smokers are a logical target for chemoprevention. However, chemoprevention should be used only in some smokers and should never be considered as an alternative to quitting. Eligible subjects should be highly motivated to quit smoking, but have failed in smoking cessation programs due to their addiction to nicotine. Chemoprevention is also likely to be most effective in smokers who have been smoking for perhaps only ten years or less. The inhibitors discussed in this paper must be present at the time of carcinogen administration in order to be effective against lung tumor induction. This raises questions about their potential efficacy, depending on how tobacco carcinogenesis is viewed. Two models of tobacco carcinogenesis are outlined in Figure 3. In the classical sequential model, initiation

by compounds such as NNK and B*a*P is followed by promotion and progression. Tobacco smoke is known to contain tumor promoters and the partial reversibility of lung cancer risk associated with quitting is consistent with the reversibility of promotion. However, it is perhaps unrealistic to think of tobacco carcinogenesis using only this model. Smokers are simultaneously exposed to carcinogens such as NNK and B*a*P, together with promoters, co-carcinogens, and toxic compounds, with every cigarette that is smoked. Therefore, the chronic exposure model may be more realistic and is consistent with multiple genetic changes associated with the carcinogenic process. These genetic changes can be decreased if inhibitors such as BITC and PEITC are present. Probably a combination of chemopreventive agents, including blocking agents such as the isothiocyanates and suppressing agents, will be necessary for effective chemoprevention of lung cancer in smokers.

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